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# Antibacterial Activity of (+) Usnic Acid against Multi Drug Resistant *Acinetobacter baumannii* from Clinical Isolates

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## Abstract

Acinetobacter baumannii (Ab) is developing resistance to a variety of common antibiotics. and become multidrug resistant, extreme drug resistant, and pan drug resistant pathogens, requiring the identification of new antibiotics as well as the identification of new plant compounds capable of acting against Ab. Recent research has revealed MDR Ab co-infections with COVID-19,, raising alarm bells. Since its isolation, Usnic acid has been investigated for a variety of pharmacological activities, including antioxidant, antitumor, antibacterial, antifungal, antiviral, antiprotozoal, and insecticidal. Many Plant-derived drugs show promising activity as new antimicrobial agents against multidrug resistant (MDR) strains. There is insufficient data to support the antibacterial activity of (+)-UA against MDR Acinetobacter baumannii.. In the present study, we evaluated the antimicrobial activity of naturally occurring compound (+) usnic acid (UA) against MDR Ab. We determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), time-kill assay in twenty multidrug resistant *Acinetobacter baumannii* clinical isolates collected from two different centers. Results revealed promising activity of (+)-UA with MIC concentration of 512–1024 µg/mL and MBC 2048 –4096 µg/mL. The MBC/MIC index indicated that the compound was bactericidal. The time-kill assay revealed a gradual decrease in the log10 value of the bacteria. Since there is a limited research available on the activity of usnic acid against MDR Acinetobacter baumannii, present study fills the gap.

**Abbreviations:** *Acinetobacter baumannii* = Ab; MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; UA = (+) - usnic acid

***Keywords:*** *Antibacterial activity, multi-drug resistant, Acinetobacter baumannii, (+) usnic acid.*

## Introduction

Antimicrobial resistance is a global issue in infectious diseases control. About 700,000 deaths occur globally in a year due to antimicrobial resistant infections. According to the Antimicrobial Resistance report, 10,000,000 deaths may occur annually worldwide by 2050 causing heavy burden on the economy [(1)]. In the present pandemic situation, the antimicrobial resistance is also aggravating. A meta-analysis from five countries showed 3.5% of co-infection and 14.3% of secondary infection with COVID–19 infection [(2)]. According to a recent study from Iran, 19 patients infected with COVID-19, Out of them 17 patients co-infected with MDR A. baumannii all of them died which demonstrating the pathogen's risk [(3)]. Efforts are being undertaken to control antimicrobial resistance by governmental organizations, giving awareness on the effect of overuse of antibiotics and its impact on health. The bacteria survive in the presence of antibiotics by adapting various mechanisms of resistance by

synthesizing proteins and developing new pathways [4]. Among various microorganisms that cause infections, a group of organisms known as ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) cause concern. These pathogens cause life threatening hospital acquired infections [5].

Multi drug resistant (MDR) *Acinetobacter baumannii* (Ab) is considered to be a hospital acquired infection globally. High mortality and prolonged hospital stay are reported in patients infected with MDR Ab [6, 7]. The bacteria undergo mutations and resistance mechanisms like efflux pump and enzyme degradation. In 2017, the World Health Organization released a list of bacteria and emphasized member countries to promote research and development for new antibiotics. In this list Ab has been categorized as one of the most critical organisms [8]. In carbapenem resistant isolates of Ab, the alternative treatment is tigecycline and colistin [9]. In some instances, resistant to these antibiotics were also reported due to uncontrolled use. Studies have reported, 74.2% and 53.1% resistance to tigecycline and colistin respectively [10, 11]. As multidrug resistance has been observed in these pathogens, there is a demand for new methods and drug treatment. Plant extracts and derivatives are widely evaluated as antimicrobial agents against MDR strains [12]. Hence there is a need to evaluate the efficacy of plant extracts for MDR Ab.

Usnic acid (UA) is a lichen derived secondary metabolite with a unique dibenzofuran skeleton and is commonly found in lichenized fungi of the genera *Usnea*, *Ramalina*, and *Cladonia*. The lichens symbiotically coexist with cyanobacteria and produce various secondary metabolites. (+) Usnic acid (UA) is one such compound isolated from various lichens and has been studied for many biological properties including antibacterial activity. The structural characteristics of UA combined with its physiochemical properties are responsible for its pleiotropic biological effects. UA has been used in medicinal products, perfumes, cosmetics. It possesses a broad spectrum of bioactivities, like antimicrobial, analgesic activity, anti-inflammatory antiviral, and anticancer. The antibacterial efficacy of lichen extracts and compounds present in them have been studied for many years. Many researchers patented the antimicrobial effect of UA [13, 14, and 15]. Usnic acid mechanism of action is not completely understood till now. Nonetheless, research indicates that Usnic acid's inhibition of bacterial nucleic acid replication and synthesis results in this action [16]. Hence, in the present study the effect of UA was evaluated for its antibacterial activity against MDR Ab.

## Materials and Methods

***Chemicals:*** Mueller Hinton agar, Mueller Hinton broth and brain heart infusion broth were purchased from M/s Himedia (Mumbai, India). Colistin was procured from M/s Cipla (Mumbai, India).

***Bacterial Strains:*** Twenty MDR Ab clinical isolates from S.V.S Medical College and Hospital (Mahabubnagar, Telangana, India) and A.C.S.R Medical College and Hospital (Nellore, Andhra Pradesh, India), collected in 2019 were used for the study. Approval from Institutional Biosafety Committee of Saveetha Medical College and Hospital (001/08/2020/IBSC/SIMATS) and Institutional Ethics Committee of Saveetha Medical College and Hospital (003/09/2020/IEC/SMCH) were obtained for carrying out the present study. All the experiments were conducted in the Biosafety Cabinet (BSL – II) and the used materials were disposed as per the Standard Operating Procedures of the institution..

**Isolation of UA:** UA was isolated following the reported procedure [17]. About 200 g of shade-dried lichen (*Roccella montagnei* Bel.) was extracted with methanol in cold. After 72 hr the methanolic extract was successively treated with petroleum ether and acetone. The acetone fraction was subjected to column chromatography on silica gel and eluted with solvents of increasing polarity. Elution of the column with benzene afforded yellow shining crystals with melting point of 196 – 198ºC. Based on spectroscopic analysis, the above compound was identified as UA (Yield = 1.0 g). The purities of the above isolated compounds were confirmed by comparison with respective authentic samples by thin layer chromatography mixed and melting point determination and super-imposable infra-red spectroscopy.

**Minimum inhibitory concentration (MIC):** Identification and estimation of the minimum inhibitory

concentration of all twenty clinical isolates of Ab were done by using Vitek2 system [18]. All the twenty MDR Ab (freshly prepared dilutions from glycerol stock isolates) were tested. Antibacterial activity of the compound was done by microbroth dilution following Clinical and Laboratory Standards Institute (CLSI) guidelines. Stock solution of the compound was prepared in dimethyl sulfoxide (DMSO). In a 96-well microtitre plate, serial dilution of the compound ranging from 2048 - 4 µg/mL was done in Mueller Hinton broth. To this, 50 µL of bacterial inoculum (1.5 x $10^5$ CFU/ mL) was added and the microtitre plate was incubated at 37°C for 18 hr. The growth of the organism in each well was visually detected and MIC was noted where the growth was inhibited. The test was performed in triplicates.

**Minimum bactericidal concentration (MBC):** 10 µL of 1x, 2x, and 4x MIC from the 96 well microtitre plate were sub-cultured on Mueller Hinton agar and incubated for 24 hr at 37°C. After incubation, the highest dilution which yielded no bacterial growth on the plates was recorded as MBC. The test was done in triplicate.

**MBC/ MIC Index:** The MBC/MIC index was calculated. Equal to or more than 4.0 was taken as bactericidal and less than 4.0 as bacteriostatic [19].

**Time kill kinetics assay:** Time kill kinetics assay was performed for 1x and 2x MIC following CLSI guidelines by broth macro dilution method [20]. A final inoculum of 1.5 x $10^5$ CFU/mL was taken in tubes. The tubes were shaken periodically and incubated at 37°C. At time intervals of 4, 8, 12, 16, 20, and 24 hr, 1 µL of inoculum was collected with a sterile loop from each tube, spread on Mueller Hinton agar, and incubated for 24 hr at 37°C. The colony counts were determined. Colistin was used as positive control and for negative control Ab alone was inoculated in brain heart infusion broth.

**Statistical Analysis:**. Linear regression analysis was used for time kill kinetics assay. A probability of 0.05 or equal was taken as statistically significant. SigmaPlot 14.5 (Systat Software, USA) was used for statistical analysis and graph plotting.

## Results

In this present study the antimicrobial activity of Usnic acid to inhibit the bacterial growth of MDR Acinetobacter baumannii were investigated by using MIC, MBC, and time kill assay. The twenty isolates of MDR-Ab used in this study were isolated from various specimens details are given in the table No. 1.

**Table No. 1: MDR *Acinetobacter* isolates isolated from various specimens**

| Sl. No. | Isolate No. | Strain type | Specimen |
|---|---|---|---|
| 1 | AI 1444 | A. baumannii | Endotracheal tube |
| 2 | AI 646-2 | A. baumannii | Cerebrospinal fluid |
| 3 | AI 646-5 | A. baumannii | Endotracheal tube |
| 4 | AI 4185 | A. baumannii | Endotracheal tube |
| 5 | AI 829 | A. baumannii | Endotracheal tube |
| 6 | AI 6142 | A. baumannii | Endotracheal tube |
| 7 | AI 7783 | A. baumannii | Endotracheal tube |
| 8 | AI 6553 | A. baumannii | Pus |
| 9 | AI 5678 | A. baumannii | Endotracheal tube |
| 10 | AI 6538 | A. baumannii | Pus |
| 11 | AI 3990 | A. baumannii | Endotracheal tube aspirate |
| 12 | AI 4888 | A. baumannii | Endotracheal tube |
| 13 | AI 3074 | A. baumannii complex | Pus |
| 14 | AI 3927 | A. baumannii complex | Sputum |
| 15 | AI 899 | A. baumannii complex | Pus |
| 16 | AI 6428 | A. baumannii complex | Pus |
| 17 | AI 2760 | A. baumannii complex | Endotracheal tube |
| 18 | AI 2540 | A. baumannii complex | Endotracheal tube |
| 19 | AI 7496 | A. baumannii | Endotracheal tube |
| 20 | AI 2368 | A. baumannii | Endotracheal tube |

**MIC, MBC and MBC/MIC ratio of isolated compound:**

In microbroth dilution method the efficacy of (+) - UA showed MIC ranged between 512 – 1024 µg/mL against all the isolates. The MBC of (+) - UA was ranged between 2048 – 4096 µg/mL. The mean of MIC and MBC concentrations of triplicate data are given in the table No. 2. The MIC/ MBC index ratio of twenty isolates were reported at 4, which indicate the bactericidal activity of compound against MDR *Acinetobacter* isolates results are given in table No. 2.

**Table No. 2: MIC and MBC of (+) - UA against MDR *Acinetobacter* isolates**

| Sl. No. | Isolate Number | Strain type | Usnic acid | | |
|---|---|---|---|---|---|
| | | | MIC µg/ ml | MBC µg/ ml | MIC / MBC Index |
| 1 | AI 1444 | A. baumannii | 512 | 2048 | 4 |
| 2 | AI 646-2 | A. baumannii | 1024 | 4096 | 4 |
| 3 | AI 646-5 | A. baumannii | 1024 | 4096 | 4 |
| 4 | AI 4185 | A. baumannii | 1024 | 4096 | 4 |
| 5 | AI 829 | A. baumannii | 1024 | 4096 | 4 |
| 6 | AI 6142 | A. baumannii | 1024 | 4096 | 4 |
| 7 | AI 7783 | A. baumannii | 1024 | 4096 | 4 |
| 8 | AI 6553 | A. baumannii | 1024 | 4096 | 4 |
| 9 | AI 5678 | A. baumannii | 1024 | 4096 | 4 |
| 10 | AI 6538 | A. baumannii | 1024 | 4096 | 4 |
| 11 | AI 3990 | A. baumannii | 1024 | 4096 | 4 |
| 12 | AI 4888 | A. baumannii | 1024 | 4096 | 4 |
| 13 | AI 3074 | A. baumannii complex | 1024 | 4096 | 4 |
| 14 | AI 3927 | A. baumannii complex | 1024 | 4096 | 4 |
| 15 | AI 899 | A. baumannii complex | 1024 | 4096 | 4 |
| 16 | AI 6428 | A. baumannii complex | 512 | 2048 | 4 |
| 17 | AI 2760 | A. baumannii complex | 1024 | 4096 | 4 |
| 18 | AI 2540 | A. baumannii complex | 1024 | 4096 | 4 |
| 19 | AI 7496 | A. baumannii | 1024 | 4096 | 4 |
| 20 | AI 2368 | A. baumannii | 1024 | 4096 | 4 |

**Time kill kinetics assay**

The time taken to reach the desired reduction for colistin (positive control), Ab alone (negative control), UA is given in Figure 1. The time-kill kinetics assay of the compound against test organism at 1x, 2x MIC concentrations showed a reduction in $\log_{10}$. For 2x MIC's of UA there was 100% bacterial growth inhibition after 8 hr. Statistical correlation between colistin, Ab alone, 1x UA was carried out to find the $\log_{10}$ reduction by linear regression analysis. The linear regression analysis as done in the study [21] was done to see the efficacy of compound when compared with positive and negative control. The correlation coefficient for colistin, Ab alone, and 1x UA were 0.990, 0.943, and 0.959 respectively. The estimated slope for colistin, Ab alone, and UA were 0.129, 0.053, and 0.082 respectively, this shows that UA is having approximately similar effect like positive control.

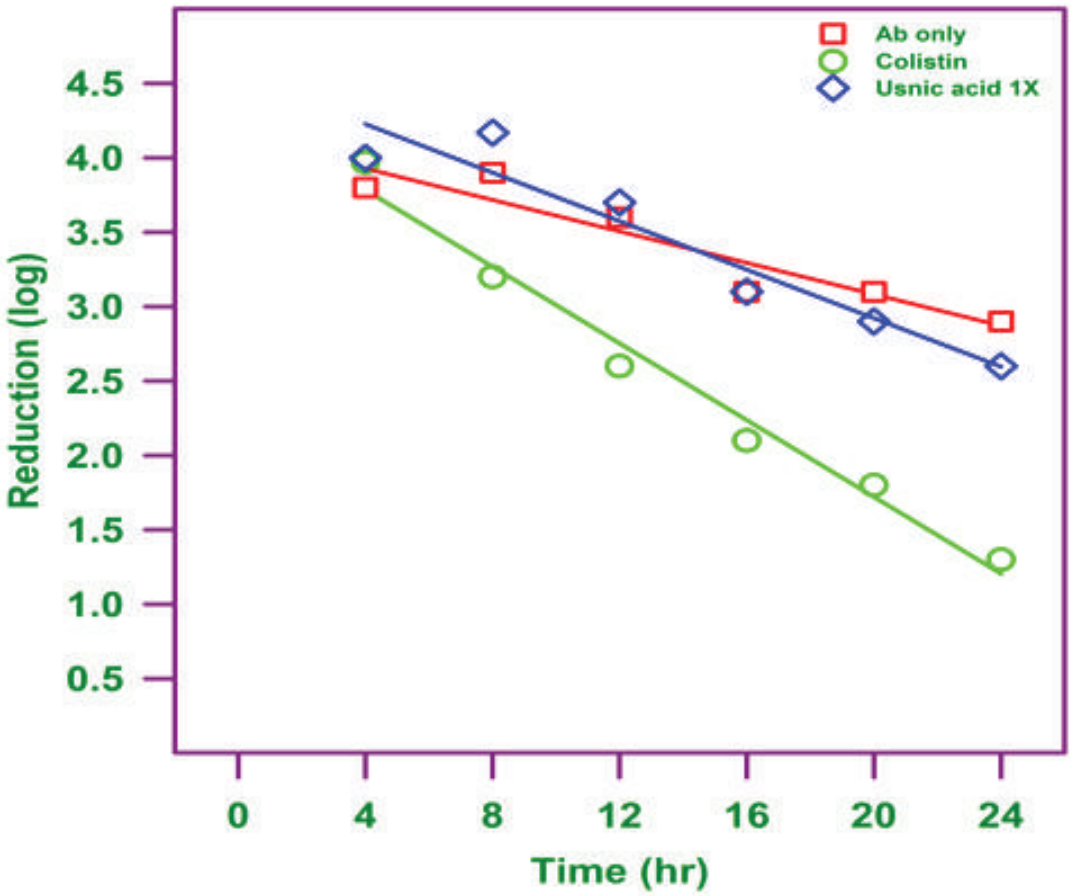


**Figure No.1 Time kill kinetics assay of UA**

## Discussion

Previous studies reported that UA possesses antimicrobial properties against not only Gram-positive but also Gram-negative microorganisms. UA has been shown to be bacteriocidal against Gram-negative bacteria such as *Bacteroides, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Proteus vulgaris, Yersinia enterocolitica*, and strong effect on *H. pylori* [15]. Due to the UA's numerous complex pharmacological properties, these findings provided a comprehensive profile of the compound, which has garnered considerable attention in recent years. The present antimicrobial effect

observed here could be linked to the compounds' previously recorded antibacterial effect; UA efficacy for *E. coli* was reported as 1000 µg/mL [22]. The possible mechanism may be due to the inhibitory activity in nucleic acid replication and synthesis of bacteria [16]. However the role of these UA in the anti-bacterial property has to be explored in detail in near feature.

## Conclusion

In the present study, the MIC, MBC and time kill assay of UA showed antibacterial efficacy of UA and will be bactericidal for *A. baumannii*. This compound can be used in combination therapy to prevent resistance development to standard antibiotics.

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# A Comparative study to Evaluate the Efficacy of Supervised Exercise Program and Cyriax Physiotherapy on Pain and Function in Lateral Epicondylitis

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## Abstract

**Background:** Lateral epicondylitis is a musculotendinous degenerative disease of the extensors origin that occurs at the humerus lateral epicondyle. Various methodologies of treatment are utilized in the board of lateral epicondylitis.

**Objectives**: The study will investigate the efficacy of the supervised exercise programme and cyriax physiotherapy on the refinement of pain and the functional within Lateral epicondylitis patients.

**Methods**: Participants (n=30) with Lateral Epicondylitis (tennis elbow) shall be recruited to participate in a comparative experimental study. Subjects will be randomized 1:1 to either (1) Supervised Exercise Programme group, or (2) Cyriax Physiotherapy group. Over a 4-week time period, a 3 times in week for the total of 12 sessions, subjects in supervised exercise programme group will receive the Extensor Carpi Radialis Brevis muscle's static stretching along with the wrist extensor's eccentric strengthening and Ultrasound. While those in group of cyriax physiotherapy will receive transverse deep friction massage for the time of 10 min instantly amid by Mill's manipulation/manoeuvre and Ultrasound. The study will be conclude at the 4 weeks.

**Discussion:** Effectiveness of the interventions on the pain and the functional improvement will be assessed by VAS (visual analogue scale) and the Tennis Elbow Function Scale (TEFS) respectively. The findings of the study will greatly contribute for the evidence on the utilization of the supervised exercise programme and therefore the cyriax physiotherapy in tennis elbow condition.

Conclusion: After the statistical analysis of the results, final statements regarding the conclusion of which treatment approaches is effective will be made.

***Keywords****: Cyriax Physiotherapy, Supervised Exercise Programme, Lateral Epicondylitis, VAS, TEFS.*

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## Introduction

The disorder essentially inside the radio humeral joint with constant impairing pain within the elbow, is named as tendonitis, tennis elbow, lateral epicondyalgia or lateral epicondylitis[1]. The precise explanation for tendonitis isn't however identified. It is quite common in people whose jobs demand recurring

forearm movements (e.g., court game sportsmen and woodworkers)[2]. It's normally because of additional fast, wearisome, recurrent eccentric contractions and gliding joint griping activities. It normally affects dominant arm. The typical amount of associate degree episode of epicondylitis in span of six months and two years. In tennis elbow macroscopic and microscopic lesions appears within the muscle named Extensor Carpi Radialis Brevis (ECRB)[3,4]. The absence of acute inflammatory cells in the pathological excision of ECRB tendons suggests that lateral epicondylitis is not a foremost inflammatory disorder. Instead, immature fibroblasts and disorganised, nonfunctional vascular elements have invaded the tissue. Angiofibroblastic hyperplasia was invented by Nirschl and Pettrone to characterise this granulation-like tissue[5,6]. The radial recurrent artery was established by Schneberger and Masquelet as the dominant vascular supply to the ECRB tendon[7,8]. Two hypovascular zones were discovered after further investigation: the first 2--3cm away from the extensor muscle insertion and the second on the lateral epicondyle. A common cause of lateral epicondylitis is repetitive microtrauma from ovework of the wrist extensor muscles. A propitous and not proven cause of lateral epicondylitis is microtrauma in the context of hypovascularity, which prevents healing[9].

The primary complaints in lateral epicondylitis includes lessened strength in grip, inflated pain and reduced functional activity. The diagnosis established by the examination which might evoke the pain, on the lateral epicondylar facet, tenderness on palpation, resisted extension of finger, resisted extension of the wrist joint, and wrist joint flexion passive in nature (cozen's test)[10]. Although various studies are directed on this clinical condition treatment, until now the foremost effectual management plan isn't in agreement whether or not it's standard or surgical. Since lateral epicondylitis is a clinical condition, radiological testing is rarely necessary and reserved for those who do not improve. X-ray is the first line of inquiry when there is a suggestion of radio-capitellar osteoarthritis or other bony pathology[11].

The treatment methods used in medicinal orthopaedic are largely dependent over the kind of condition, cyriax includes manipulations and deep friction massage. Techniques of manipulation (small-amplitude, rapid and passive movement with thrust, also called "C grade mobilisation") are accustomed to reduce tiny gristly displaced particles in the spine in joints of periphery (slack objects). Manipulation is often requited to reinstate usual movability in a joint that has been limited by ligamentous adhesion or when bones have become subluxed. Deep friction is an effective treatment for soft tissue lesions caused by trauma or overuse. The logic for incorporating deep friction (a form of soft tissue mobilisation) is backed up by decades of research that confirms and explains the positive effect of movement on muscle and skeletal tissues healing.[12] Supervised exercise program comprehensive of static stretching with eccentric strengthening where, Static stretching is described as slowly placing the muscle tendon unit in a maximum stretch and keeping it there for an expanded period of time. The optimum position of stretching is evaluated by the patient's mild discomfort and/or pain. It is difficult to activate the stretch reflex, which induces contraction of the muscle tendon unit rather than relaxation, when static stretching is done slowly. Furthermore, since the viscoelastic structure of muscle tendon unit that elongates, the muscle tendon unit's resistance is low in slow static stretching than in fast static stretching. Only the harmed tendon, not all tendons around the skeletal field, should be stretched with static stretching exercises, according to therapists.[13]

## Methods/Design

### Aim

To evaluate the efficacy of the Supervised Exercise Programme and therefore the Cyriax Physiotherapy on pain and functional improvement in tennis elbow.

**Study setting:**

This study will be carried out in the Musculoskeletal Physiotherapy OPD of Ravi Nair Physiotherapy College, Sawangi (Meghe), Wardha, after approval from Institutional Ethics Committee of Datta Meghe Institute Of Medical Sciences, Deemed to be University. Before inclusion, all the participants will be informed regarding the aim and procedure of research. Those participants who will meet the inclusion criteria must give the written informed consent. In a comparative study, the participants (N=30) diagnosed with lateral epicondylitis (LE) will be enrolled for 4 weeks[14]. Fig. 1. Show's the flow chart of the study.

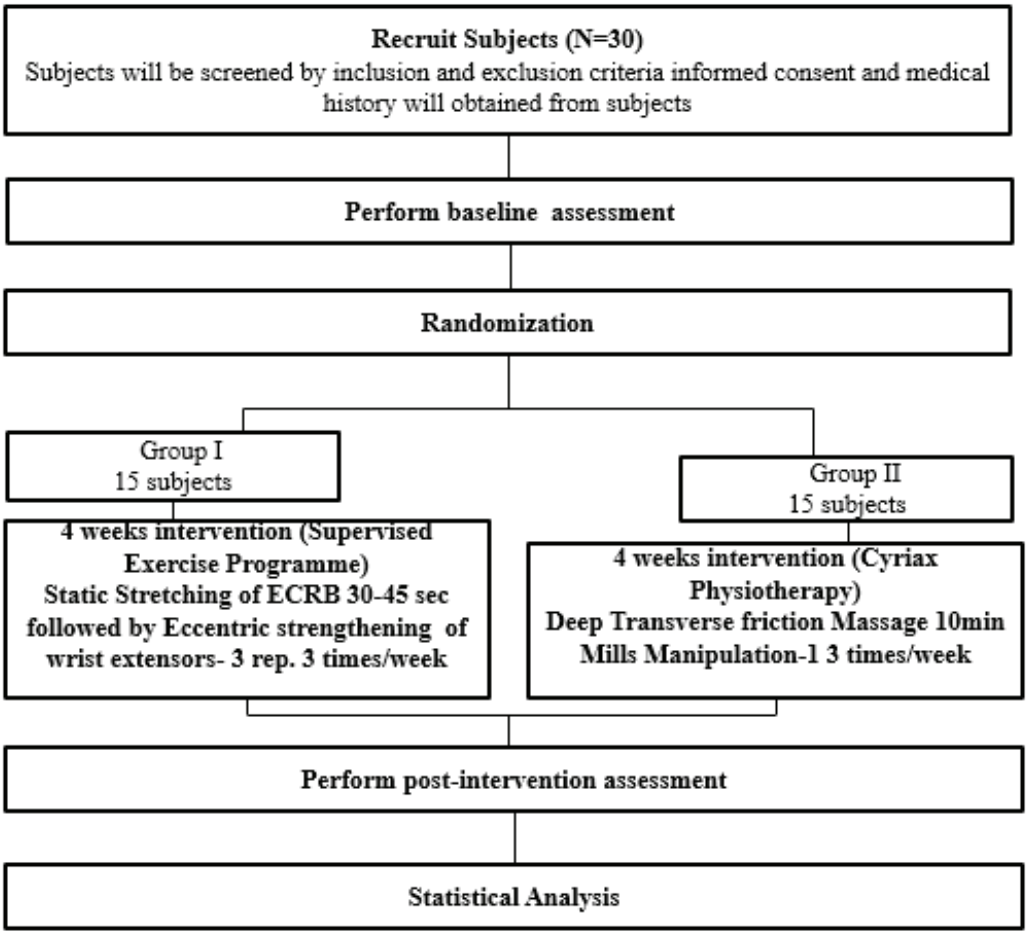


**Fig. 1: Study plan**

**TRIAL DESIGN:**

A comparative study in which the patients will be randomized into two group independent design. Group I will be Supervised Exercise Programme group and Group II will be assigned with Cyriax physiotherapy which basically includes Deep Fraction Massage and Mill's manipulation.

**PARTICIPANTS:**

Based on the criteria of inclusion and exclusion participants will be involved in the study. The subjects in the 20-50 years of age group of both the genders will be included. Subjects who are going to be having pain on passive wrist flexion with the elbow extension also with wrist pain on resisted extension incorporated in study[15]. Also the participants with chronic lateral

epicondylitis and will be having tenderness on the lateral epicondyle of humerus on palpation will be encompassed.

Subjects who will be having cervical radiculopathy, neurological impairments, any preceding trauma to elbow region, any surgeries to elbow region and elbow pain acquired from any other pathology than tennis elbow (lateral epicondylitis) will be eliminated from the study. The subjects who are getting to be having neuromuscular diseases, peripheral nervous disorder and any of them who are getting to be receiving corticosteroid injection in previous 6 months before the study period begins, will not be considered in to avoid further complications.

PARTICIPANT TIMELINE:

Each patient will be required to complete 4 weeks of interventions like supervised exercise programme and cyriax physiotherapy after enrolment in the study.

**Recruitment:**

The patients who are already undergoing rehabilitation in our IPD or coming to the physiotherapy OPD and diagnosed with lateral epicondylitis will be systematically assessed for the eligibility in the study according to the criteria of inclusion and exclusion. After fulfilling the eligibility criteria for their enrollment in the study, the informed patient consent will be cumulated taken after elaborating the purpose and procedure of the study.

SAMPLE SIZE CONSIDERATION:

This comparative study is an experimental two-group design that examines efficacy of the two interventions – the supervised exercise programme and physiotherapy of cyriax in lateral epicondylitis (tennis elbow) treatment[16]. 30 participants will be enrolled to the group I and group II and will be randomized by cheat method.

INTERVENTION DESIGN:

**Group I - Supervised Exercise Programme**

All the participants of administered supervised exercise programme will undergo static stretching of Extensor Carpi Radialis Brevis (ECRB) in a flash followed by the wrist extensors eccentric strengthening. The position of the steady stretch will be held for 30-45 seconds, this intervention will be carried out 3 counts prior and 3 counts after the segment of strengthening eccentric in nature treatment for 6 repetitions in total[17]. In each repetition of stretching there will be 30 seconds of interim. After the manual treatment there will be 5 min application of therapeutic ultrasound with parameters of1MHz frequency, 1:4 ratio of pulsed mode with 0.8 W/cm$^2$ intensity.[18]

Group II – Cyriax Physiotherapy

All the participants will get Cyriax physiotherapy that involves transverse deep friction massage for ten minute which will be straight away accompanied by Mill's manipulation/ manoeuvre. With the side of the fingertip of the thumb on the area of tenderness after palpating the anterior and lateral side of the lateral epicondylar surface of the humerus transverse deep friction massage will be applied. After achieving the effect of numbing, the tendon is made ready for Mill's manipulation. Manoeuvre of Mill's is thrust of a low in amplitude and high in velocity in the end range of elbow extension. It will be single application[19]. With therapeutic ultrasound parameters as above for 5 min.

OUTCOME MEASURES:

1) Visual Analogue Scale - VAS will evaluate the pain, in which is a horizontal bar of 10 cm with two ends zero cm is a 'minimum imaginable pain" and ten cm "worst pain imaginable". Patient will have to draw out a upright line on the horizontal scale according to their present level of pain.

2) Tennis Elbow Function Scale (TEFS) – The Functional activity of elbow will be evaluated on Tennis Elbow function Scale which have ten group of activities and their rating will be based on discomfort i.e. 'No discomfort' to 'Extreme discomfort'. This

scale is scores in relation with VAS in combination for pain related function.

**Data Collection and Management:**

The assessment data will be collected from a predetermined spreadsheet with the baseline characteristics variable. The hard copies of assessment forms and signed consent forms will be stored securely at the study site. Data collection and documentation will be done under the guidance of the principal investigators. The study documentation will be evaluated thoroughly for accuracy.

## Statistical Analysis

To perform the comparison between the two groups, t-tests will be used for the demographic measures and initial scores on outcome measures. For the interpretation of the results, we will significant differences. Significance will be set at P less than 0.05. Data will be coded and entered in MS excel worksheet and analysed using appropriate statistical software.

## Discussion

The study is aimed at assessing the efficacy on pain and functional improvement of the interventions like cyriax physiotherapy and supervised exercise programme with therapeutic ultrasound in lateral epicondylitis (tennis elbow)[20,21]. Earlier studies have proved that both these interventions are beneficial in pain reduction and function improvement[22]. But amongst the two which one is more better and well tolerated by the patients is not known. Also above mentioned each intervention along with therapeutic ultrasound not yet performed in any studies published till now. Henceforth which intervention amongst the two will be helpful for the patients of lateral epicondyalgia is the main aim of this research.

To conclude we aim at finding out improvement in pain and the functional efficacy through supervised exercise programme and cyriax physiotherapy on lateral epicondylitis. Hence which intervention is more beneficial on these outcome measures is to be analysed.

**Ethics and Dissemination:**

The ethical clearance will be obtained from Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences, Deemed to be University. The main findings regarding the efficacy of the Supervised exercise program and Cyriax physiotherapy in patients with Lateral Epicondylitis on pain and function will be published in a peer-reviewed journal.

**Patient Consent:** The informed consent will be obtained from the patient on a printed form with signatures and give the proof of confidentiality.

**Author's contribution:** The study was created and designed by all of the authors, and the final manuscript was approved for publication by all of them.

**Declaration of interests:** The authors declare no conflicting interest.

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# A Study to Assess Respiratory Health Status among Bricks Workers

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## Abstract

**Introduction:** Respiratory health problem is one of the major health problem among bricks industry workers those who are working in dusty area. These problem can be reduce when proper intervention taken like use of mask, hanky and other devices during working time.

**Objectives:**

1. To assess respiratory health status of bricks workers working in brisk industry.
2. To find the association among respiratory health status and selected demographic variables.

**Methodology:** Quantitative research approach utilized with cross sectional research study among Bricks workers .91 Samples were collected by non probability convenient sampling technique. Demographic and respiratory health assessment done through questionnaire.

**Results:** Result showed that Average age of participants was 33.20 ± 5.123(mean ± SD),Average monthly income of participants were 12659.34,Average year of experience was 7.35 ± 4.510 (mean ± SD), Gender majority of participants were males 82(91.1%), Disease or any issue other then respiratory health,89 (97.8 %) had no any diseases, 67(73.6%) were not using any devices during working time, 47(51.6%) were smoker. In respiratory assessment symmetrical chest movement had seen in 91(100%) participants. 21(23.1%) had Acute Cough. 8(8.8%) had white colour phlegm and 4(4.4%) had black colour phlegm. 23(25.3) had Grade I of shortness of breath,1(1.1%) had history of self reported asthma. there was significant Association found between cough and Age in year, Experience in year, Other Health problem and Issue , Habits of smoking.

There was significant Association found between phlegm and Age in years , Other Health Issue , Habits of smoking, drug and other supplements.

There was significant Association found between shortness of breath and Age , Qualification, Experience in year , Habits of smoking.

**Conclusion:** The study concludes that factors like years of experience, habits of smoking ,age are more responsible for respiratory health problem among bricks industry workers.

***Key words** : Respiratory health status , Bricks workers.*

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## Introduction

The bricks industry is one of the traditional industry giving work to huge number of semiskilled and untalented laborers. This manufacturing has been taking interest in production of various products like hollow bricks, country bricks, hollow block bricks and fly ash bricks. The enhance in building activities in urban and rural areas has more encouraged this industry to have its stake in the market. The Brick making process includes burrowing, forming, and stacking, stacking and emptying, terminating of blocks and transportation of blocks to the normal goal. Every one of these procedures include unskilled workers.[1]

Workers at brick kiln might connected with conveying clay dust and bricks, molding or baking (Shaikh et al, 2012) release from brick kilns contains tiny dust particles, hydrocarbons, Oxides of Nitrogen (NOx), Sulphur Dioxide (SO2), Carbon Monoxide (CO), Fluoride compounds and limited quantity of carcinogenic dioxins if rubber tyres were utilized. Clay dust include a blend of non-living compound including silica, lime, magnesium carbonate, iron oxide, alkalis, calcium sulfate, calcium carbonate and sodium chloride and changeable amount of organic materials.[2][3][4]

Most of the brick kiln use woods and coal for boiling the bricks that make the brick kiln people at risk to high exposure to air pollution and it has an adverse health effect on the workers. Due to air pollution at the kiln, a significantly higher proportion of chest symptomatic diseases have been found among brick kiln workers compare to the general population. [4][5]

One of the major threats of brick dust inhalation is crystalline silica exposure. Silica is usually seen in quartz rock and minerals; when cut, crushed and ground, particles small sufficient to respire are released into the air. As a known carcinogen, long duration of inhalation of crystalline silica can decrease the breathing ability of the lungs.[6]

Objectives of the study

· To assess respiratory health status of bricks workers working in brisk industry.

· To find the association between respiratory health status and selected demographic variables.

## Material and Methods

Quantitative research approach utilized with Non-experimental research study among the population of Bricks workers in selected areas of Gujarat. Sample size will be based on sample calculation and feasibility of the study. By using the formula,

$n=4pq/L^2$ where $p=0.224$ ,$q=0.776$,$L=0.0448$

$= 4\times0.224\times0.776/ 0.0448\times0.0448$

$= 0.695/ 0.0019$

$=365$

Estimated sample size was 365 due to time constraint and global pandemic situation investigator consider 25% of sample size. hence 91 participant in the study were recruited .The Research variable was respiratory health status among bricks workers and demographic variable was Age, Education, Occupation, Monthly Family Income, Working Area etc. About data collection procedure, Ethical approval obtained by IEC- CHARUSAT-91 Samples chosen by Non Probability Convenient technique of Sampling from selected areas. The investigator introduced the self Before collecting data and informed about the importance of this Research process or interest of the bricks workers. Demographic and respiratory assessment tool was given in vernacular language to workers for filled up.

## Statistical Analysis

Data was analyzed through software of Statistical Package of Social Sciences (SPSS version 21). Descriptive statistics of socio-demographic variables were computed as mean, standard deviation or frequency percentages. Incidence was possible

for presence of chronic respiratory symptoms and illnesses by calculating the frequency percentages of the incidence of symptoms and illnesses in the sample. The secondary objective of the study was to find association among respiratory health status and selected demographic variables.

## Results

Result shows that Average age of participants was 33.20 ± 5.123(mean ± SD),Average monthly income of participants were 12659.34,Average year of experience were 7.35 ± 4.510 (mean ± SD). regarding Gender majority of participants were males 82(91.1%) Educational detail 38(41.8%) were having Primary education and 1(1.1%) were having higher secondary education, all 91(100%) were belongs to Hindus, Place of residence 83(91.2%) were residing in rural area, 5(5.5%) were residing in semi urban area. Disease or any issue other then respiratory health,89 (97.8 %) had no any diseases, 67(73.6%) were not using any devices during working time,47(51.6%) were smoker. 90(98.9%) were not taking any drugs, 38(41.8%) were using Chula, 37(40.7%) were using gas stove. In respiratory assessment symmetrical chest movement had seen in 91(100%) participants. 21(23.1%) had Acute Cough. 8(8.8%) had white colour phlegm and 4(4.4%) had black colour phlegm. 23(25.3) had Grade I of shortness of breath,1(1.1%) had history of self reported asthma.

1. Association between demographic variables and cough.

Suggest that there was significant Association found between **Age in year** ($\chi^2$ value 13.680,p value-0.003), **Experience in year** ($\chi^2$ value 20.612,p value-.000),**Other Health problem and Issue** ($\chi^2$ value 6.618,p value-0.009) and **Habits of smoking** ($\chi^2$ value 12.686,p value-.000) with cough.

**2. Association between demographic variables and phlegm**

Suggest that there was significant Association found between Age in years ($\chi^2$ value 15.180,p value-0.002),Other Health Issue ($\chi^2$ value 13.463,p value-0.000), Habits of smoking ($\chi^2$ value 8.864,p value-0.003) and drug and other supplements ($\chi^2$ value 6.656,p value-0.010)with phlegm.

**3. Association between demographic variables and shortness of breath.**

suggest that there was significant Association found between **Age** ($\chi^2$ value 23.514,p value-0.000), **Qualification** ($\chi^2$ value 12.427,p value-.006), **Experience in year** ($\chi^2$ value 17.292,p value-0.001) and **Habits of smoking** ($\chi^2$ value 15.365,p value-.000) with shortness of breath.

**4. Association between demographic variables and asthma** suggest that there was No significant Association found among demographic variables and asthma.

## Conclusion

The study concludes that respiratory health problem are more common in bricks worker. The importance of the research process to assess respiratory health status of bricks workers . 91 participants were selected from bricks industry by Non Probability Convenient technique of sampling. This information was collected through questionnaire and interpreted by applying descriptive and inferential statistical method. Result showed that factors like years of experience, habits of smoking ,age are more responsible for respiratory health problem among bricks workers.

**Conflict of Intrest:** Nil Source of funding: No Ethical clearance:

**Ethical consideration** was taken from Institutional ethics Committee- IEC CHARUSAT, Charotar University of Science and Technology and permission given on 24th July 2020. Proposal ID: CHA/IEC/ADM/20/07/599.05.

**Statement of Informed Consent:**

Informed consent was acquired from the

participants.

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# Study of Ossification Centres Around the Elbow and Wrist of Adolescent Age Group 15-18 Years

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**Abstract**

**Intoduction:** Determination of age in both living and dead plays a pivotal role in medicolegal cases. There are various parameters to assess the age by physical characteristics, odontological development but the most valuable method seems to be the osteological changes observed by radiological examination ie., the appearance and fusion of secondary ossification centres around respective joints The present study was conducted on 131 subjects out of which 110 were males and 21 were females. The main aim of the study was to find the age of fusion of secondary ossification centres around elbow and wrist joint, to compare the distribution of fusion of ossification centres between males and females

**Methods:** Firstly informed consent was taken. Preliminary details of each individual was noted that included general examination, physical examination, dental examination followed by taking X-ray AP view of elbow and wrist joint for radiological examination. The results were noted based on the degree of fusion as No Union (O), Beginning union(B), Recent union(R), Complete union(C).

**Results:** The present study showed among males, the fusion for each ossification centre around elbow joint started by 15-16 years and was completed by 18-19 years whereas in females it started fusion by 15-16 years but complete fusion occurred around 16-17 years itself . In wrist joint the fusion starts at 16-17 years in males but only fusion of lower end of ulna is completed by 18-19 years, however both lower end of radius and ulna are fused by 18-19 years in females.

**Conclusion**: Based on the analysis of fusion of ossification centres it shows that both thecentres around elbow and wrist joint fuses earlier in females by 1-2 years than males.

***Keywords:*** *Age, X-rays, ossification, fusion.*

## Introduction

Age estimation in the living is one of the most important tasks especially in developing countries where both birth records are often not available or not well maintained[4].Despite the fact that there are a number of laws requiring registrations of births(Eg: Registration of Births and Deaths Act 1969) most births are not properly recorded. Though there are many general developmental factors in assessing the age, changes in bones specially time related appearance and fusion of different ossification centres in growing period are valuable indices for assessing the age [4.] The variation in the appearance and the fusion of ossification centres is mainly attributed to various factors like climate, hereditary,race,nutrition,dietary

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habits and gender,socioeconomic status of the population[8]. Scientific estimation of age of an individual whether living or dead or from human remains is a vexing problem for medical jurist in both civil and criminal matters, Age estimation cases are often referred to forensic experts as it plays a vital role in deciding upon the quantum of punishment given to the accused and where to execute the same in a Reformation or juvenile court school or jail.. As the age between 15 to 17 years is very important medicolegally especially in the females, it is important to differentiate between 14-15 years in employment and 17-18 years in connection with Hindu Marriage Act. Amongst all the parameters of age determination, radiological examination of bones ends has shown accuracy and reliability acceptable to medical profession and legal fraternity.So the objective of this study is the to find out the age of an individual from the fusion of secondary ossification centers around Elbow joint and Wrist joint, to compare the age of fusion of ossification centers around elbow and wrist joints between males and females .

## Materials and Methods

The present study was conducted in Government Junior College, Nemmikal, Suryapet, Telanagna. The study was carried on a total of 131 subjects out of which 110 were males and 21 were females. Subjects with deformities of elbow and wrist joint, signs of malnutrition, congenital anomalies, infections and metabolic disorders were excluded. We obtained written consent for every individual subject for their radiological examination. The X-rays of AP view were taken in an outside lab. The persons selected for the study were grouped as per their stated age viz, 15-16 years, 16-17years, 17-18years & 18-19 years . Age as stated by them was further confirmed by birth certificate or entry in their school record .The Xerox copy of proof of birth certificate was collected. The persons belonging to the age group selected for either gender are included in the study irrespective of their socio- economic, religion. We prepared a proforma with particulars containing Name, sex ,date of birth ,address, height, weight and Identification marks. In males the colour and growth of scalp hair, beard, moustache, axillary and pubic hair were examined and noted. In females, development of breast was noted and complete menstrual history was taken. Dental examination was done by noting the number of temporary and permanent teeth and a dental chart was prepared. Radiological assessment of various ossification centres, their appearence, process of fusion were noted. The observations were based on the following grades of stages of fusion.

DEGREE 0: A dark radiolucent line seen throughout the length of the epiphyseal and metaphyseal joining surface (Centre not appeared, union not commenced).

DEGREE 1: Radio opaque area is seen in the middle of or on either side of, but occupies less than half of, the epiphyseal and metaphyseal joining surface (Centre appeared but incomplete, union commenced ).

DEGREE 2: Radio opaque area is seen in more than half of epiphyseal and metaphyseal joining surfaces, but the cortical shadow is not continuous ( Union started but in complete).

DEGREE 3: Radio opaque area is seen in the entire length of the epiphyseal and metaphyseal joining surface and the cortical surface is continuous without any notch (Complete union).

For tabulating the findings the stages of fusion were noted in the form of following abbreviations.

1. No union (O).

2. Beginning union (B).

3. Recent union (R).

4. Complete union(C).

## Results

**TABLE 1: COMPLETE FUSION OF OSSIFICATION CENTRES AROUND ELBOW JOINT IN MALES AND FEMALES**

| AGE | TROCHLEA | | CAPITULUM | | LATERAL EPICONDYLE | | MEDIAL EPICONDYLE | | UPPER END OF RADIUS | | UPPER END OF ULNA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| 15-16 | 6(21.4% | 8 (80%) | 10(25.7%) | 2(20%) | 12(42.8%) | 2(20%) | 8(28.5%) | 3(30%) | 10(35.7%) | 7(70%) | 12(42.8%) | 5(50%) |
| 16-17 | 12(37.5%) | 4 (100%) | 20(62.5%) | 4(100%) | 22(68.7%) | 4(100%) | 22(68.7%) | 4(100%) | 24(75%) | 4(100%) | 26(81.2%) | 4(100%) |
| 17-18 | 30(93.7%) | 6(100%) | 28(87.5%) | 6(100%) | 26(81.2%) | 6(100%) | 28(87.5%) | 6(100%) | 30(93.7%) | 6(100%) | 30(93.7%) | 6 (100%) |
| 18-19 | 18(100%) | 1(100%) | 18(100%) | 1(100%) | 18(100%) | 1(100%) | 18(100%) | 1(100%) | 18(100%) | 1(100%) | 18(100%) | 1 (100%) |

Table 1 shows that the complete fusion of ossification centres around elbow joint in males starts at the age of 15-16 years followed by individuals of 17-18 years showing complete fusion from a range of 80-90% and all the 18 subjects between 18-19 years shows 100% complete fusion.In females it showed that the complete fusion of all ossification centres around elbow joint started at the age group of 15-16 years but was not complete and it was followed by appearance of 100% complete fusion in 16-19 years age group of individuals .

**TABLE 2: COMPLETE FUSION OF OSSIFICATION CENTRES AROUND WRIST JOINT IN MALES AND FEMALES**

| AGE | LOWER END OF RADIUS | | LOWER END OF ULNA | |
|---|---|---|---|---|
| | M | F | M | F |
| 15-16 | 0(0%) | 0(0%) | 0(0%) | 0(0%) |
| 16-17 | 2(6.2%) | 0(0%) | 4(12.5%) | 1(25%) |
| 17-18 | 20(62.5%) | 4(66.6%) | 26(81.2%) | 4(66.6%) |
| 18-19 | 14(77.7%) | 1(100%) | 18(100%) | 1(100%) |

Table 2 shows that the complete fusion of lower end of radius and ulna in males starts by the age of 16-17 years .The age group of 18-19 years shows only 77.7% of complete fusion of lower end of radius but 100% complete fusion was seen only in lower end of ulna. It implies that the lower end of ulna fuses earlier, followed by the lower end of radius in males.Among females complete fusion of lower end of radius starts at the age of 17-18 years and the lower end of ulna starts by 16-17 years. The age group of 18-19 years shows 100% complete fusion of both lower end of radius and lower end of ulna. It implies that the lower end of radius completely fuses in females earlier when compared to males but fuses in line at the same age for lower end of ulna .

**TABLE 3: DISTRIBUTION OF FUSION OF OSSIFICATION CENTRE AROUND ELBOW JOINT IN MALES AND FEMALES**

| AGE | TROCHLEA | | CAPITULUM | | LATERAL EPICONDYLE | | MEDIAL EPICONDYLE | | UPPER END OF RADIUS | | UPPER END OF ULNA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | M | F | M | F | M | F | M | F |
| 15-16 | 6 | 8 | 10 | 2 | 12 | 2 | 8 | 3 | 10 | 7 | 12 | 5 |
| 16-17 | 12 | 4 | 20 | 4 | 22 | 4 | 22 | 4 | 24 | 4 | 26 | 4 |
| 17-18 | 30 | 6 | 28 | 6 | 26 | 6 | 28 | 6 | 30 | 6 | 30 | 6 |
| 18-19 | 18 | 1 | 18 | 1 | 18 | 1 | 18 | 1 | 18 | 1 | 18 | 1 |
| p value | 0.049 | 0.050 | 0.014 | 0.061 | 0.007 | 0.061 | 0.020 | 0.044 | 0.017 | 0.042 | 0.013 | 0.034 |

**TABLE 4: DISTRIBUTION OF FUSION OF OSSIFICATION CENTRE AROUND WRIST JOINT IN MALES**

| AGE | LOWER END OF RADIUS | | LOWER END OF ULNA | |
|---|---|---|---|---|
| | M | F | M | F |
| 15-16 | 0 | 0 | 0 | 0 |
| 16-17 | 2 | 0 | 4 | 1 |
| 17-18 | 20 | 4 | 26 | 4 |
| 18-19 | 14 | 1 | 18 | 1 |
| p value | 0.157 | 0.278 | 0.142 | 0.182 |

**FIGURE 1:**

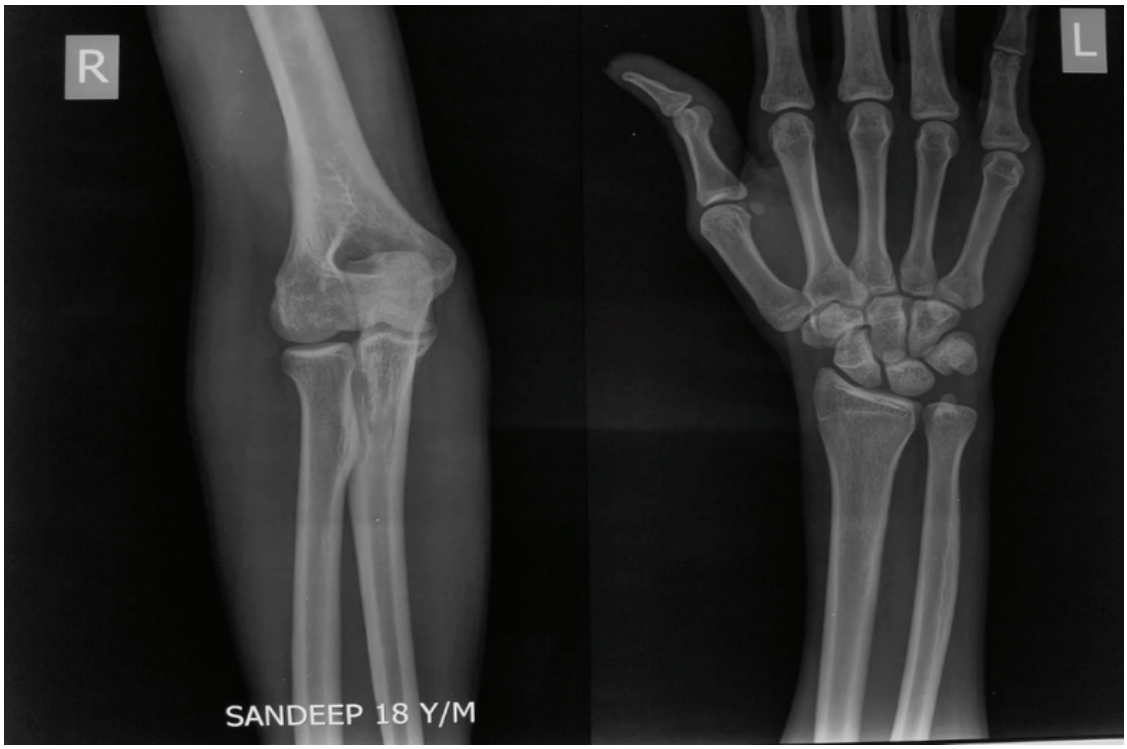


**Figure 1 depicts X-ray AP view of elbow and wrist joint of 17 years male showing complete fusion of all ossification centres around elbow joint and beginning of fusion in lower end of radius and ulna in the wrist joint.**

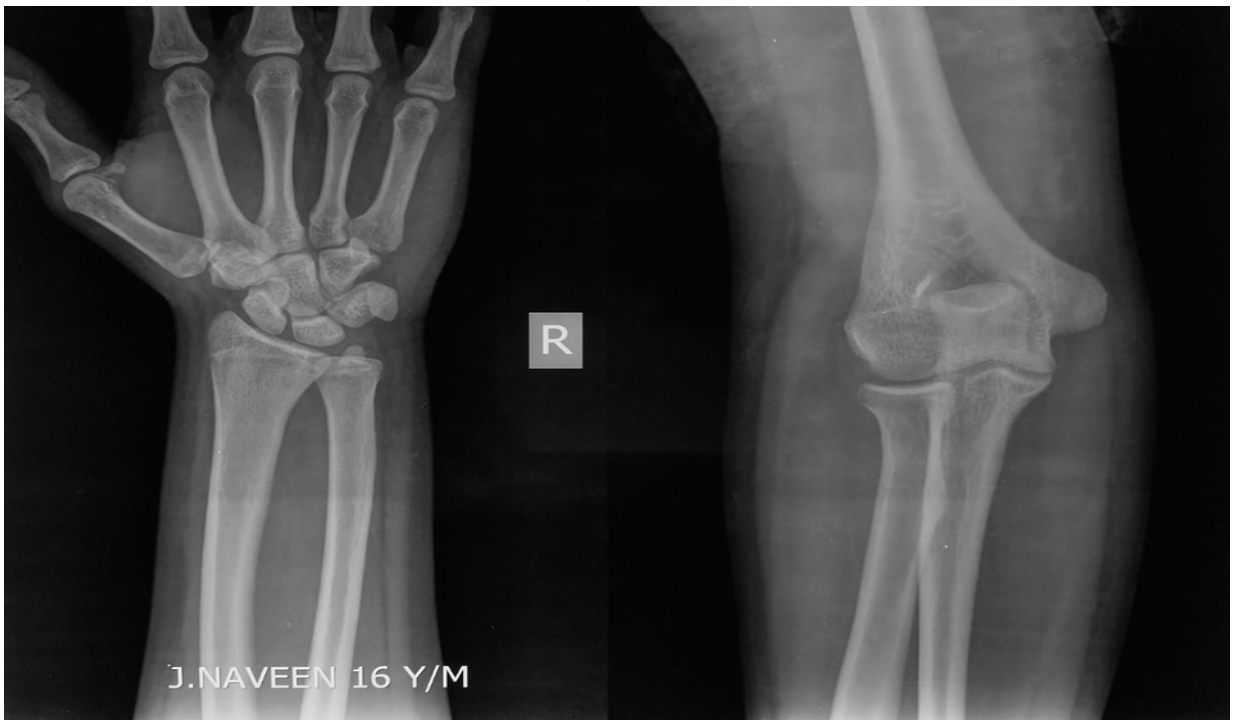


**FIGURE 2:**

**Figure 2 depicts X-ray AP view of wrist and elbow joint of 16 years female showing complete fusion of all ossification centres around elbow joint and recent fusion of lower end of radius and ulna in the wrist joint.**

**FIGURE 3:**




**Figure 3 depicts X-ray AP view of wrist and elbow joint of 17 years female showing complete fusion of all ossification centres around elbow joint and recent fusion of lower end of radius and ulna in the wrist joint.**

## Discussion:

Age determination is important in law of attaining maturity and in criminal case where the disposal of body is done by dismembering the body parts by using various methods or when only skeletal remains is available for examination. Whereas in living it helps in solving medicolegal cases comprising civil cases like those related to employment, education, sports and in criminal cases like robbery, sexual assaults, abduction or kidnapping etc .,

The present study was conducted on 131 subjects out of which 110 were males and 21 were females. It shows that fusion of trochlea in age group of 15-16 years the complete fusion is seen only 21% rest population shows recent fusion in males and in age group 17-18 years 93% were completely fused. In females age group of 15-16 years 8% completely fused. Fusion of capitulum in age group 15-16 years the complete fusion is seen only 35% rest population shows recent fusion in males and in age group 17-18 years 87% completely fused whereas In females of the age group 16-17 years 100% was completely fused.

The fusion of lateral epicondyle in the age group 15-16 years complete fusion is seen only in 42% and rest of population shows recent fusion in males, in age group 17-18 years ,81% completely fused whereas in females the age group 16-17 years completely fused . The fusion of medial epicondyle in age group 15-16 years the complete fusion is seen only in 28% rest population shows recent fusion in males and in age group 17-18 years, 87.5% completely fused whereas in females of age group 16-17 years ,100% was completely fused.

Fusion of upper end of radius in age group of 15-16 years complete fusion is seen in only 35% rest of the population shows recent fusion in males and in

the age group 17-18 years 93% was completely fused however in females the age group of 16-17 years showed 100% complete fusion. Fusion of upper end of ulna in age group of 15-16 years , the complete fusion is seen only 42% rest of the population shows recent fusion in males and in age group 17-18 years , 93% was completely fused whereas in female age group of 16-17 years ,100% complete fusion was seen.

Ossification centresaround lower end of radius in males in the age group of 16-17 years shows only 6% complete fusion, in 18-19 years 77% shows complete fusion. In females the age group of 18-19 years shows 100% complete fusion. Ossification centresaround lower end of ulna in males in the age group of 16-17 years shows only 12 % complete fusion, in 18-19 years only 81% shows complete fusion.In females the age group of 18-19 years shows 100% complete fusion.

The olecranon centre showed a tendency to ossify earlier than trochlea in girls and boys in this study similar results was observed by the study conducted by Cesar Satosh,Miyazaki Daniel et al in November 2017 [1]. The fusion of epiphyseal centre of medial epicondyle with the shaft was seen between 15-17 years in males which were concordant with the study conducted by UmeshChoudary et al in march 2017 [3].The fusion of lower end of radius and ulna shows complete fusion in 100% population at 18-19 year group individuals which were similar to the results of the study done by Hassan noor et al in June 2016 [2].

It is observed that the ossification centres in females fuse earlier than males in both elbow and wrist joint. With reference to the theory of null hypothesis considering p=0.05 as the statistical significant value. The age of fusion of ossification centresaround elbow and wrist joint between males and females were observed and it showed that the p value calculated for females was >0.05 when compared to males which were <0.05 (0.013-0.020) and is the probability that the null hypothesis is true.

## Conclusion:

It shows that the complete fusion of ossification centres around elbow and wrist joint in females occurs earlier than in males by 1-2 years. 100% complete fusion around elbow joint in females occurs at 16-17 years whereas in males it is seen at 18-19years. 100% complete fusion around wrist joint is seen in females of age group 18-19 years whereas only lower end of ulna shows complete fusion in males at 18-19 years.

**Conflicts of Interest:** Nil.

**Source of Funding:** Self.

**Ethical Clearence:** Institute ethical committee, Osmania medical college.

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# Cheiloscopy - A Tool For Identification in Twins

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## Abstract

**Introduction :** Lip prints are an adjuvant tool for identification in forensic medicine. The grooves on the lips have discrete characteristics to make it different from one other. This study was conducted on 24 pairs of twins out of which 22 pairs were Monozygotic twins and 2 pairs were Dizygotic twins. Dizygotic twins are unique in all aspects while Monozygotic twins are similar phenotypically and genotypically. The objective of this study is to find the most common pattern in the twins , the commonest pattern among the male and female twins and to know the variations in the pattern of lip prints among Monozygotic and Dizygotic twins.

**Materials and Methods:** A lipstick, drawing chart and magnifying lens were the materials used to record the imprints of the lips. A four compartment method with a clockwise direction approach was used and the analysis was done based on Suzuki and Tsuchihashi classification.

**Results:** The present study shows Type 1' was the most common lip prints observed among the 24 pairs of twins. This pattern was predominantly present in the right lower lip quadrant. Out of the study subjects of 24 pairs of twins, 38 were males and 10 were females in which Type I ' is common in males and type I is common in females. Among the monozygotic twins those which had < 50 % resemblance were 14 pairs, 50% resemblance were 6 pairs, >50% resemblance were 2 pairs in which Type is the commonest. Among the dizygotic twins, both the pairs were totally different from each other yet Type was the common pattern.

**Conclusion:** Based on the analysis of the lip print patterns among monozygotic twins, though similarities were prevailing between them , they are not identical .Whereas among dizygotic twins both the pairs of twins are unique. Hence Lip print can be utilized as a differentiating tool for identification.

***Keywords:*** *Lip prints, patterns, twins, identification.*

## Introduction

Identification is a crucial and important role in forensic medicine from the ancient times till date as there are many tools for personal identification which includes Anthropometry, Dactylography, sex determination, differentiation by blood groups, DNA profiling, Odontology etc[5]. Among the various tools the most commonly used is the fingerprints because of its uniqueness in each individual[12]. Yet another tool which can be used based on its uniqueness is the Lip prints, but its contribution in identification is still in trials and not yet confirmed about its feasibility and reliability because of its never

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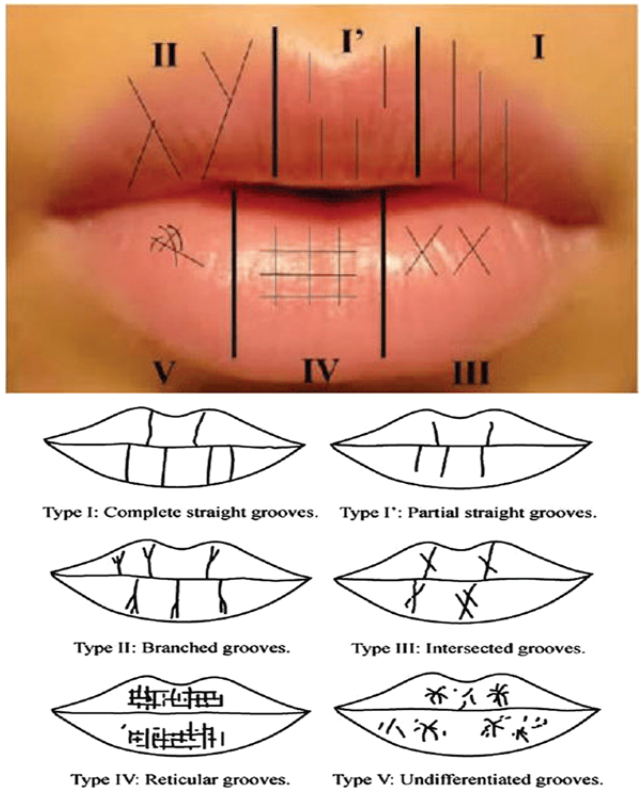
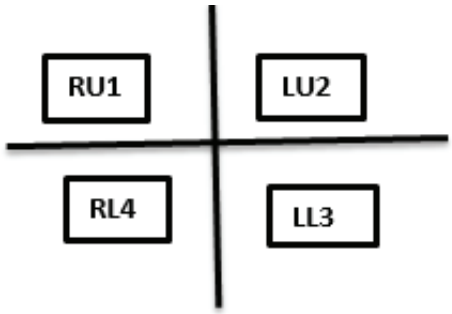
ending debate. Moreover it is used as an adjuvant technique[12]. The patterns of wrinkles on the lips have discrete characteristics as that of finger prints. The wrinkles and the grooves on the labial mucosa form a characteristic pattern called lip prints[12]. It is least invasive and is easily available method to study. The study of lip prints is called Cheiloscopy. The term is derived from the word Cheilos meaning Lips and Scopy meaning to see[5]. Among the various studies done based on the lip prints, those which are done on twins are comparitively less and still an area of research with lacunae.The present study aims at determining the most common pattern of lip print among the twins, the variations and differences among the patterns ,whether they are identical or not, so that it gives a better understanding and a clear way in the approach of identification among the twins. So the study of Lip print pattern in twins would be a significant contribution, since it has been mentioned in previous studies , that uniovular twins share the same proteins , same genetic information but have different finger prints[5] . So any major differences found in the lip print patterns would be of great importance in the field of forensic medicine.

## Methods

The present study was conducted on 24 pairs of monozygotic twins. Twin A was elder, and Twin B was younger. Subjects with inflammation trauma, congenital abnormalities, and surgical scars and other abnormalities of the lips were excluded because of their unsuitability for this study[5]. Lip prints were collected from the subjects after obtaining informed consent from the parents of the family. The subject was made to sit on a stool in front of a table and was advised not to move so that the recording of the lip prints will be accurate. First the observer demonstrated how to imprint the lips on the drawing chart. Then the observer stood in front of the subject and asked to keep the mouth closed, lip muscles relaxed and record the lip print. A dark colour lip stick was applied all over the lips upto the lip line and the individual was made to bend forwards and imprint the lips on to the drawing chart, press it firmly forwards and then roll it sidewards to right and left side respectively. The drawing chart was made to air dry for few minutes and marked with serial number at the back of it for its identity. The lip prints were visualized using a magnifying lens , grooves, wrinkles and various patterns were appreciated and noted. The lip prints obtained were entered in a proforma along with name and sex of the individual. The analysis of lip prints was based on Suzuki and Tsuchihashi Classification[16].




The Analysis of lip prints can be done by compartment methods like 1,4,6,8,10 compartment methods. In this study it was done by using a Four compartment method .The lips were divided into four quadrants and allotted the digits 1-4 in a clockwise direction starting from upper right corner of the lip, The upper lip is divided into right and left upper quadrants and the lower lip is divided into right and left lower quadrants . For each quadrant there may be more than one type of lip print. This is the most commonly used on the literature and so followed in this study.




## Results

**TABLE 1: Distribution of Lip print patterns**

| | TWIN A | TWIN B |
|---|---|---|
| TYPE I | | |
| First Quadrant | 05 (20%) | 04 (16%) |
| Second Quadrant | 03 (12%) | 03 (12%) |
| Third Quadrant | 04 (16%) | 06 (25%) |
| Fourth Quadrant | 04 (16%) | 01 (04%) |
| | | |
| TYPE I’ | | |
| First Quadrant | 10 (41%) | 12 (50%) |
| Second Quadrant | 11 (48%) | 12 (50%) |
| Third Quadrant | 09 (37%) | 07 (29%) |
| Fourth Quadrant | 12 (50%) | 14 (58%) |
| | | |
| TYPE II | | |
| First Quadrant | 01 (04%) | 02 (08%) |
| Second Quadrant | 05 (20%) | 03 (12%) |
| Third Quadrant | 06 (25%) | 03 (12%) |
| Fourth Quadrant | 03 (12%) | 04 (16%) |
| | | |
| TYPE III | | |
| First Quadrant | 07 (29%) | 05 (20%) |
| Second Quadrant | 04 (16%) | 05 (20%) |
| Third Quadrant | 05 (20%) | 08 (33%) |
| Fourth Quadrant | 05 (20%) | 05 (20%) |
| | | |

***Cont...*** **TABLE 1: Distribution of Lip print patterns**

| | | |
|---|---|---|
| TYPE IV | | |
| First Quadrant | 01 (04%) | 01 (04%) |
| Second Quadrant | 01 (04%) | 01 (04%) |
| Third Quadrant | - | - |
| Fourth Quadrant | - | - |
| | | |
| TYPE V | | |
| First Quadrant | - | - |
| Second Quadrant | - | - |
| Third Quadrant | - | - |
| Fourth Quadrant | - | - |

Table 1 shows the overall distribution of lip print patterns observed in all the four quadrants on the total subjects of the study.

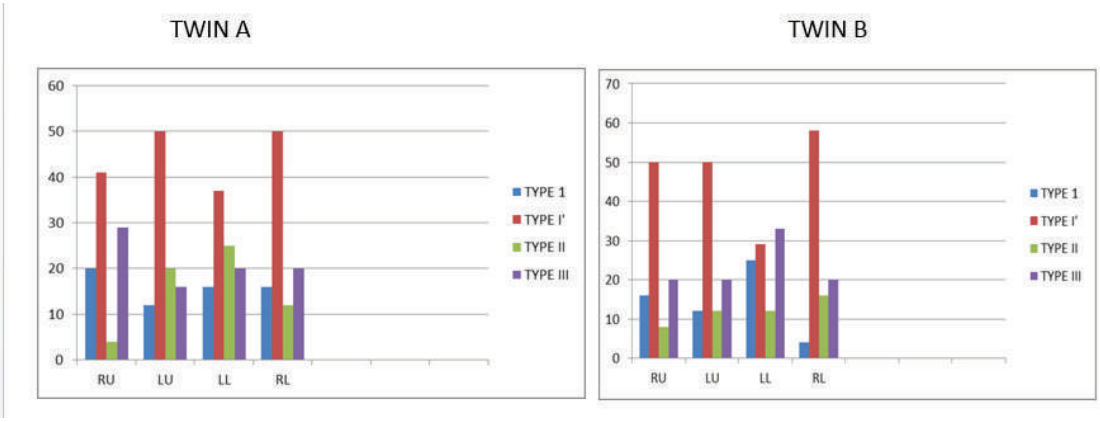



**FIGURE 2 & 3 : Common lip print in TWIN A and TWIN B**

Figure 2 & 3 shows Type I ' is the predominant lip print pattern noted in the right lower quadrant among both TWIN A and TWIN B with 50% and 58 % respectively.

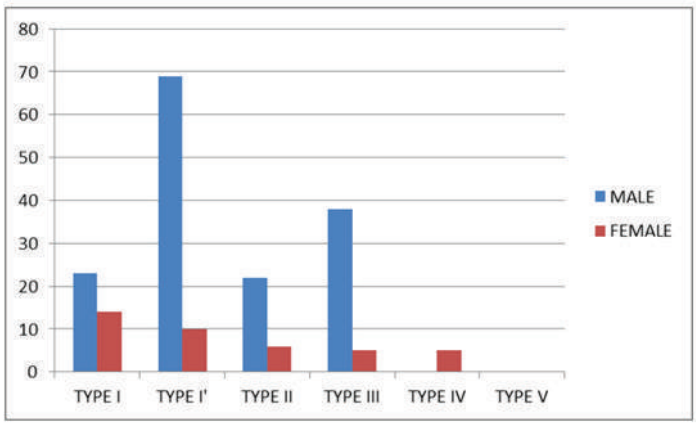



**FIGURE 4: COMMON LIP PRINT PATTERN IN MALES AND FEMALES**

Figure 4 shows Type I ' is the common pattern observed in males and Type I in females .

**TABLE 2 : PERCENTAGE OF RESEMBLNACE AMONG THE TWINS**

| PERCENTAGE OF RESEMBLANCE | NUMBER OF TWINS (N) |
|---|---|
| 0% | 02 |
| 50% | 14 |
| <50% | 06 |
| >50% | 02 |

Table 2 : The results shows that among 24 pairs of twins , 14 pairs have 50% of resemblance , 6 pairs have < 50 % resemblance , 2 pairs have > 50% of resemblance, 2 pairs have no resemblance and are completely different.

## Discussion

Cheiloscopy is a simple yet useful technique which aids in identification by thorough and meticulous observation of the lip traces. Many studies state that the lip prints are unique and permanent throughout the life, like fingerprints[12].The imprints that are left over a glass, clothing , body ,crime scene or any object handled by the individual can have direct or latent imprints. Commonly, direct lip prints can be picked up by cellophane tape method, but the untraced lip prints can be lifted by using aluminium and magnetic powder[14]. In criminal cases it is a boon for the forensic expert to present it as evidence in the court . In cases like sexual assaults, thefts, the link between the victim and the criminal can be established whereas in mass disasters and other civil cases identification of the individual can be made[15].

The present study was conducted on 24 pairs of twins out of which 22 pairs were Monozygotic twins and 2 pairs were Dizygotic twins .The commonest type of lip print was Type I ' among the study population and it was observed to be predominant in the right lower quadrant with 50% and 58% in Twin A and Twin B respectively followed by Type III pattern. Type V was not present in any of the subjects. Among the 24 pairs of twins 38 were males and 10 were females, on observation the common pattern noted in males were Type I' and Type I in females. However the study done by Bhawna Thakur et al on 40 pairs of twins showed that Type III was the most common pattern in the left lower lip Quadrant[5].Another study done by Suzuki and Tsuchhashi on 18 pairs of uniovular twins showed Type III was the most common pattern[6] .

Our study also makes us to understand the similarities and differences between the pairs of twins. In 22 pairs of Monozygotic twins 14 pairs of twins showed < 50 % resemblance , 6 pairs showed 50% resemblance, 2 pairs showed >50% resemblance . Among the dizygotic twins, both the pairs were totally different from each other without being alike. The results seems to be concordant with the study conducted on 5 pairs of twins by Venkatesh R et al showed that the lip print patterns were similar but none of them were identical[7].

## Conclusion

On analysis, the lip print patterns irrespective of monozygotic or dizygotic twins it shows that they are different from each other and hold its uniqueness in place. Since they are common and better accessible evidence in the crime scene, it can be considered as a higher preferential tool for identification. However a further detailed study with a bigger sample size , study within the family to find the inheritance,

the correlation study of Lip prints with other tools of identification may give an accurate theory of uniqueness about Cheiloscopy.

**Conflictof Interest:** Nil

**Source of Funding**: Self

**Ethical Clearence:** Not required

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# Synthesis, Characterization, Molecular Docking of Sulphamethoxazole Schiff Base Metal Complexes and Its Antibacterial, Anti-Inflammatory and Anti Depressant Activity

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## Abstract

In our study we have a tendency to synthesized schiff' base of bactericide drug sulphamethoxazole on treating with aromatic aldehydes like p-diethyl amino benzyldehyde and p-dimethyl amino benzyldehyde. The synthesized schiff's bases were regenerate to its ion Schiff bases by treating with methyl group halide. The ion Schiff bases were regenerate to metal complexes by treating with metals like CuCl2, ZnCl2 and CdCl2. All the synthesized compounds were characterised by Elemental analysis, IR and $^1$H proton magnetic resonance. Docking study was performed to know the interaction of binding sites with protein receptor using MAO-B enzymes (PDB ID: 2BK5) and COX-2 enzyme (PDB ID: 5IKR) by Virtual Screening software for Computational Drug Discovery. Synthesized metal complexes were evaluated for antibacterial, anti inflammatory and antidepressant activity. Copper metal complexes showed potent antibacterial and anti-inflammatory activity. Significant anti-depressant activity was shown for 2A2 and 2B2 zinc metal complexes

***Key words:*** *Sulphamethoxazole, antibacterial, anti-inflammatory, antidepressant activity*

## Introduction

Schiff bases area unit the vital compounds within the field of healthful chemistry due to their wide selection of biological activities and industrial applications. Many studies showed that the presence of a lone combine of electrons during a sp2 hybridized orbital area unit of biological importance. Development of a replacement chemotherapeutical Schiff base could be a new space of analysis. Several studies are according relating to the biological activities of Schiff base viz. Anticancer, antibacterial drug antifungal and herbicidal activities. They conjointly function a back base for the synthesis of assorted heterocyclic compounds[1, 2]. We all need iron, copper and atomic number 30 for traditional brain perform however metal metabolism becomes dysregulated during a sort of neurodegenerative diseases. Metals accumulate in Alzheimer's {disease|Alzheimer's|Alzheimers|presenile insanity} dementia and shaking palsy and are deficient in Menkes disease. Whether or not excess metals seem as a cause or a consequence of the illness method isn't sure, however accumulation of metals have the potential to trigger cellular harm. During a healthy brain, metals ar tightly regulated. Through a sublime system of copper chaperones that taxi copper from the cell surface to specific living thing destinations,

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there's primarily no free copper in cells. Cellular iron isn't regulated by chaperones however rather by iron regulative proteins that orchestrate and synchronize iron uptake with iron storage to cut back the supply of free iron. In distinction to copper and iron, atomic number 30 is that the 'wild card' of brain metal metabolism as a result of not solely will it contribute to the site of key antioxidative metalloenzymes, like the Cu-Zn SOD, however it additionally exists as free atomic number 30 in some colligation vesicles and acts as a neurochemical. Once brain tissue is broken, like following a stroke, free atomic number 30 will flood the abraded areas leading to necrobiosis thanks to atomic number 30 excitotoxicity. Transition metals perform an outsized vary of biological functions at intervals the brain. a standard feature is their ability to exist during a sort of chemical reaction states and participate in reaction reactions; therefore copper, iron, and metal ar all catalytically active metals during a category of enzymes that sequester free radicals. it's helpful to appear at the common and ranging functions of transition metals within the brain to higher perceive what mechanisms ar noncontinuous in metal dyshomeostasis and the way this could cause necrobiosis in diseases of the central nervous system[3-5].

Literature review reveals that synthesized schiff's base metal complexes possess sensible medicine property. Antibacterial medicines are accepted antimicrobial agents, which are wide utilized in varied diseases. up to now antibacterial drug metal advanced has not been synthesized. Thus we have a tendency to aim to synthesize schiff's base of antibacterial medicine with aromatic organic compound like p-diethyl amino benzyldehyde and p-dimethyl amino benzyldehyde and to make metal complexes higher than schiff's base with metals like copper, metal and metallic element.

## Materials and Methods

### Syntheis of schiff's base

The schiff's base 2A has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30ml)of Sulphamethoxazole (mol wt. 253.28) with hot ethanolic solution (30 ml) of (0.01 mole) Para diethyl amino benzaldehyde " ( mol wt 177.24) for about 2 – 3 hours at 60 – $70^0$C. The resulting solution was concentrated, allowed to stand overnight and recrystallized with ethanol. The schiff's base 2B has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 ml0.01 mole) Sulphamethoxazole (mol wt 253.28) with hot ethanolic solution of para dimethyl amino benzyldehyde ( mol wt 149.19) for about 2 – 3 hours at 60 – $70^0$c The resulting solution was concentrated, allowed to stand overnight and recrystallized with ethanol (Figure-1)[6, 7].

### Synthesis of transition metal complexes

Anhydrous $CuCl_2$, $ZnCl_2$ and $CdCl_2$ (0.0005 mol) was dissolved in 50ml ethanol added with cationic derivative of Schiff base refluxed for 6 hours. The reaction mixture was left overnight and recrystallized with $C_2H_5OH$. (Figure-2)[8].

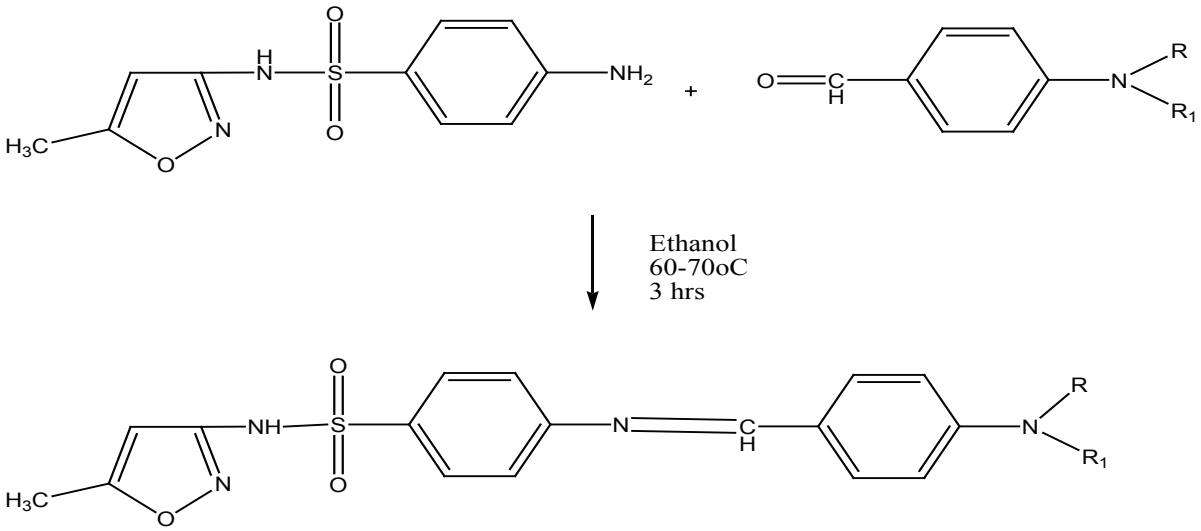


**Figure 1- Scheme-1 (Synthesis of Sulphamethoxazole Schiff base)**

**Compound 2A R-$C_2H_5$, $R_1$-$C_2H_5$**

**Compound 2B R-$CH_3$, $R_1$-$CH_3$**

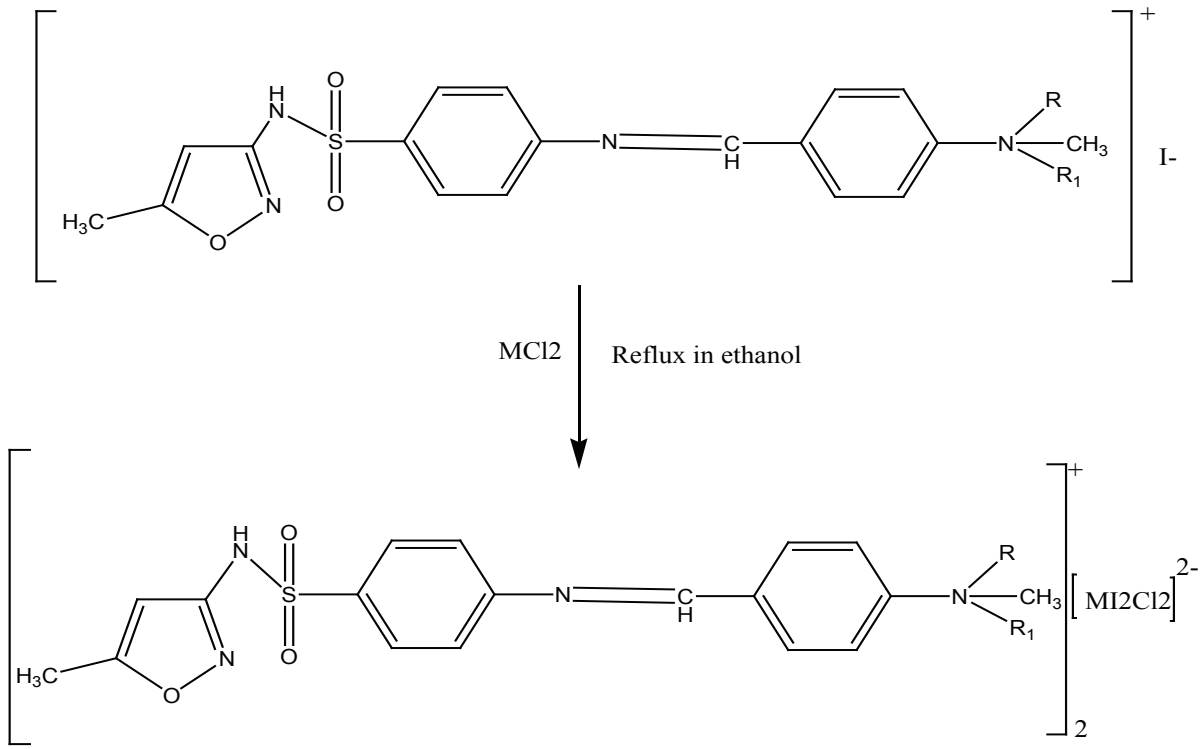


**Figure 2-Scheme-2 (Synthesis of cationic Schiff base metal complex)**

**Compound 2A R-$C_2H_5$, $R_1$-$C_2H_5$**

**Compound 2B R-$CH_3$, $R_1$-$CH_3$**

**$MCl_2$- $CuCl_2$, $Zncl_2$ and $CdCl_2$**

**Docking study**

Docking study was performed to know the interaction of binding sites with protein receptor using MAO-B enzymes (PDB ID: 2BK5) and COX-2 enzyme (PDB ID: 5IKR) by by Virtual Screening software for Computational Drug Discovery[9].

**Anti bacterial activity**

Required range of Muller agar plates were ready and divided into range of quadrant.Then the plates were inoculated with acknowledged take a look at organism. Sterile discs were placed with in every quadrant.Using small measuring device ten small cubic decimeter of saturated resolution of the derivatives is applied on the individual discs. Then the plates square measure incubated at 37°C for eighteen to twenty four hrs. Once incubation for every spinoff against completely different organisms was measured and tabulated[10, 11].

**Determination of median lethal doses (LD50 )**

Animal's Swiss mice (20-25gm) and Male Sprague - Dawley rats (160-180) were maintained at customary diet and ad physical attraction. The experiment protocol was approved from institutional moral committee. LD50 values were calculable by the "acute toxicity test" as delineate elsewhere. The take a look at compounds were dissolved in three nothing DMSO administered orally to completely different teams with increasing doses. Six animals were taken in every cluster. Mortality make up my mind once twenty four hours of treatment. The dose, at that the fifty nothing mice survived, was thought of as LD50 worth of the compound[12].

**Anti inflammatory activity**

Swiss mice were divided into 5 teams of six animals every. The take a look at teams received orally twenty mg/kg of every sample. The reference cluster received diclofenac sodium (10 mg/kg, p.o) whereas the management cluster received vehicle (tween 80). After 1h, 0.1 mL, 1 Chronicles w/v carrageenin suspension in traditional saline was injected into the subplanatar tissue of the correct hind paw. The paw volume was measured at 30min. 1, 2,3 and 4hr once carageenan injection employing a micrometer screw gauge. The proportion inhibition of the inflammation was calculated[13].

**Anti depressant activity**

Male Sprague - Dawley rats consideration 160-180 grams are used. They are brought to the laboratory at least one day before the experiment and are housed one by one in makrolon cages with free access to food and water. Naïve rats are one by one forced to swim within a vertical Plexiglas. Rats placed in cylinders for the 1st time are at first extremely active, smartly swimming in circles, attempting to climb the wall or diving. Once 5-6 minutes immobility reaches a tableland wherever the rats stay immobile for around eightieth of the time. Once quarter-hour in the water the rats are removed and allowed to dry in a heated enclosure (32 c) before came back to their home cages. They are once more placed in the cylinder twenty four hours later and the total period of immobility is measured throughout a 5minute take a look at. Floating behaviour throughout this five minutes amount has been found to be duplicable in totally different teams of rats. Associate in Nursing animal is judged to be immobile whenever it remains floating passively in the water in a slightly round-backed however up-right position, its nose simply on top of the surface. take a look at medicine or commonplace are administered one hour before testing. Since experiments with the commonplace drug (Imipramine) showed that injections one,5 and twenty four previous the take a look at gave the most stable results in reducing floating these times are chosen for the experiment[14].

## Results and Discussion

**Characterization of synthesized compounds**

**2A1- Copper metal complex of *(E)*- N-(4-(diethyl, methyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{22}H_{27}Cl_2CuI_2N_4O_3S$. M.wt: 815.8. IR (KBr) cm-1: NH bond stretching at 3424 cm-1, stretching at 1690 cm-1 for C=N , S=O stretching at 1140 cm-1, C=C stretching at 1650 and 1475 cm-1. H1 proton magnetic resonance (CDCl3) δ values: Multiplet at 7.54-7.68 for aromatic nucleus, undergarment at 8.39 for N=CH peak, undergarment at 4.2 for aromatic NH peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undergarment at 2.42 for N-methyl substitution and undergarment at 2.35 for methyl radical hooked up in oxazole nucleus. Elem Anal Calc: C, 32.39; H, 3.34; Cl, 8.69; Cu, 7.79; I, 31.11; N, 6.87; O, 5.88; S, 3.93. Elem Anal Found: C, 32.29; H, 3.44; Cl, 8.65; Cu, 7.77; I, 31.31; N, 6.87; O, 5.88; S, 3.93.

**2A2- Zinc metal complex of *(E)*- N-(4-(diethyl, methyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{22}H_{27}Cl_2I_2N_4O_3SZn$. M.wt: 817.6. IR (KBr) $cm^{-1}$: NH bond stretching at 3464 cm-1, stretching at 1690 cm-1 for C=N, S=O stretching at 1140 cm-1, C=C stretching at 1630 and 1475 cm-1. H1 magnetic resonance (CDCl3) δ values: Multiplet at 7.44-7.69 for aromatic nucleus, undershirt at 8.39 for N=CH peak, undershirt at 4.2 for aromatic American state peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undershirt at 2.42 for N-methyl substitution and undershirt at 2.35 for alkyl radical connected in oxazole nucleus. Elem Anal Calc: C, 32.32; H, 3.33; Cl, 8.67; I, 31.04; N, 6.85; O, 5.87; S, 3.92; Zn, 8.00. Elem Anal Found: C, 32.22; H, 3.43; Cl, 8.64; I, 31.08; N, 6.82; O, 5.89; S, 3.97; Zn, 8.00.

**2A3- Cadmium metal complex of *(E)*- N-(4-(diethyl, methyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{22}H_{27}CdCl_2I_2N_4O_3S$. M.wt: 864.7. IR (KBr) $cm^{-1}$: NH bond stretching at 3405 cm-1, stretching at 1690 cm-1 for C=N, S=O stretching at 1140 cm-1, C=C stretching at 1640 and 1475 cm-1. H1 nuclear magnetic resonance (CDCl3) δ values: Multiplet at 7.46-7.70 for aromatic nucleus, undergarment at 8.39 for N=CH peak, undergarment at 4.2 for aromatic NH peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undergarment at 2.42 for N-methyl substitution and undergarment at 2.35 for alkyl radical hooked up in oxazole nucleus. Elem Anal Calc: C, 30.56; H, 3.15; Cd, 13.00; Cl, 8.20; I, 29.35; N, 6.48; O, 5.55; S, 3.71. Elem Anal Found: C, 30.52; H, 3.19; Cd, 13.01; Cl, 8.21; I, 29.37; N, 6.47; O, 5.56; S, 3.71.

**2B1- Copper metal complex of *(E)*- N-(4-(trimethyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{20}H_{23}Cl_2CuI_2N_4O_3S$. M.wt: 787.7. IR (KBr) $cm^{-1}$: NH bond stretching at 3415 cm-1, stretching at 1690 cm-1 for C=N, S=O stretching at 1140 cm-1, C=C stretching at 1640 and 1475 cm-1. H1 nuclear magnetic resonance (CDCl3) δ values: Multiplet at 7.46-7.70 for aromatic nucleus, undergarment at 8.39 for N=CH peak, undergarment at 4.2 for aromatic NH peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undergarment at 2.42 for N-methyl substitution and undergarment at 2.35 for alkyl radical hooked up in oxazole nucleus. Elem Anal Calc: C, 30.49; H, 2.94; Cl, 9.00; Cu, 8.07; I, 32.22; N, 7.11; O, 6.09; S, 4.27. Elem Anal Found: C, 30.59; H, 2.98; Cl, 9.05; Cu, 8.17; I, 32.12; N, 7.01; O, 6.07; S, 4.25.

**2B2- Zinc metal complex of *(E)*- N-(4-(trimethyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{20}H_{23}Cl_2I_2N_4O_3SZn$. M.wt: 789.6. IR (KBr) $cm^{-1}$: NH bond stretching at 3455 cm-1, stretching at 1690 cm-1 for C=N, S=O stretching at 1140 cm-1, C=C stretching at 1630 and 1475 cm-1. H1 magnetic resonance (CDCl3) δ values: Multiplet at 7.44-7.69 for aromatic nucleus, 8 undershirt at 8.39 for N=CH peak, undershirt at 4.2 for aromatic

American state peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undershirt at 2.42 for N-methyl substitution and undershirt at 2.35 for alkyl radical connected in oxazole nucleus. Elem Anal Calc: C, 30.42; H, 2.94; Cl, 8.98; I, 32.14; N, 7.10; O, 6.08; S, 4.26; Zn, 8.28. Elem Anal Found: C, 30.32; H, 2.98; Cl, 8.98; I, 32.24; N, 7.00; O, 6.18; S, 4.29; Zn, 8.24.

**2B3- Cadmium metal complex of *(E)*- N-(4-(trimethyl amino) benzylidene)-4-(5-methyl isoxazol-3-sulfonamidyl) benzenamine**

M.F: $C_{20}H_{23}CdCl_2I_2N_4O_3S$. M.wt: 836.6. IR (KBr) $cm^{-1}$: NH bond stretching at 3455 cm-1, stretching at 1690 cm-1 for C=N, S=O stretching at 1140 cm-1, C=C stretching at 1630 and 1475 cm-1. H1 magnetic resonance (CDCl3) δ values: Multiplet at 7.40-7.68 for aromatic nucleus, undershirt at 8.39 for N=CH peak, undershirt at 4.2 for aromatic American state peak, 2 triplet at 1.23 for 2 CH3 teams in N-ethyl substitution, 2 quadret at 3.39 for 2 CH2 cluster in N-ethyl substitution, undershirt at 2.42 for N-methyl substitution and undershirt at 2.35 for alkyl radical connected in oxazole nucleus. Elem Anal Calc: C, 28.71; H, 2.77; Cd, 13.44; Cl, 8.48; I, 30.34; N, 6.70; O, 5.74; S, 3.83. Elem Anal Found: C, 28.70; H, 2.78; Cd, 13.46; Cl, 8.47; I, 30.38; N, 6.71; O, 5.75; S, 3.84.

**Molecular Docking Studies of synthesized Schiff's base metal complexes compounds**

To understand the interaction of all the synthesized compounds (2A1-2A3 and 2B1-2B3 ) with MAO-B enzyme and COX-2 enzyme, the crystal structure of MAO-B enzyme and COX-2 enzyme was downloaded from Protein Data Bank (PDB ID: 2BK5 and 5IKR) and the molecular docking studies were performed.

The ligands 2A1 had a -score value of −9.3 to −8.5 against COX-2 enzyme (PDB ID: 5IKR) (Table-1 and figure-3). Out of all synthesized compounds 2A1 gave good score value than the standard. The minimum Glide energy required for the formation of complex between ligand and the receptor indicates excellent binding affinity.

**Table-1 docking score values of synthesized metal complex 2A1**

| Ligand | Docking score binding affinity | Rmsd/ub | Rmsd/lb |
|---|---|---|---|
| 5ikr_macro_prepared_2A1_ligand | -9.3 | 0 | 0 |
| 5ikr_macro_prepared_2A1_ligand | -9.1 | 25.864 | 24.43 |
| 5ikr_macro_prepared_2A1_ligand | -9 | 25.877 | 24.364 |
| 5ikr_macro_prepared_2A1_ligand | -9 | 54.922 | 52.502 |
| 5ikr_macro_prepared_2A1_ligand | -8.8 | 2.985 | 1.616 |
| 5ikr_macro_prepared_2A1_ligand | -8.7 | 28.398 | 24.268 |
| 5ikr_macro_prepared_2A1_ligand | -8.6 | 25.803 | 24.445 |
| 5ikr_macro_prepared_2A1_ligand | -8.6 | 25.42 | 23.292 |
| 5ikr_macro_prepared_2A1_ligand | -8.5 | 40.226 | 31.697 |

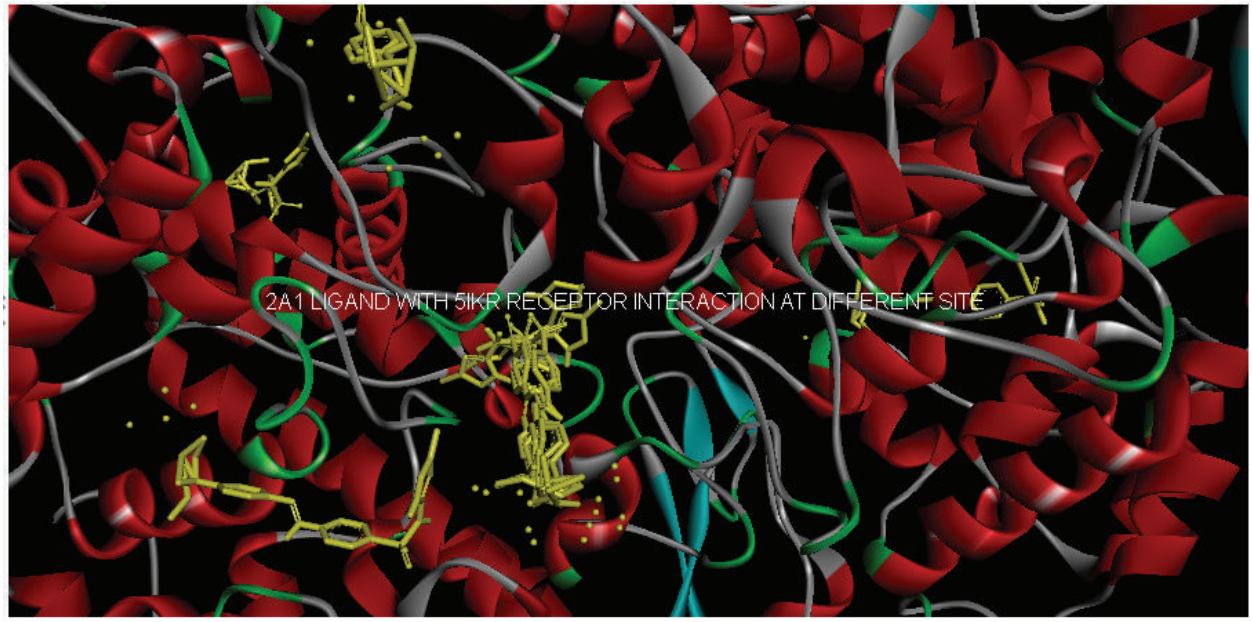


**Figure 3-2A1 ligand with COX-2 (5IKR) receptor interaction at different active binding sites**

The ligands 2A2 and 2B2 had a -score value of −9.0 to −7.5 against MAO-B enzyme (PDB ID: 2BK5) (Table-2 and figure-4). Out of all synthesized compounds 2A2 and 2B2 gave good score value than the standard imipramine.

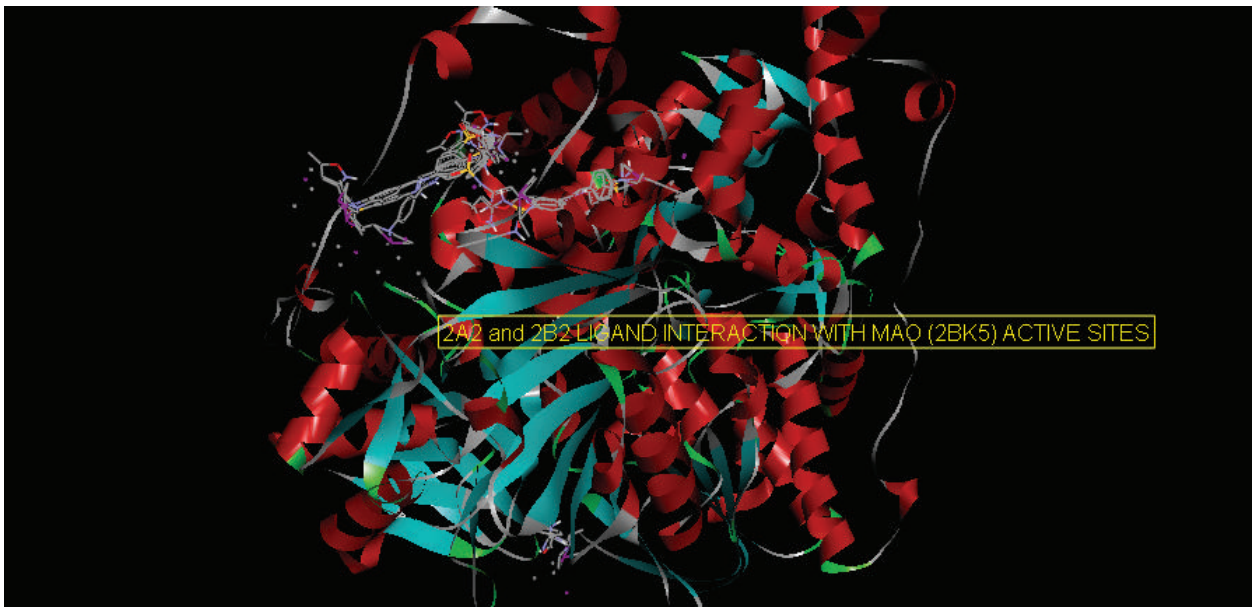


**Figure 4-2A2 and 2B2 ligand with MAO (2BK5) receptor interaction at different active binding sites**

**Table-2 docking score values of synthesized metal complex 2A2**

| Ligand | Docking scoring binding affinity | Rmsd/ub | Rmsd/lb |
|---|---|---|---|
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -9 | 0 | 0 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.3 | 43.145 | 40.927 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.2 | 43.858 | 41.481 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.2 | 45.801 | 42.408 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.1 | 45.859 | 43.915 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.1 | 45.765 | 43.917 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -8.1 | 44.644 | 42.207 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -7.8 | 43.928 | 42.025 |
| 2bk5_MACRO_PREPARED_NEW_2A2_ligand | -7.5 | 47.727 | 44.352 |

**Antibacterial activity**

Antibacterial activity was carried out by disc plate method using *B.cereus, E.coli, and P. aeruginosa* microorganism. The results are calculated and given in the table-3. The results of antibacterial activity revealed that the ligand with the Cd, Zn complexes does not exhibit antibacterial activities. However it is important to note that ligand with Cu complex exhibits potent antimicrobial activities. The Cu complex shows more activity than the Cd and Zn complex. This perhaps because of the upper stability of copper advanced than the Cd and metal advanced. The microorganisms take up metal ions on their cell walls and as a result respiration processes of cells square measure disturbed and macromolecule synthesis is blocked that is that the demand for more growth of organisms. The expansion inhibition effects of metal ions square measure goodly[15].

**Table-3 Results of antibacterial activity of Schiff's base metal complexes of Sulphamethoxazole**

| S.No | Compounds | Zone of inhibition (mm) | | |
|---|---|---|---|---|
| | | B. cereus | E. coli | P.aeruginosa |
| 1 | 2A1 | 25 | 24 | 22 |
| 2 | 2A2 | 25 | 18 | 24 |
| 3 | 2A3 | 21 | 20 | 20 |
| 4 | 2B1 | 20 | 24 | 22 |
| 5 | 2B2 | 22 | 17 | 18 |
| 6 | 2B3 | 19 | 17 | 19 |
| 7 | Streptomycin | 24 | 24 | 21 |

**Anti inflammatory activity of synthesized compounds**

Anti inflammatory activity was carried by paw oedema method and results shows that compound 2A1 and 2B1 at dose level of 20mg/kg.b.wt. significant activity (81 and 84 % respectively) when compared to standard diclofenac 10mg/kg.b.wt (84%). In our study results revealed that copper complexes showed potent anti-inflammatory activity but zinc complexes posses moderate anti-inflammatory activity, whereas cadmium complexes has not shown considerable activity. It was confirmed that the elevation of plasma copper-containing elements represents a physical response which can result in remission. Promotion of this physical response may be a valid approach to the treatment of the diseases with inflammatory elements. it absolutely was therefore confirmed that copper complexes, a singular category of doubtless additional therapeutically helpful for anti inflammatory medication[16].

**Anti depressant activity of synthesized compounds**

Anti depressant activity was evaluated by force swim test method, the animals which are immobile for less time considered as active and results showed significant percentage response such as 60%, 63% of the compound 2A2 (20mg/kg.b.wt.) and compound 2B2 (20mg/kg.b.wt.) respectively than the standard Imipramine (5mg/kg.b.wt). The most probable causes for depression area unit connected with the loss of physiological condition of the strain hormones, neurotransmitters, and disturbed trace components levels. It's rumored that youth stress could be a major risk issue for development of later depression because of affected maturation in brain, particularly in hippocampus. On the molecular level, these methods are also zinc-dependent via antioxidative activity changes and its influence on correct course of brain development process[17].

## Conclusion

In conclusion we synthesized Schiff base by reacting Sulphamethoxazole with p-diethyl amino benzyldehyde and p-dimethyl amino benzyldehyde, six metal complexes of Schiff base by treating with $CuCl_2$, $ZnCl_2$ and $CdCl_2$. *In silico* studies results showed that docked complexes are the results of accumulative result of these interactions that are expected to own higher pharmacological activities such as anti inflammatory and anti depressant activity. Copper metal complexes 2A1 showed potent antibacterial activity against *B.cereus* and copper metal complexes 2A1 and 2B1 showed potent antibacterial activity against *E.coli* and copper metal complexes 2A1 and 2B1showed potent antibacterial activity against *P.aeruginosa* strains. Copper metal complexes 2A1 and 2B1 showed excellent anti-inflammatory activity. Significant anti-depressant activity was shown for 2A2 and 2B2 zinc metal complexes. The above 2A1, 2B1, 2A2 and 2B2 compounds activities are supported by results of *in silico* docking studies. Hence we tend to conclude that Schiff's base metal complexes offer a flexible platform for etymologizing numerous pharmacologically active medicines.

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**Financial Disclosure:** None

**Conflict of Interest:** The authors declare no conflicts of interest

**Ethical Clearance:** All experimental protocols were approved under the Vel's University and all experiments were carried out in accordance with approved guidelines.

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# Aortic Atherosclerosis and Risk Factors: A Clinico-Demographic Autopsy-Based Study

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## Abstract

**Backdrop**: Aortic atherosclerosis continues to be a complex process, with the genetic factors, personal lifestyles, alterations in local hemodynamics, vascular injury, age related changes, endothelial inflammation and autoimmune diseases, all contributing to its pathogenesis. The scenario of atherosclerosis in India is appalling with highest loss in potentially productive years of life. As study of atherosclerosis in living population is difficult, invasive and expensive especially in the developing countries, autopsy studies have been proved to be a good method for assessing atherosclerosis. So, the present study was planned to evaluate the prevalence of atherosclerosis, with the clinic-demographic profiles of the victims of aortic atherosclerosis and analysing the risk factors associated with it.

**Material and Methods:** This is a cross-sectional descriptive study which was conducted to detect aortic atherosclerosis among all the deceased who got referred to R.G. Kar Medical College Police Mortuary for autopsy between 1st April to 30th September, 2014.

**Resusts:** Majority of the victims belonged to 4th to 8th decades, with male preponderance in all the age groups. Though the residence has got no significant implication on atherosclerosis, age turned out to be the single most culprit in the pathogenesis of atherosclerosis, with hypertwnsion, hyperlipidemia, hyperglycemia and smoking playing their significant parts.

**Conclusion:** The authors feel, in most cases, atherosclerosis can be prevented as most risk factors associated with this illness are directly connected with the lifestyle of an individual. In this regard, educational campaign that focuses on informing the members of the society about the dangers of atherosclerosis, its causes, and the ways of its prevention is likely to reduce the occurrence of this disease and thus improve the overall health level of the nation.

***Keywords:*** *Atherosclerosis, Hypertension, Hyperlipidemia, Hyperglycemia, Smoking, Cardiovascular Disease*

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## Introduction

Atherosclerosis continues to be one of the hot topics in pathology research. Cardiovascular disease (CVD) has emerged as a major health burden worldwide, with atherosclerosis being the major

cause, contributing to the mortality and morbidity.[1] It is defined as thickening of artery wall due to accumulation of fatty materials and is one of the leading causes of CVD worldwide.[2] Atherosclerosis, being a disease of the large elastic and muscular arteries, the aorta is the most frequently involved structure than the rest of the body.[3] Atherosclerosis in the thoracic aorta is closely related to the degree of coronary and carotid disease.[4] The etiology of atherosclerosis comprises of genetic factors, personal lifestyles, alterations in local hemodynamics, vascular injury, age related changes, endothelial inflammation and autoimmune diseases.

The scenario of atherosclerosis in India is appalling with highest loss in potentially productive years of life (deaths in people aged 35 to 64 years of life).[5] The reported loss was 9.2 million in the year 2000 and projected to reach 17.9 million by 2030 which is 9.4 times greater than loss in the USA. The incidence of CVD is reported to be 2–3 times higher in urban than in rural people.

As study of atherosclerosis in living population is difficult, invasive and expensive especially in the developing countries, autopsy studies have been proved to be a good method for assessing atherosclerosis.[2] So, the present study was planned to evaluate the prevalence of atherosclerosis, with the clinic-demographic profiles of the victims of aortic atherosclerosis and risk factors, and thus to formulate preventive strategies to curtail this ascend of morbidity and mortality due to aortic atherosclerosis.

## Aims and Objectives

1. To estimate the prevalence of aortic atherosclerosis

2. To study the clinic-demographic profiles of the victims

3. To analyse the correlation between atherosclerosis with hypertension, hyperlipidemia, hyperglycemia and smoking

## Material and Methods

a. Place of study: R.G. Kar Medical College Police Mortuary, Kolkata, West Bengal.

b. Period of study: 1st April to 30th September, 2014

c. Study population: All the patients sent for autopsy examination at R.G. Kar Medical College Police Mortuary in the study period

· *Inclusion criteria: All the deceased which were sent for autopsy examination at R.G. Kar Medical College Mortuary.*

· *Exclusion criteria:*

*i. Mutilated and decomposed bodies*

*ii. Cases with incomplete or inadequate history*

d. Sample size: All the 406 cases sent for autopsy at R.G. Kar Medical College Mortuary in the defined study period

e. Study design: Cross-sectional descriptive study

f. Procedure: Autopsy was conducted only after proper identification of the body. Thoracic cavity was opened and the lungs were dissected out. Then aorta is dissected out from its origin, up to the aortic opening of the diaphragm. Then diaphragm is divided and aorta is dissected up to the renal arteries. The dissected out aorta is cut open along its posterior surface and presence of atheromatous plaques, if any, were noted and grading was done as per the operational definition.

g. Source of data:

i. Police or Magistrate inquests

ii. Statements of the patient relatives

h. Statistical analysis: Details regarding the cases were obtained from the inquest report, interviewing the eyewitnesses and the family members of the deceased. All the data were manually checked and

edited for completeness in a pre-determined format and were then coded for computer entry. Collected data was recorded in Microsoft Excel worksheet and SPSS IBM 19. The data was collected, tabulated and statistically analyzed by applying student's t-test. The $p < 0.01$ was considered as statistically significant.

### i. Operational Definition:

**Grading system for severity of aortic atherosclerosis[6]**

| Grade | Severity | Description |
|---|---|---|
| 1 | Normal | Intimal thickness < 2mm |
| 2 | Mild | Mild (focal or diffuse) intimal thickening of 2-3mm |
| 3 | Moderate | Atheroma > 3-5mm (no mobile or ulcerated components) |
| 4 | Severe | Atheroma > 5mm (no mobile or ulcerated components) |
| 5 | Complex | Grade 2, 3 or 4 atheroma with mobile or ulcerated components |

## Results

### 1. Prevalence of Aortic Atherosclerosis

156 (38.4%) of 406 cases, who reported for autopsy, presented with aortic atherosclerosis.

### 2. Distribution according to age and gender

Among the deceased presenting with aortic atherosclerosis, 55.8% were males, and people of age group 60-80 years were mostly affected, followed by the deceased between 50-60 years of age [Fig 1].

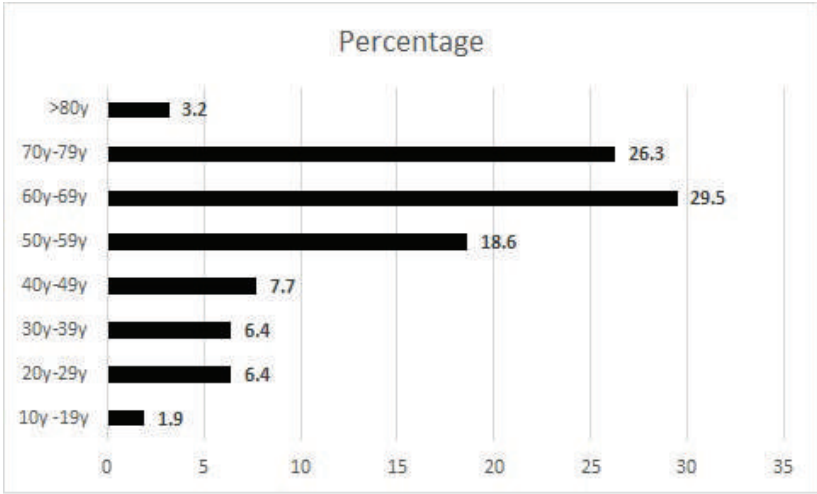


**Fig 1 Age distribution**

### 3. Distribution according to residence

Out of 156 cases presenting with aortic atherosclerosis, 49.7% were from the urban areas and the rest from the rural background.

### 4. Distribution according to the history of Hypertension, Hyperlipidemia, Hyperglycemia and Smoking habits

**Table 1 Distribution according to Hypertension, Hyperlipidemia, Hyperglycemia and Smoking**

| Variables | Hypertension | Hyperlipidemia | Hyperglycemia | Smoking |
|---|---|---|---|---|
| Present | 40.4% | 65.4% | 35.9% | 33.3% |
| Absent | 59.6% | 34.6% | 64.1% | 66.7% |

### 5. Distribution according to Grading of aortic atherosclerosis

Grade1 and Grade 2 atheromatous plaques were noted in almost 56% of the population [Fig 2].

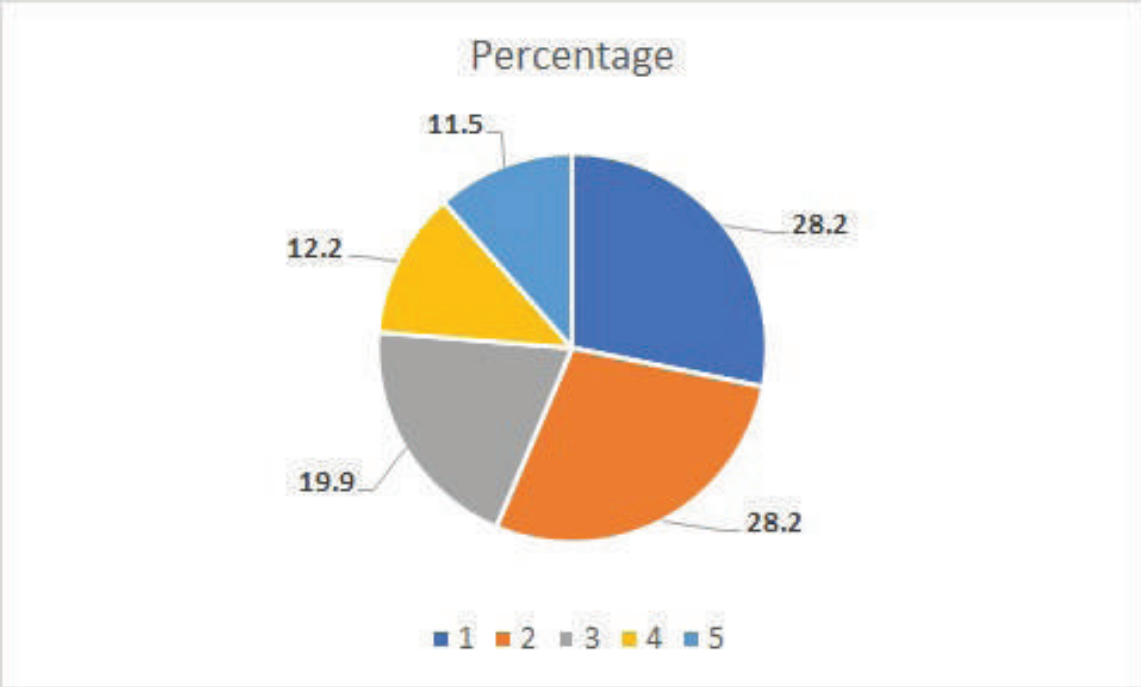


**Fig 2 Distribution according to grading of Aortic Atherosclerosis**

### 6. Correlation of Grading with Hypertension, Smoking, Hyperlipidemia and Hyperglycaemia

Grading of aortic atherosclerosis is seen to be positively correlated with hypertension, hyperlipidemia, hyperglycemia and smoking habits, with a significance level of $p<0.001$ in all the instances [Table 2].

**Table 2 Correlation of Grading with Hypertension, Hyperlipidemia, Hyperglycemia and Smoking**

| Variables | Correlation | Hypertension | Hyperlipidemia | Hyperglycemia | Smoking |
|---|---|---|---|---|---|
| Grading | Pearson Correlation | 0.702 | 0.801 | 0.815 | 0.798 |
| | Sig. (2-tailed) | <0.001 | <0.001 | <0.001 | <0.001 |

**7. Distribution according to the cause of death**

Out of 156 patients presented with aortic atherosclerosis, 54 patients (34.6%) died due to cardiac causes and the rest of the patients due to non-cardiac causes.

## Discussion

Atherosclerosis is a systemic inflammatory disease, which characteristically starts from the branching points of the arteries.[7] Hemodynamic factors play a pivotal role in the localisation of atherosclerosis, with abdominal aorta being the site of earliest and most severe atherosclerotic disease. The extent of the lesions in aorta may reflect the propensity of the patient to develop atheroma. The incidence of atherosclerosis is decreasing in Western Europe, the United States, and in Australia, but is steeply increasing in Central and Eastern Europe, Asia and Africa.[1] The greater cause of concern is the early age of CVD related deaths in the developing countries as compared to the developed countries, which cripple the major workforce of the nation. A study from Scotland supports this with their study within a population considered to be at low or intermediate risk of CVD reflecting a disease prevalence of 5.3%.[8] However, 49.4% of participants presented at least one vessel with stenotic disease and 27% with multivessel disease. Similarly, though the present study reported a prevalence of 38.4%, only 34.6% of the patients with aortic atherosclerosis succumbed due to cardiac causes. This difference in the people affected may be explained by the fact that autopsy-based naked eye macroscopic examination was considered as a methodology as compared to the whole-body MR imaging in living patients. The prevalence and extent of the atherosclerosis increase rapidly with increasing age.[9] DeBakey et al. even reported that aging was a risk factor for atherosclerotic occlusive disease in Grades 1, 2 and 4, with a strong association between aging and aortic atherosclerosis,[10] which is consistent to our study depicting greater involvement among the people belonging from 5$^{th}$ to 8$^{th}$ decades. Atherosclerosis is classed as a disease of aging, with age being a single most independent risk factor for the development of atherosclerosis.[11] Atherosclerosis is also associated with premature biological aging, as atherosclerotic plaques show evidence of cellular senescence characterized by reduced cell proliferation, irreversible growth arrest and apoptosis, elevated DNA damage, epigenetic modifications, and telomere shortening and disfunction. There is a growing evidence that cellular senescence promotes atherosclerosis, shooting a possibility to retaining the cellular senescence as a therapeutic target in atherosclerosis. Though according to DeBakey et al.[10], there is a predominance of males in all the categories of ages, our study failed to find a significant difference between the genders. Similar gender-based results were also seen across the globe, which stated no relationship between aortic atherosclerosis and gender.[7,12] Taking into consideration the place of residence, our study depicted almost equitable distribution of aortic atherosclerosis among rural and urban population, with no significant difference. However, the minimal edging of rural people over the urban ones can be explained by the limited medical and diagnostic facilities, coupled with awareness of the common mass in the rural India.

Several authors have shown a strong association between aortic atherosclerosis and other risk factors for stroke. Age, hypertension, atrial fibrillation, carotid artery disease and diabetes mellitus are well known risk factors for cardiac events and stroke.[13] Similar to our study, age has been shown to a strong independent predictor of aortic atherosclerosis, which suggests that atherosclerosis may be a marker of aging, rather than a true risk factor of stroke. One of the most serious health problems related to untreated high blood pressure is atherosclerosis, or plaque build-up in the arteries. Clinical trials have shown that, in the highest quintile of diastolic pressure, even with the added risks of high cholesterol and smoking, hypertension still contributes significantly to the risk for atherosclerosis.[14] This is may be due to the fact that high blood pressure puts added force to the artery wall. Overtime, this extra pressure can damage the arteries, making them more vulnerable to narrowing and plaque build up associated with atherosclerosis. The narrowed artery limits or blocks the flow of blood to heart muscle, thus depriving the heart oxygen. In conjunction to this, we also detected a significant positive correlation between aortic atherosclerosis and hypertension, with the 'p' value being less than 0.001. Similarly, our study is of a vision that smoking represents one of the most important preventable risk factors for the development of atherosclerosis, the correlation being statistically significant. Together with the aggressive merchandising practices and the highly addictive nature of nicotine, cigarettes are currently one of the most dangerous drugs freely available to human beings.[15] Smoking plays a strong role not only in CVD initiation but also significantly contributes to and causes disease progression and fatal cardiovascular outcomes. The key processes in smoking-induced atherogenesis initiation are endothelial dysfunction and damage, increase in and oxidation of proatherogenic lipids, as well as decrease of high-density lipoprotein, induction of inflammation, and the shift toward a procoagulant state in the circulation. Current data clearly show that also second-hand smoking can trigger life-threatening conditions (including in children). Just like smoking and hypertension, hyperlipidemia is considered to be a very dangerous factor leading to cardiovascular and cerebrovascular diseases, especially atherosclerosis.[16] The authors even noticed a statistically significant relation between the two. Other studies provide new insight into the complex mechanisms where hyperlipidemia causes progressive atherosclerosis.[17] It has been shown that physical injury to the endothelial lining of arteries sets off a process which probably is an attempt at healing the injury but which can lead to atherosclerosis. It has also been found that chemical agents such as homocystine can produce a similar series of events leading to atherosclerosis. Again, diabetes mellitus and atherosclerosis appear to be connected through several pathological pathways.[18] Authors too found a significant relation between hyperglycemia and atherosclerosis. Both types of diabetes mellitus have been shown to be independent risk factors for accelerated atherosclerosis development. Among the known pathological mechanisms connecting diabetes and atherosclerosis are dyslipidemia, hyperglycemia, increased oxidative stress, and inflammation. Adequate glycemic control and reduction of known risk factors remain the most frequently used strategies for protecting such patients.

Thus, atherosclerosis is a multifactorial disease. The impact of traditional risk factors such as age, sex, elevated blood pressure, smoking and hyperlipidemia coupled to hyperglycemia has long being demonstrated beyond any doubt, with the emergence of new risk factors like elevated triglyceride levels. The combination of traditional and emerging risk factors is expected to facilitate the assessment of patients' global risk, thereby allowing optical use of diagnostic and therapeutic efforts in high-risk subjects.

## Conclusion

Atherosclerosis is a vast varied and intricate topic, yet our study is a tangible attempt of exploring the socio-demographic profiles of victims and the triggering effects leading to aortic atherosclerosis.

The present study indicated a prevalence of 38.4% in a comparatively urban population, with males dominating in all the age groups. Though residence was not seen to play a pivotal role in the pathogenesis of atherosclerosis, aging was depicted as the single most important factor in the causation of atherosclerosis, with victims mostly from the age group of 4th to 8th decade. Statistically significant correlation was seen between atherosclerosis to that of hypertension, hyperglycemia, hyperlipidemia and smoking, thus emerging with a multifactorial causation.

Thus, atherosclerosis has become one of the major health issues encountered by the modern society. Apart from being harmful on its own, this disorder can provide a basis for the development of a wider array of diseases, which may have a devastating effect on one's health. At the same time, in most cases, atherosclerosis can be prevented as most risk factors associated with this illness are directly connected with the lifestyle of an individual. In this regard, educational campaign that focuses on informing the members of the society about the dangers of atherosclerosis, its causes, and the ways of its prevention is likely to reduce the occurrence of this disease and thus improve the overall health level of the nation.

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# Estimation of Stature & Gender from Thumb Indices in Indian Population

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## Abstract

**Background:** Human characteristics like stature and gender identification on basis of human remains is a crucial element of any medicolegal investigation and is especially important in the field of forensic anthropometry. Thus, this study was undertaken with the aim of determining stature and gender by using thumb indices in the Indian population.

**Material & Methods:** 568 adults (294 males and 274 females) belonging age between 18-25 years participated in the present study. Thumb length, breadth, thickness and circumference were measured by time to time calibrated measuring tape and digital vernier caliper. Unpaired 't' test, Person Correlation Coefficient, Linear and Logistic regression methods used to analyze the data.

**Results:** This study showed significant correlation of thumb indices with stature & gender.

**Conclusion:** The stature and gender identification models are helpful to forensic experts and crime scene authorities to determine the stature and gender of an isolated thumb.

***Key Words:*** *Stature, Gender, Thumb length, breadth, thickness & circumference*

## Introduction

Stature or body height is one of the most important and useful anthropometric parameters that determines an individual's physical identity [1]. When a complete dead body is discovered, determining the individual's stature is relatively simple; however, when only parts of the body are available, determining the individual's stature is difficult [2]. Estimating an individual's stature from skeletal material or mutilated or amputated limbs or parts of limbs has obvious importance in personal identification in the events of murders, accidents, or natural disasters primarily concerned with forensic identification analysis. Many factors, such as racial and nutritional factors, play an important role in human development and growth, necessitating the use of different nomograms for different populations [3]. Until now, most studies on stature estimation have focused on the length of bones such as the femur, tibia, humerus, radius, and so on. There is very little data on work done in an Indian population to determine gender and stature using dimensions of different

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parts of the upper limb. As a result, the purpose of this research is to fill those gaps. This research will look into the possibility of using thumb indices to determine gender and stature in an Indian population.

## Material and Methods

This cross-sectional study was conducted in the Krishna Institute of Medical Sciences (KIMS), Karad from year 2018-2020. According to standard ethics drawn by ethical committee for human experimentation, 568 adults (294 male and 274 female) aged 18-25 years participated in the present study. Individuals with no obvious deformities, injury, fracture, amputation or history of any surgical procedure of hand or previous history of trauma to hands, feet, spine and limbs were excluded from the study. Data on age, sex and height were collected from each of the participant. Height, thumb length and thumb circumference were measured by measuring tape in centimeters while thumb breadth and thumb thickness were measured by Digital Vernier Caliper in millimeters. The measurements in millimeters were further converted into centimeters. Thumb length was measured from proximal flexion crease of the thumb to tip [4]. Thumb breadth was measured from the most lateral to the most medial point of interphalangeal joint of thumb [4]. Thumb thickness was measured from middle of the dorsal aspect of interphalangeal joint to middle of palmar aspect of interphalangeal joint. Thumb circumference was measured from the superficial distance around the edge of interphalangeal joint [4].Stature i.e. natural height of person was measured with the individual standing barefoot on platform of stadiometer with the upper back buttock and heels pressed against the upright position of the instrument. Head was positioned in Frankfort plane, and head plate was brought into firm contact with vertex [1].

## Statistical Analysis

All measurements are summarized into mean and standard deviation (SD). Correlation of height with each of the thumb measurements was determined by Pearson's Correlation Coefficient (r). Backward Linear Regression analysis was performed to estimate the height of the individual with most significant and statistically important thumb indices. Receiver Operating Characteristic (ROC) Curve analysis was performed to determine the cut-off value for each of the thumb measurement, with high sensitivity and specificity, that discriminating between male and female population. The measurements were categorized on basis of these cut-off values. These categorized variables were utilized in identification of gender. Backward-Wald Binary Logistic Regression analysis was further carried out to develop the model estimating gender. Data was analyzed using SPSS-20 version.

## Results

The data collected from 294 males and 274 females aged between 18-25 years were analyzed using SPSS software. The mean height of 294 male participants was 172.4 cm with standard derivation 7.0 cm, while the mean height of 274 female participants was 159.8cm with standard derivation 7.1 cm, as seen in the graph (Fig.1).Males were significantly taller than females (Unpaired t test = 21.419, $p<0.001$), according to the comparison.

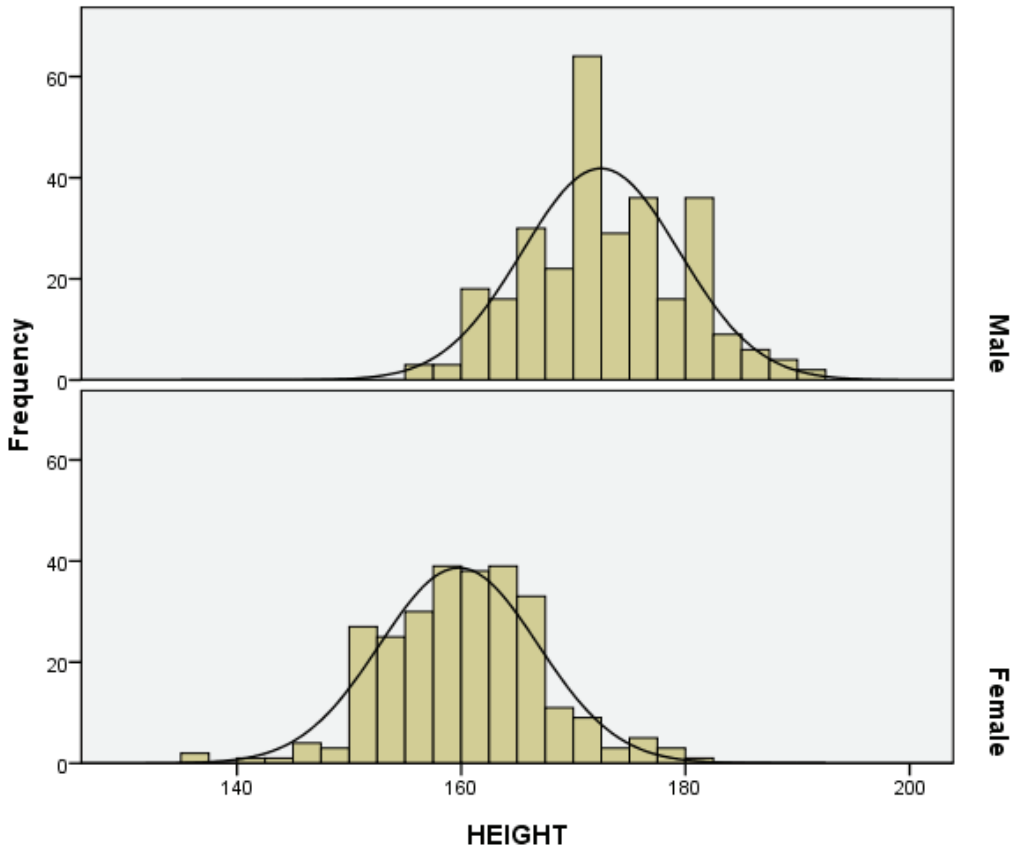


**Fig 1: Graphical presentation of heights of males & females**

In both male and female populations, there was a strong significant correlation between stature and right and left thumb lengths (Table1). Male stature was also correlated to the circumferences of the right and left thumbs, while female stature was correlated to the breadth and thickness of the right and left thumbs.

**Table1: Correlation coefficient (r) between stature & thumb indices of males & female.**

| Gender | Stature | TLRT | TLLT | TBRT | TBLT | TTRT | TTLT | TCRT | TCLT |
|---|---|---|---|---|---|---|---|---|---|
| Male | r | .380** | .382** | -.010 | -.017 | .000 | -.001 | .171** | .169** |
| | p | .000 | .000 | .860 | .768 | .987 | .985 | .003 | .004 |
| Female | r | .370** | .374** | .121* | .126* | .121* | .126* | .070 | .063 |
| | p | .000 | .000 | .046 | .037 | .045 | .038 | .249 | .301 |

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
**TLRT**: Right Thumb Length, **TLLT**: Left Thumb Length, **TBRT**: Right Thumb Breadth, **TBLT**: Left Thumb Breadth, **TTRT**: Right Thumb Thickness, **TTLT**: Left Thumb Thickness, **TCRT**: Right Thumb Circumference, **TCLT**: Left Thumb Circumference.

The male population had significantly higher values in all dimensions than female population. Males have significantly higher right and left thumb lengths, breadths, thicknesses, and circumferences than females (Table 2).

**Table 2: Gender wise Mean and SD of thumb indices.**

| Thumb Indices | Male | | Female | | Unpaired test t value | p value |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | |
| TLRT | 6.86 | 0.59 | 6.04 | 0.44 | 18.698 | <0.001 |
| TLLT | 6.84 | 0.58 | 6.02 | 0.44 | 18.744 | <0.001 |
| TBRT | 1.91 | 0.42 | 1.52 | 0.13 | 14.830 | <0.001 |
| TBLT | 1.91 | 0.43 | 1.52 | 0.13 | 14.793 | <0.001 |
| TTRT | 1.67 | 0.37 | 1.36 | 0.11 | 13.621 | <0.001 |
| TTLT | 1.68 | 0.37 | 1.36 | 0.11 | 13.769 | <0.001 |
| TCRT | 6.51 | 0.54 | 5.62 | 0.36 | 23.155 | <0.001 |
| TCLT | 6.48 | 0.53 | 5.59 | 0.37 | 23.125 | <0.001 |

Using right and left thumb indices, backward linear regression analysis was used to estimate male and female stature. The ANOVA F results for all regression models revealed that for both genders, the estimation of the dependent variable 'Stature' using right and left thumb indices is statistically significant ($p<0.05$), indicating that it is significantly most accurate.

**Male:**

Stature = 141.438 + 4.519×TLRT; ANOVA F=49.415, $p<0.001$; Adjusted $R^2$=0.142

Stature = 140.836 + 4.623×TLLT; ANOVA F=49.856, $p<0.001$; Adjusted $R^2$=0.143

Stature = 142.373 + 51.859×TBRT + 4.565×TLLT – 52.460×TBLT; ANOVA F=19.307, $p<0.001$; Adjusted $R^2$=0.158

**Female:**

Stature = 123.835 + 5.951×TLRT; ANOVA F=43.156, $p<0.001$; Adjusted $R^2$=0.134

Stature = 123.658 + 5.998×TLLT; ANOVA F=44.289, $p<0.001$; Adjusted $R^2$=0.137

Stature = 119.732 – 126.711×TBRT + 6.082×TLLT + 128.927×TBLT; ANOVA F=17.168, $p<0.001$; Adjusted $R^2$=0.151

**Irrespective of Gender:**

Stature = 101.229 + 6.774×TLRT + 1.992×TBRT + 2.941×TCRT; ANOVA F=144.985, $p<0.001$; Adjusted $R^2$=0.432

Stature = 100.704 + 6.928×TLLT + 2.636×TTLT + 2.807×TCLT; ANOVA F=145.889, $p<0.001$; Adjusted $R^2$=0.434

Stature = 100.691 – 32.216×TTRT + 2.876×TCRT + 6.894×TLLT + 34.658×TTLT; ANOVA F=110.755, p<0.001; Adjusted $R^2$=0.436

In each above, the first model is based on right thumb measurements, second model is based on left thumb measurements and third model is based on both thumb measurements to estimate the stature.

The gender discriminating demarking points, i.e. cut-off values of each right and left thumb measurement, were determined using Receiver Operating Characteristic (ROC) Curve analysis (Table 3). These are the values that indicate a high level of sensitivity and specificity.

**Table 3: Classification of observed female and male as per cut-off value of thumb indices.**

| Thumb Indices | Cut-off as per ROC Curve | Gender as per cut-off | Observed Gender | | Total n=568 |
|---|---|---|---|---|---|
| | | | Female n=274 | Male n=294 | |
| TLRT | <6.35 | Female | 218 | 45 | 263 |
| | ≥6.35 | Male | 56 | 249 | 305 |
| TBRT | <1.68 | Female | 250 | 38 | 288 |
| | ≥1.68 | Male | 24 | 256 | 280 |
| TTRT | <1.49 | Female | 235 | 42 | 277 |
| | ≥1.49 | Male | 39 | 252 | 291 |
| TCRT | <6.05 | Female | 230 | 49 | 279 |
| | ≥6.05 | Male | 44 | 245 | 289 |
| TLLT | <6.4 | Female | 219 | 51 | 270 |
| | ≥6.4 | Male | 55 | 243 | 298 |
| TBLT | <1.68 | Female | 248 | 38 | 286 |
| | ≥1.68 | Male | 26 | 256 | 282 |
| TTLT | <1.48 | Female | 231 | 34 | 265 |
| | ≥1.48 | Male | 43 | 260 | 303 |
| TCLT | <5.95 | Female | 210 | 26 | 236 |
| | ≥5.95 | Male | 64 | 268 | 332 |
| | | | | | |

To estimate the gender, a logistic regression model was created using categorical thumb indices (Table 3)

as independent variables. These models were created using the backward Wald method.

The model skeleton for gender estimation from thumb indices is as follows:

$$\text{GENDER PROBABILITY} = \frac{e^{g(x)}}{1+e^{g(x)}}$$

**Model 1: Estimation of gender from right thumb indices.**

g(x) = 1.645×TLRT + 2.562×TBRT + 2.007×TTRT + 1.72×TCRT – 3.611

The length, breadth, thickness, and circumference of the right thumb were found to be significantly correlated with gender in model 1.

The gender estimates based on right thumb indices were very similar to the real gender values. This model 1 had an overall correct classification percentage of 91.9 %, with 93.1% of females and 90.8 % of males correctly classified. (Table 4)

**Model 2: Estimation of gender from left thumb indices.**

g(x) = 1.091×TLLT + 2.182×TBLT + 2.327×TTLT + 2.213×TCLT – 4.109

Gender was also found to be significantly correlated with the length, breadth, thickness, and circumference of the left thumb in model 2.

The gender estimates based on left thumb indices were very similar to the real gender values. Model 2 had an overall accurate classification percentage of 91.7 %, with 90.9 % for females and 92.5 % for males. (Table 4)

**Model 3: Estimation of gender from right and left thumb indices.**

g(x) = 1.206×TLRT + 2.307×TBRT + 2.298×TTLT + 2.136×TCLT – 4.128

Gender was found to be significantly correlated with right thumb length, breadth, thickness, and circumference in model 3.

The gender estimates based on right and left thumb indices were very similar to the real gender values. Model 3 had an overall accurate classification percentage of 91.7 %, with 91.2 % for females and 92.2 % for males. (Table 4)

**Table4: Predicted gender percentage by thumb indices.**

| Observed Gender | Predicted Gender | | | | | | Percentage Correct | | |
|---|---|---|---|---|---|---|---|---|---|
| | Female | | | Male | | | | | |
| | RTI | LTI | RTI & LTI | RTI | LTI | RTI & LTI | RTI | LTI | RTI & LTI |
| Female | 255 | 249 | 250 | 19 | 25 | 24 | 93.1 | 90.9 | 91.2 |
| Male | 27 | 22 | 23 | 267 | 272 | 271 | 90.8 | 92.5 | 92.2 |
| Overall Percentage | | | | | | | 91.9 | 91.7 | 91.7 |
| RTI : Right Thumb Indices, LTI : Left Thumb Indices. | | | | | | | | | |

The values of the independent variables, i.e. of thumb indices, should be entered in the model as per specified in the Table 5. If calculated GENDER PROBABILITY value turns<0.5, it implies the thumb measurements represents Female; otherwise Male. All the three logistic regression models developed for gender estimation revealed that they could estimate gender with more than 91% of accuracy.

**Table 5 : Gender probability in male and female**

| Thumb Indices | Measurement | Value to be entered in calculation of g(x) |
|---|---|---|
| TLRT | <6.35 | 0 |
| | ≥6.35 | 1 |
| TBRT | <1.68 | 0 |
| | ≥1.68 | 1 |
| TTRT | <1.49 | 0 |
| | ≥1.49 | 1 |
| TCRT | <6.05 | 0 |
| | ≥6.05 | 1 |
| TLLT | <6.4 | 0 |
| | ≥6.4 | 1 |
| TBLT | <1.68 | 0 |
| | ≥1.68 | 1 |
| TTLT | <1.48 | 0 |
| | ≥1.48 | 1 |
| TCLT | <5.95 | 0 |
| | ≥5.95 | 1 |

## Discussions

Mass disasters, such as wars, acts of terrorism, and natural disasters, are becoming more common, posing challenges for investigators in establishing identification from isolated upper extremity long bones. There has been a lot of work put into identifying the bones. Many studies have used hand as well as the lengths of the middle, index, and ring fingers, to measure stature [4-6]. Vijaya Kumari N discovered an important association between stature and the length of both thumbs. Males have a slightly higher

correlation between length of left thumb and stature, while Females have a greater correlation between the length of their right thumb and their stature [7]. Kumar L. noticed a correlation between the length of thumb and stature. The correlation coefficient varied between 0.240 and 0.256[8]. In a study by Prerna et al., the right thumb had a correlation coefficient of 0.635 and the left thumb had a correlation coefficient of 0.245 in males, while the right thumb had a correlation coefficient of 0.212 and the left thumb had a correlation coefficient of 0.197 in females [9]. In contrast, Sen et al. found that index finger length had a higher correlation coefficient to estimate height than ring finger length [6,7]. Habib et al. analyzed 159 Egyptians aged 18 to 25 years and observed that males' little fingers were unrelated to stature [10]. Just a few studies have used the length of the thumb to estimate stature and gender [7,11]. And the bulk of the studies were confined to the teenage population. As a result, research into the relationship between stature and thumb length in various geographical locations is required, which could be extended to the adult population.

In this analysis, we looked at both the right and left thumb indices when estimating gender and stature. In an Indian population, we found an important correlation between stature and thumb indices, as well as a correlation between gender and thumb indices. Our findings revealed a strong significant correlation between stature and right and left thumb lengths in both male and female populations. Male stature was also correlated to the circumferences of the right and left thumbs, while female stature was correlated to the width and thickness of the right and left thumbs. The ANOVA F results for both regression models revealed that the estimation of the dependent variable 'Stature' using left and right thumb indices is statistically significant ($p<0.05$) for both genders. In this analysis, statistically significant differences were found between the male and female groups in all parameters, with male measurements being higher than female measurements.

To estimate gender from index and ring finger lengths, Krishan et al. used the sectioning point and binary logistic regression methods. The accurate predictive percentages for the right and left hands were 80.7% and 82.2%, respectively [12]. Jee et al. examined 29 hand measurements in 321 Koreans for gender estimation. When using the sectioning point process, accuracy from index finger length was found to be 65.9% for males and 70.1% for females. Discriminant feature analysis yielded 83.2% accuracy when the three lengths of the thumb, index, and middle finger were used [4].

When analyzed according to gender, all of the parameters in present study were strongly correlated with the right and left thumb indices. All of the gender prediction models produced provided a strong and most accurate estimate of the study population. Anthropometric measurements of the right and left thumb indices for the Indian population are highlighted in this study. These measurements were used to create models for gender estimation. The model's success rates are discussed. Model 1 had a 91.9% overall correct classified percentage, with 93.1 % of females and 90.8 % of males, using right thumb indices to estimate gender (Table 4). Model 2 had a 91.7% overall correct classified percentage, with 90.9 % for females and 92.5 % for males, using left thumb indices to estimate gender (Table 4). Model 3 correctly classified 91.2% of females and 92.2% of males using right and left thumb indices, resulting in an overall accurate classification percentage of 91.7% (Table 4).

Receiver Operating Characteristic Curve analysis was performed to identify the gender cut-off values of each right and left thumb measurements. These categorized variables were used in identification of gender (Table 3).

It was possible to achieve the aim of this research, which was to find correct stature and gender discriminators using thumb indices. The length, breadth, circumferences, and thickness of all thumb parameters were all found to be significantly related to gender. Furthermore, previous research did not consider the breadth, thickness, and circumference of thumb indices when deciding gender. When the breadth, thickness, and circumferences of thumb indices are considered in addition to thumb length, enhanced gender determination accuracy is achieved. This was rarely studied in previous research.

## Conclusion

Various anthropometric criteria with different levels of precision are used to assess gender and stature. As a result, we tested the accuracy of thumb indices to predict stature and gender.

All three gender estimation logistic regression models showed that they were able to estimate gender with greater than 91% accuracy.

Receiver Operating Characteristic Curve analysis found useful to identify the gender cut-off values of each right and left thumb measurements.

The length, width, thickness, and circumference of thumb indices can be used for more precise gender and stature determination, according to a study conducted on an Indian population.

Furthermore, the regression model equations obtained are valid for stature and gender estimations for a given value of thumb measurement.

So, even if only the thumb is available, regression model equations are capable to estimate an individual's stature and gender. To verify the accuracy of regression model equations in various geographical locations more research must be conducted.

These models are useful for anatomists, forensic anthropologists, forensic pathologists, archaeologists, and forensic medicine investigators in gender and stature identification purposes, which may be considered as surrogate method in situations where DNA analysis is difficult for economic or other reasons, such as war and mass disasters.

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# Sudden Death with Dual Organ Pathologies–An Autopsy -based Case Report

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## Abstract

Sudden death due to cardiac cause is considered as a major health problem worldwide accounting for 15–20% of all deaths and cardiomyopathies account for 10–15% of the cases.According to the 2016 WHO classification, angiomatous meningioma is a rare subtype of meningioma classified as Grade I. It is an aggressive variety with a fair prognosis, with typical symptoms including headache and seizures. We present a case of a 60-year-old man brought to the morgue for autopsy with a history of progressive left-sided weakness and headache for several months with no prior diagnosis or treatment for the same because of current pandemic of COVID-19.On conducting medicolegal autopsy significant pathologies in heart and brain were found which could have contributed to the cause of death.

***Key words:*** *Sudden death,hypertensive cardiomyopathy,Angiomatousmeningioma, Intracranialtumor, Space occupying lesion, Autopsy.*

## Introduction

Sudden cardiac death (SCD) is a term used to describe an unpredicted death of a person as a consequence of a cardiovascular event, with or without an existence of an underlying cardiac pathology[1]. It is considered as a major health problem worldwide accounting for 15–20% of all deaths[2]. Coronary artery diseases (CAD), valvular heart diseases, cardiomyopathy syndromes, infiltrative diseases of the myocardium, myocarditis, infective endocarditis, hereditary ion channel abnormalities, and congenital heart illnesses are some of the underlying cardiac causes of SCD.CAD is responsible for 80 percent of SCD cases, cardiomyopathies for 10–15 percent, and hereditary heart abnormalities such as coronary artery abnormalities or cardiac channelopathies for 5–10 percent of SCD cases[3]. In people over the age of 35, coronary atherosclerosis is the most common cause of SCD.Meningiomas are primary central nervous system tumours that account for 15 to 18 percent of intracranial tumours and 33% of all incidental neoplasms in adults[4].The Angiomatous meningiomas are an uncommon (2.1 percent) kind of meningioma identified by the World Health Organization (WHO) based on morphology.Although they appear aggressive, these are benign in nature, and the meningiothelial type 1 variety is the most frequent[5]. Main differential is hemangiopericytoma, which also goes by the label "angioblastic meningioma." Hemangiopericytoma and angioblastic meningioma are difficult to distinguish radiologically, however textural analysis can aid.In radiology,

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histology, immunohistochemistry, and even clinical presentation, these have distinct properties. Therefore, a multidisciplinary approach is required to distinguish this unique meningioma from other space-occupying vascular lesions[6].Literature searchprovided adequate research in radiology, immunohistochemistry, pathology but lacked autopsy-related documentation of this variety in Indian scenario.

We present a case of a 60yearold man who suddenly collapsed at the home after fall from the bed right after the dinner. He had past medical history of hypertension on irregular medication. Autopsy examination showed acute myocardial infarction (AMI) of the left ventricle with atherosclerotic changes of the coronaries. Apart from that, there was an incidental finding of a space occupying lesion in the brain and anterior cerebral artery atherosclerosis.

Case Details:According to the inquest and documents provided by the investigating officer, the deceased had been suffering from left-sided weakness for a few months, he was known to be hypertensive and was on irregular medication for hypertension, and on the day of the incident, 05/01/2021 at late night 13:00 while his daughter saw the deceased lying unconscious on the floor with bleeding from his nose and mouth, he was shifted to a hospital.

**Autopsy findings:** There was a 2cmx1cm contusion present over nasal bridge red in colorand there was no other external injury. The face was congested with bluish appearance of nailbed and lips. On internal dissection, lungs were oedematous,heart weighed 518gm and fat deposits were found around the heart. The right and left ventricular wall thicknesses were found 2mm and 15mm, respectively. The left ventricle showed acute myocardial infarction (AMI) which was demonstrated by the haemorrhagic myocardium seen in the papillary muscles (Fig.1). The stomach had around 300gm semi-digested food material with visible grain particles having no particular smell, the mucosa was congested without submucosal haemorrhages. The liver weighed 1500gms and it was congested; both kidneyswere congested, left kidney weighs 158gms and the right kidney weighs 160gms with intact corticomedullary junction. On removing the skull cap the dura was adherent to inner table of the skull and it was bluish in colour, left side frontal region had an organised space-occupying lesion adherent to dura with visible vessels over the surface. The space occupying mass weigh 50gms and the brain tissue adjacent to the lesion had a depression of 4cmx3cmx2cm (Fig. 2). The brain was oedematous and vessels were engorged and weigh 1346gms. Gross dissection of brain showed atherosclerotic changes in multiple areas of circle of Willis, more commonly in the right anterior cerebral artery.The heart and space-occupying lesion were preserved in formalin solution and sent for histopathological analysis.

**Histopathology laboratory report**: Angiomatous meningioma grade I was discovered in the space occupying mass (Fig-3), and cardiac tissue revealed striking hypertrophy of the myofibres (Fig-4). Transverse diameters of affected myofibres increased three to five times, with nuclear enlargement and hyperchromasia.In addition, anterior Cerebral blood vessels exhibited straightforward atherosclerotic alterations but no essential luminal constriction.

## Discussion

One of the less prevalent types of primary cardiomyopathies is hypertrophic cardiomyopathy (HCM).Although the illness is becoming more widely recognised in India, there is little information on its incidence and mortality rates.The primary goal of this case report was to look into HCM that was discovered after an autopsy.The role of myofibre disarray as a diagnostic sign for the disease has been severely examined.Our goal was also to demonstrate the necessity of histological analysis in confirming or excluding the diagnosis of HCM,

particularly in the case of sudden death.In most cases, SCD autopsy techniques begin with a macroscopic examination of the heart; if the aetiology is found, no more testing is required.However, depending on the victim's age, a negative macroscopic test is followed by either histological or genetic testing.If a victim is over 30 years old and has had a negative macroscopic exam, histological testing is the next step; if the test is positive, no further action is required[7].After the heart has been cleared of blood, other measures are obtained, including the heart's weight, thickness, and dimensions[8].After staining the tissues with hematoxylin and eosin also the Masson trichrome stain, histological studies are carried out[9]. Hypertrophic cardiomyopathy (HCM), an autosomal dominant illness caused by mutations in sarcomere protein-coding genes, can be found in young people with SCD[10].It has thickened left ventricular (LV) walls, asymmetrical septal hypertrophy, and mid-ventricular blockage morphologically.Myocardial fibre disarray and myocyte hypertrophy, which is associated with a fourfold increase in cell and nuclear size, are microscopic observations in HCM.There is also an aberrant thickening of the coronary arterioles[11]. Meningiomas account for 36.1 percent of primary cerebral tumours and are more common in people in their medium to late years of life[12].Meningiomas develop from arachnoid villi meningiothelial cells[13].Divya et al provided a case of agiomatous meningioma with an intraparenchymal origin (angiomatous meningioma, WHO Grade 1 of meningiomas)[14].The most common symptom of angiomatous meningioma is headache[15].Juyoung et al and Dietzmann K et al stated on radiological examination, it is difficult to distinguish the type of meningioma due to the presence of distinctive perilesional oedema.Immunohistochemistry and electron microscopy can be used to distinguish angiomatous meningioma from hemangioblastoma and hemangiopericytoma[16,17]. Angiomatous meningiomas have a vascular component that accounts for more than half of the tumour size and 2.1 percent of all meningiomas[18]. Daniel et al found these are more common in the area of cerebral convexity and connected to the dura[19].The female/male ratio of meningiomas is higher, with a male preponderance in thesubtype angiomatous meningioma[15]. Martin et al classified angiomatous meningiomas based on the diameter of vascular channels, macrovascular (more than 50% of vessels with a diameter greater than 30 micrometre) and microvascular (less than 50% of vessels with a diameter greater than 30 micrometre), (more than 50 percent of vessels diameter smaller than 30micrometer)[15]. Meningiomas are normally asymptomatic, however they can cause seizures, focal deficits, and neuropsychiatric symptoms such as depression or psychosis in some cases[5].Metastasis is uncommon in meningiomas, occurring in only 0.1 percent of cases[15].






Fig-Papillary muscle HaemorrhageFig-2 Space Occupying Lesion

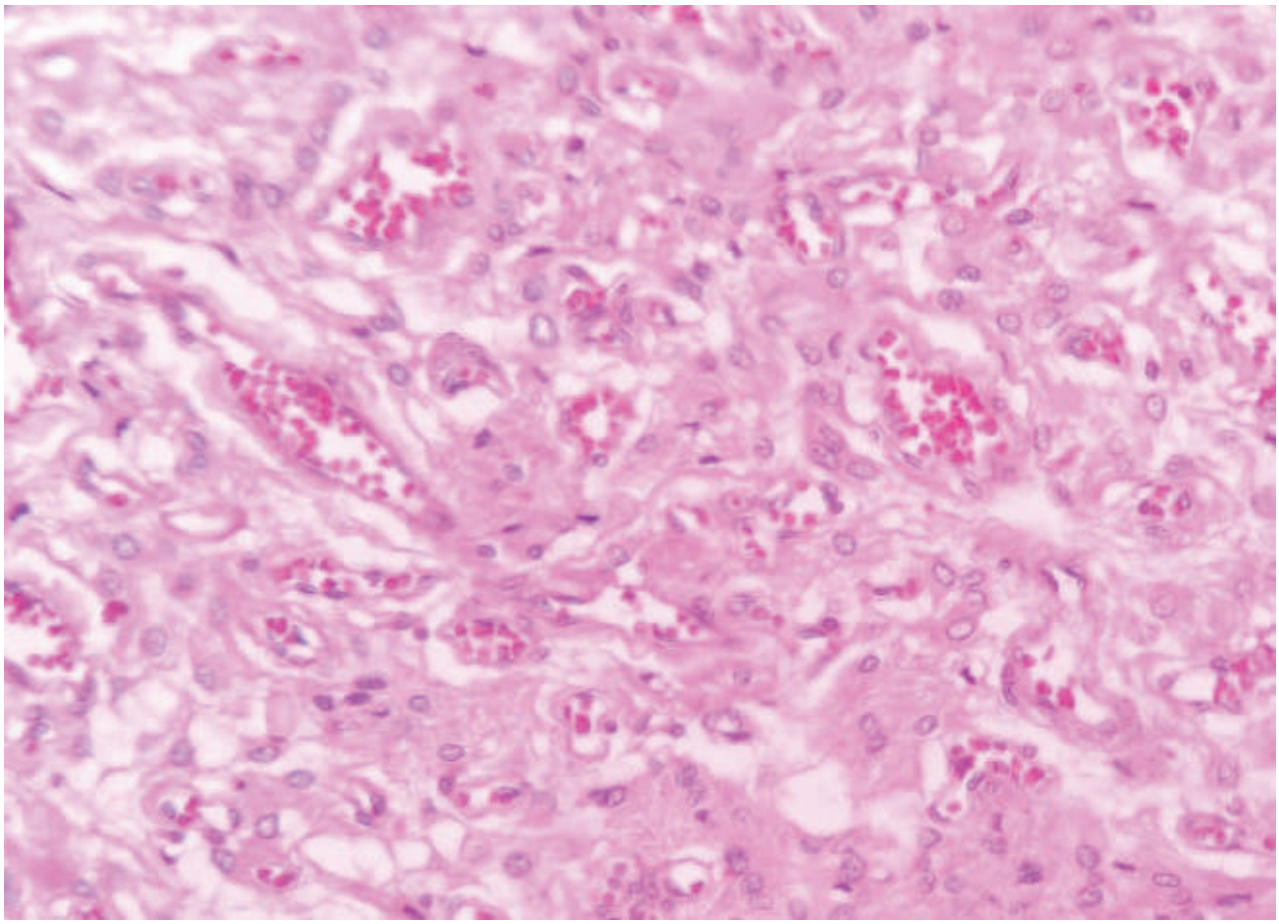

**Fig- 3 AngiomatousMeningioma**

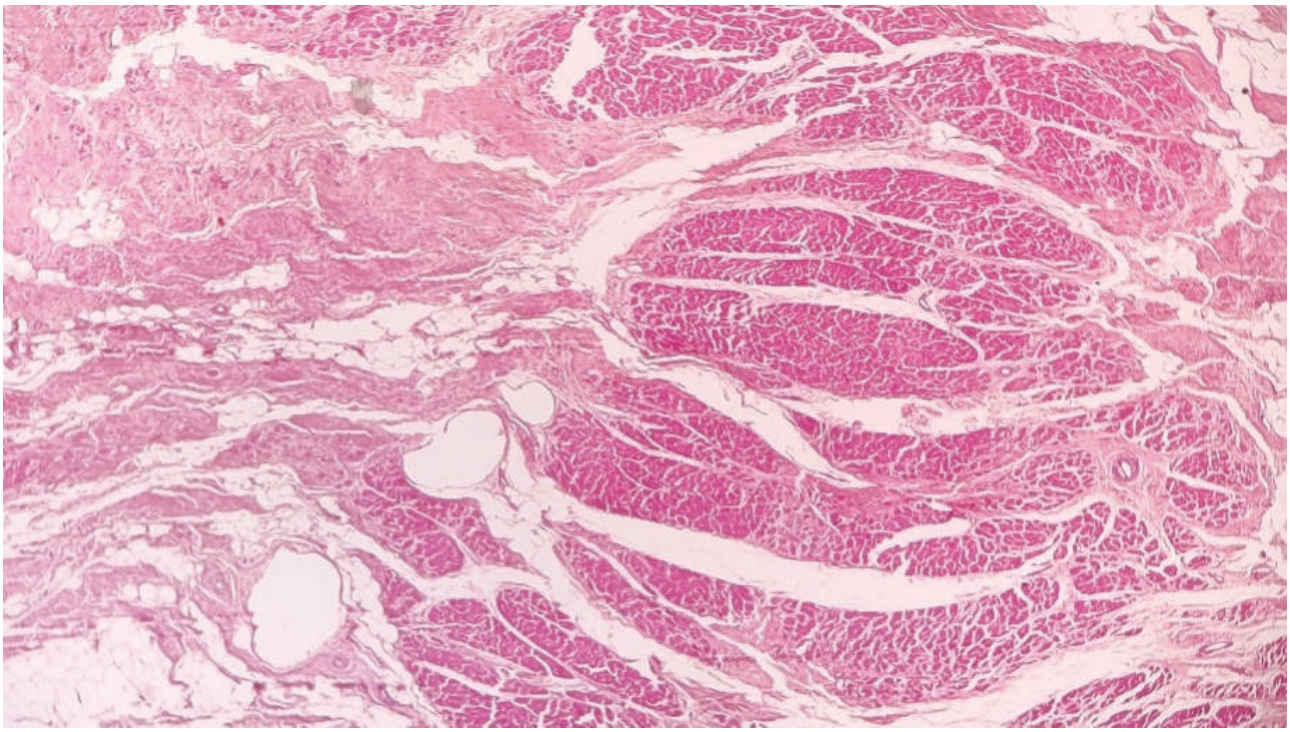

**Fig-4 Myocardial Hypertrophy**

## Conclusion

Sudden death is unquestionably one of the most unforeseen and tragic consequences.The use of myofibre disarray as a diagnostic for the diagnosis of HCM in biopsy specimens is limited.This is due to the varying amount and distribution of disorder in the myocardium, as well as sample restrictions.Disarray is pathognomonic of HCM when measured quantitatively alongside other histological characteristics.The value of a histological investigation cannot be overstated, particularly in the case of a sudden death.Angiomatous meningioma is characterised by a high blood supply and specific features. It is more prevalent in males and is frequently seen connected to the dura in cerebral convexities, with headache being the most prevalent symptom.Autopsy report in ourcase holds a significant value in detailing the exact cause of death.

**Conflict of Interest-** None

**Source of Funding-** None

**Ethical Clearance**: Taken from Institutional Ethical Committee, AIIMS Bhubaneswar. The identity of the deceased was not revealed in the manuscript.

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# Demographic Study of Unnatural Deaths in Paediatric Age Group in General Hospital, Khammam

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## Abstract

**Introduction:** The data regarding pediatric deaths are very limited particularly in developing countries due to lacunae in death registration system. The present study is a retrospective study regarding the causes which resulted in the deaths of children in pediatric age group from 0-12 yrs with an aim to know the overall incidence, the causes of death and manner of death. Unnatural childhood deaths are not only associated with intense trauma and distress, but also indicates a sense of self neglect to protect children from harm. The unnatural deaths may be due to unintentional or intentional acts.

**Materials and Methods:** This study is a retrospective demographic study of unnatural deaths among pediatric age group children between 0-12 years. All the unnatural deaths that were autopsied at the mortuary, General Hospital, Khammam of Telangana state for a period of 2 years from August 2012 to July 2014 were studied based on inquest, post mortem examination findings to know the profile of paediatric deaths.

**Results and Conclusion:** A total of 1283 cases were autopsied during the study period and out of that 2.4% of cases are unnatural deaths in pediatric age group of 0-12 years. The most commonly involved are male children in the age groups of the cases observed were between 3- 6 years and 9-12 years, the least involved age group was between 0- 3 years. Most common manner of death is accidental amounting to about 93.3%.Most common cause of death was due to road traffic accidents. These deaths are common in the rural population belonging to the low socio economic status.

***Key words:*** *Demographic study, Unnatural pediatric deaths, Post mortem examination, Road traffic accients, Socio economic status.*

## Introduction

There is a substantial rise in unnatural causes of deaths in paediatric age group in the last few decades. Death is unnatural when caused prematurely against the order of nature due to injury, accident, poison or other means of violence. Many cultural and socio-economic factors of a country are usually related to causation of unnatural deaths. The unnatural deaths may be due to unintentional or intentional acts. Unintentional acts are the leading cause of unnatural deaths among children which includes mainly accidental causes. Intentional acts leading to death include suicidal and homicidal deaths. Trauma related deaths and sudden infant death syndromes also quite

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common beyond neonatal period.

Drowning, electrocution, burns, fall from height, explosions, collapse of structures, animal attacks, mass disasters and mechanical asphyxial deaths are the other leading causes of paediatric unnatural deaths in our country [1]. Homicidal pediatric deaths are uncommon.

Police investigation records provide a valuable source of information on the events leading to the death of an individual. Most of the unnatural deaths will report to the mortuary and autopsies are important in the investigation of childhood deaths. Analysis of these records along with postmortem examination reports may help us in understanding the cause and manner of death, to find out potential areas of intervention and also to develop preventive measures.

## Aim of the Study

The present study is taken up to know the demographic profile of unnatural deaths in pediatric age group and to suggest preventive measures to reduce such unfortunate unnatural deaths.

## Materials and Methods

This study is a demographic study of unnatural deaths among pediatric age group of 0-12 years autopsied at mortuary, General Hospital, Khammam of Telangana state for a period of 2 years from August 2012 to July. Study Criteria includes all cases of unnatural deaths in the age group of 0-12 years and excludes all cases of natural deaths in the age group of 0-12 years and all cases of unnatural deaths in the age group above 12 years.

Relevant autopsy findings related to each of these cases were taken for analysis. The various epidemiological factors involved such as age, sex, socio-economic status and others were noted down. These were then correlated with the post-mortem findings to conclude the analysis of each case. Further details of clinical data of the victim including the investigations and procedure done, survival period, time and cause of death were obtained from hospital records. All the findings those were obtained were noted down in a separate proforma for each case. The statistical analysis of the data collected is presented in the tabular form and bar diagrams.

## Observations & Discussion

During the study period, out of 1283 cases brought for autopsy at General Hospital, Khammam, 30 (2.4%) cases were unnatural deaths seen in pediatric age group of 0-12 years and remaining 97.6% cases belong to the children above 12 years and adult population.

| Age group | No of Deaths | Percentage (%) |
|---|---|---|
| 0-3 (years) | 3 | 10 |
| 3-6 (years) | 11 | 36.6 |
| 6-9 (years) | 5 | 16.6 |
| 9-12 (years) | 11 | 36.6 |
| Total (years) | 30 | 100 |

**Table 1: Table showing age wise distribution of paediatric death cases.**

As shown in the table 1, among 30 cases of unnatural pediatric deaths, maximum number of cases belongs to the age groups of 3-6 years and 9-12 years and most of the victims were male children with sex distribution of 25(80%) deaths in males and 6(20%) deaths in females. this is in concurrence with studies done at Manipal, South India (55.5% male and 44.5% female) and studies done by John R Hall and coworkers (50.5% male and 49.5% female), Kim A Collins and coworkers (57.5%maleand42.5%female), Jonathan P Wyatt and co workers(60.5% male and 43.5% female).

Cause of death based on inquest and post mortem examination findings, as shown in the diagram 1, the study finds that 16 (53.3%) cases were due to Road traffic accidents followed by 16.6% due to accidental drowning, 2 (6.6%) cases were due to accidental electrocution, 1(3.3%) are due to poisonous snake bite, 5 (16.6%) cases are due to organophosphate poisoning and 1(3.3%) case was due to strangulation. In the present study, road traffic accidents were the most common cause of unnatural deaths among the pediatric age group.

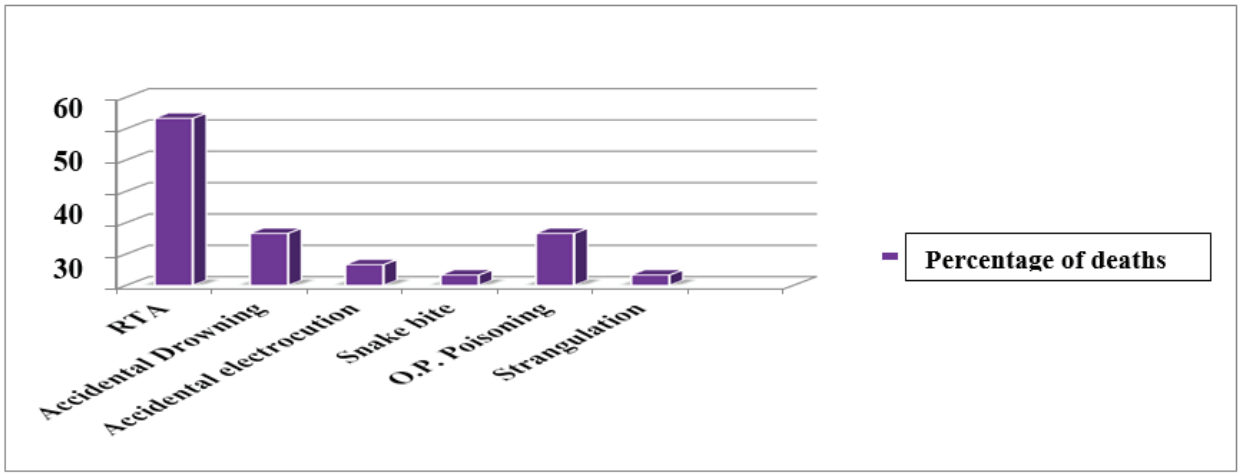


**Diagram 1: Bar diagram showing percentages of cases based on cause of death.**

The manner of death is of prime importance, in this study, the maximum number of deaths that were reported are accidental deaths topped in the list with 28 in number (93.3%) and cases followed by 1 (3.3%) which was homicidal and 1 (3.3%) was due to unknown manner of death. The present study did not encounter any suicidal deaths correlated with the studies by Palimar V, Arun M [11].

**Table 2: Table showing the Manner of death in cases of Paediatric deaths**

| Manner of Death | No of Deaths | Percentage (%) |
|---|---|---|
| Accidental | 28 | 93.3 |
| Suicidal | 0 | 0 |
| Homicidal | 1 | 3.3 |
| Natural | 0 | 0 |
| Unknown | 1 | 3.3 |
| Total | 30 | 100 |

In this study, 1 case of homicidal death was reported 4 years old male child was kidnapped and strangulated. These findings are correlating with the studies done by Meel B L [12].

Based on circumstantial evidence from inquest, place of occurrence of death in these cases was 6 deaths occurred indoors, 24 deaths occurred outdoors, with a distribution of 20%, 80% in indoors and outdoors respectively. This can be attributed to the care and observation of parents or guardians usually have over the child while they are at home. House does also provide protection from extreme of temperature, protection from animals, snake bites and vehicular accidents. When the child is outside the house, there is a risk of vehicular accidents as well as rough play among the children. The mortality rate was quite higher in boys compared to girls because, boys do have more aggressive behavior are active in outside sports and are interested in going outside the house alone, while girls are usually restricted to stay at home and while going out are accompanied by an elder.

In this study, maximum numbers of deaths were reported in Rural population i.e., 15 (50%) deaths, followed by 5 (16.6%) deaths in Semi-urban and 10 (33.3%) deaths in urban population. These findings correlate with the studies done by Soori H, Naghavi M et al which also says that unnatural deaths are more common in rural areas. According to this study the crude mortality rate was 4.33 per 1000 and the number of deaths from unintentional injuries was 5213 (10.7%) of all deaths [19] . Low socio economic status, poor hygienic conditions of surroundings, scarcity of proper medical attention in rural areas contributes to the increase in mortality of the pediatric age group.

**Table 3: Table showing the distribution of cases based on the socio economic status.**

| Socioeconomic status (Modified Prasad's Classification- Per Capita Income in Rs./Month) | No of Deaths | Percentage (%) |
|---|---|---|
| I (2200 and above) | 2 | 6.6 |
| II (1,100-2199) | 4 | 13.3 |
| III (660-1099 | 6 | 20 |
| IV (330-659) | 8 | 26.6 |
| V (Below 330) | 10 | 33.3 |
| Total | 30 | 100 |

In the present study, maximum number of victims were from social class V i.e, 10 (33.3%) cases, followed by social class IV i.e. 8 (26.6%), followed by social class III i.e. 6 (20%) social class II i.e, 4(13.3%) and followed by least among social classes I i.e, 2 (6.6%) cases. Children belonging to low socio economic status usually have less parental care and vigilance as the parents tend to have more children, are usually illiterate or low liter and have to do laborious work so they tend to leave their children unattended.

**Table 4: Table showing the time of death based on inquest & Circumstances.**

| Time of Death | No of Deaths | Percentage (%) |
|---|---|---|
| Day(6 am to 6 pm) | 25 | 83.3 |
| Night(6.01pm to 5.59 am) | 5 | 16.6 |
| Total | 30 | 100 |

Another important aspect of our study is to determine the relation of time and unnatural deaths in children. As shown in the table 4, in vast number of cases, deaths were reported during day time and only 16.6% of cases were reported during night.

Conclusion & Recommendations: From this study, it is evident that the male children are most commonly involved in the age groups of between 3- 6 years and 9-12 years and the least involved age group were between 0- 3 years. In the present study, maximum number of cases manner of death was accidental due to road traffic accidents in children belonging to low socio economic status living in the rural areas during day time. As majority of deaths in pediatric age group are accidental, Creation of awareness in the community to recognize the special responsibility towards kids and children is important. RTA can be reduced by following some simple precautions and strict rules should be framed and implemented for drivers regarding their duty hours and driving under influence of drugs. Poisons and hazardous substances should always be kept out of reach of children and preferably under lock. Surroundings of houses, parks and playgrounds must be maintained properly to prevent snakes from living there as they prefer living in bushes and in anthills. Open defecation should be avoided and people should be advised to construct toilets near their homes as open defecation at night time can lead to snakebites. Children should be taught to swim from a young age to prevent death due to drowning. Children should always be sent along with a responsible adult who knows how to swim when they go to play in water bodies.

**Ethical Committee Clearance-** Taken.

**Source of funding:** Nil

**Conflict of interests** – Nil

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# Emergence of Synthetic Cannabinoids as Drugs of Abuse

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## Abstract

Cannabis has been the most commonly used drug throughout the world for centuries. The psychoactive properties of cannabis are largely attributed to Δ9-tetrahydrocannabinol or THC, the active psychoactive ingredient in the cannabis plant. Lately, new psychoactive substances (NPS) have appeared that are mostly ruled by cathinone's and synthetic cannabinoids (SCs). SC's have emerged as drugs of abuse because of their ability to mimic the euphoric effects of THC. Sprayed on natural herb mixtures, they were initially sold as 'herbal incense' or 'herbal smoking blends' as substitutes for cannabis. These synthetic drugs became popular as 'legal highs' under brand names such as Spice, K2, Mojo and many others in the early 2000's. SC's stimulate the same CB1 and CB2 receptors as THC but they are linked to higher toxicity in terms of duration and severity than cannabis. This is because SC's act as direct agonist of cannabinoid receptors, whereas THC being a partial agonist. Reports suggest that SC's are associated with a range of undesired pulmonary, cardiovascular, gastrointestinal effects. Long term SC use is also linked to severe cognitive deficits. With the global rise in use of SC products, it is important to develop and validate the screening procedures and investigate the toxicological and pharmacological aspects and risk factors associated with its use and abuse.

***Keywords****: Cannabis; THC; drug abuse; toxicity*

## Introduction

The intoxicating properties of cannabis has been known since quite ancient. It is the most widely trafficked and abused illicit drug of this century, especially among teens and young adults [1,2].

Also, a recent report by UNODC indicated that the lockdown is increasing the demand for cannabis [3]. Since the legal status of cannabis is quite questionable, a new class of alternative chemically modified psychoactive substances gained popularity in the 2000's [4]. The UNODC defines NPS (New psychoactive substances) as 'substances of abuse, either in pure form or preparation, that are not controlled by the 1961 single convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat'. NPS comprises of chemical derivatives and analogues (with one or more chemical modifications) or mimetics that are designed to mimic the psychotropic effects of already established illicit drugs [5]. Over these years there has been a tremendous rise in the availability and demand of NPS all over the world. Adolescents and young adults of age group 18-25 account for the majority of SC users because they mistakenly believe

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that its safer than cannabis and due to its controversial legal status. With the rise in hospital admissions and deaths due to these drugs, they have become a public health concern globally.[6,7] After the discovery of Δ9-THC in 1960's, many analogues were synthesized for experimental purposes attributed to its therapeutic benefits and to enhance the understanding of cannabinoid receptor pharmacology [8,9]. CP 55, 940 was discovered by Charles Pfizer in the mid 1970's as potential analgesics, HU-210, discovered by a group led by Raphael Mechoulam at the Hebrew University in the 1980's. However, JWH-018, the most important chemical in the JWH series were developed by John W. Huffman at Clemson University in 1994 [10,11]. However, its use as a drug of abuse started in 2000's when it was first marketed as herbal incense or herbal mixtures, known as 'Spice' as a substitute of cannabis over the internet [12,13]. Thereafter, many competitive brands entered the market and became prevalent worldwide by 2008. The products were sold in headshops, local tobacco shops, gas stations and online under a number of brand names such as K2, black mamba, mojo, etc. Synthetic cannabinoids are also sold as aphrodisiac tea, potpourri, bath salts and air fresheners. These products are often described as "herbal highs" or "legal highs" due to their legal status and supposed herbal make-up. These were sold legally in the streets, labeled as 'Not for human consumption' [14-16]. The main mind-altering component in marijuana responsible for most of its intoxicating effects is *delta-9-tetrahydrocannabinol* (THC). Similar to THC, SC's bind to the cannabinoid type-1 (CB1) and cannabinoid type-2 (CB2) receptors and cause their psychoactive and addictive effects by CB1 receptor agonism [17,18]. Categorized as synthetic cannabinoid receptor agonists (SCRA's), these synthetic analogs have much higher binding affinity and potency to CB1 receptors than THC does [19-21]. Due to the similarity, SC's are sometimes misleadingly referred to synthetic marijuana or fake weed, and are often marketed as safe and legal alternatives to natural cannabis. But in real, synthetic cannabinoids have been linked to severe adverse and unpredictable effects and also presents a major risk of abuse and dependence than cannabis [13,22,23].

**What does it contain**

Packaging labels of synthetic cannabinoid products often claim to contain several herbs like *Leonotis leonurus* (Wild dagga), *Pedicularis densiflora* (Indian warrior), *Nymphacea caerulea*, *Leonurus sibiricus*, *Carnavalia maritima* and *Zornia latifolia*, giving rise to a wide range of drug combinations [15,24,25]. However, none of the listed ingredients have been detectable. It is difficult to determine the exact composition of these products due to lack of any reference samples and presence of masking agents such as eugenol, fatty acids, tocopherol which are added to confute identification. Tocopherol, a form of vitamin- A, is a blood- thinner which is said to hamper drug- testing [26,27].

**Misleading advertising**

The packaging of these products is misleading as only the herbal ingredients are listed with no mention of its addictive constituents and their amounts, which can increase the risk of overdose. Manufacturers use catchy foil packaging and colorful plastic bottles to attract customers. They may try to elevate sales by adding various flavors or by mixing other drugs like-methamphetamine, phencyclidine, ecstasy, making it more lethal [28].

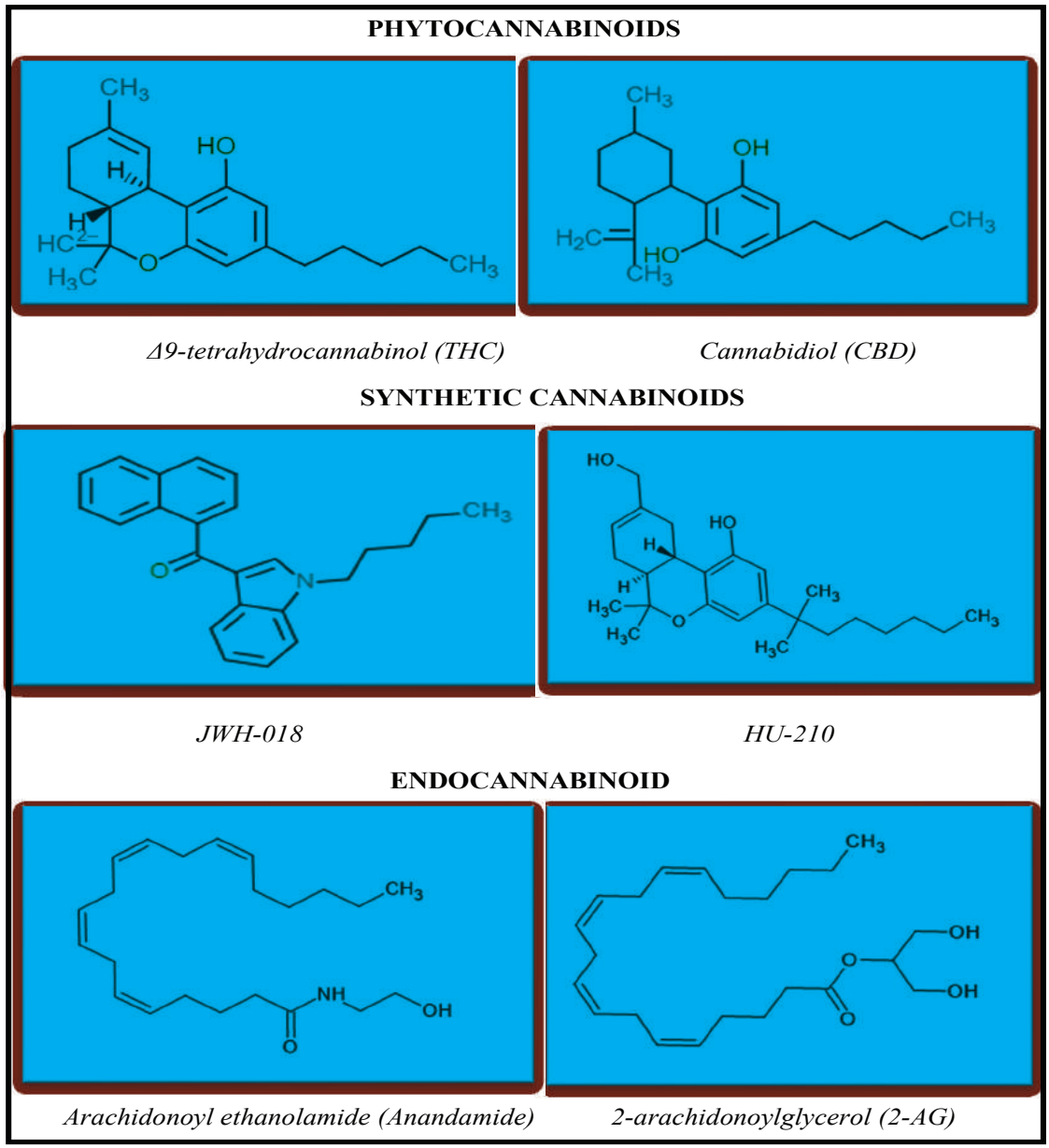
**Usage and Availability**

Initially, SCB products could only be purchased on the internet. However, due to its increased popularity and usage, they are available on headshops and local tobacco shops. In comparison to the organic marijuana, synthetic cannabinoid products are relatively low-cost and easy to purchase with no age restriction [15].

Cannabinoids and The Endocannabinoid System

Cannabis has a very complex cannabinoid profile. It contains approximately 113 different cannabinoids, the two most significant of them are THC and CBD

(cannabidiol). With the discovery of these compounds, an important neurotransmitter system called the endocannabinoid system (ECS) was discovered in the early 1990's [29,30]. The ECS is a unique communication system in the brain and body responsible for regulating and maintaining many essential physiological functions of the body such as immune response, temperature regulation, sleep, memory, movement etc. [31,32]. The human body produces its own kind of chemicals, called as endocannabinoids, and these interact with receptors in the body to regulate these body processes. Several health conditions may also occur if enough endocannabinoids are not produced by the body. Cannabinoids occurring in cannabis can efficiently fill this gap in humans by generating what the body isn't producing in sufficient quantities. Thus, the endocannabinoid system comprises of cannabinoid receptors (CB1 & CB2), endocannabinoids such as anandamide, 2-arachidonoylglycerol(2-AG) and the enzymes that synthesize and degrade the endocannabinoids




[33,34].

**Figure 1: Depiction of chemical structure of phytocannabinoids ($\Delta_9$-tetrahydrocannabinol and cannabidiol), most common synthetic cannabinoid (JWH-018 and HU-210) and endocannabinoids (anandamide and 2-AG).**

**Cannabinoids are Different from other Neurotransmitters**

Neurons communicate with each other in the form of chemical and electrical signals across the synapse. These messages are responsible for governing both the cognitive and motor functions of the body. Chemicals called neurotransmitters are released from one neuron at the presynaptic nerve terminal, travels across the synaptic cleft, then binds and activates the receptor on the adjacent neuron. THC and other cannabinoids mimic or block the functions of neurotransmitters and so hinder normal communication between neurons [35]. However, the endocannabinoid (EC) system communicates its signals in a 'backward' way i.e., when the postsynaptic neuron is activated, cannabinoids are made on demand from lipid precursors or fat cells present in the neuron. Once released from that cell, they travel backwards to the presynaptic neuron, where they get attached to the cannabinoid receptors [35,36]. Δ9-THC and other cannabinoid agonists (chemicals that act on receptors in brain) exert their psychoactive effects by acting on two specific endogenous cannabinoid receptors - CB1, CB2 [37].

**Cannabinoid receptors**

Cannabinoid receptors are 7- transmembrane receptors of the G- protein coupled receptor family [38]. Most psychoactive effects of cannabinoids and endocannabinoids are mediated by two receptors- CB (1) and CB (2). The CB1 receptors are predominantly present in very high levels in areas of the brain such as cerebellum, hippocampus, basal ganglia and dorsal spinal cord regions. The CB2 receptors have a comparatively restricted distribution in peripheral nervous system, especially in immune and gastrointestinal cells and is responsible for modulating anti-inflammatory response [39]. Cannabinoid receptors are activated by a neurotransmitter called anandamide; a natural cannabinoid produced by the body. The chemical structure of THC is quite similar to this endocannabinoid (anandamide). This similarity allows the human body to recognize THC and bind to cannabinoid receptors that activates neurons in the brain thus exerting its effects in the mind and body [40].

**Mode of administration**

The synthetic cannabinoids are mostly administered by smoking. They are used in a similar way as cannabis. The most common mode of administration is smoking as a spliff or joint or using a pipe or bong. After the development of vaping devices or electronic nicotine delivery systems, they have also become a new way of consumption. There has been reports of oral consumption as well, however the effects may be delayed [41].

**Effects of synthetic cannabinoids on humans**

Most synthetic cannabinoid receptors act as full agonists at CB1 receptor and have a much higher potency than that of THC which is a partial agonist [42,16]. Synthetic cannabinoids are mistaken to produce similar effects or 'high' caused by marijuana, but there are remarkable differences between the toxicity of these two. SC's are usually more detrimental if compared to plant- based marijuana. Adverse effects of smoking marijuana are relatively mild and acute whereas synthetic cannabinoids were reported to cause dangerous consequences such as hallucinations, psychosis, hypertension, seizures, panic attacks, etc. The effects of synthetic marijuana are quite unpredictable and the impact on the patients' health can be tremendous and fatal [43,44].

**[A]. Physiological effects**

The physical effects may vary from mild to severe depending upon the exposure and quantity. SC's intoxication can cause a variety of physical or somatic functions such as altered mood, nausea, vomiting, fever, sweating, dilated pupils, shortness of breath, pallor [45].

**[B]. Pulmonary effects**

Common effects include shortness of breath, dry cough, wheezing, producing phlegm with abnormal lung examination. Reports have shown patients with a condition called pulmonary infiltrates (abnormal accumulation of pus/ fluid in the lungs) after chronic inhalation of multiple products of synthetic cannabinoids. Since $CB_1$ receptor is expressed in multiple cell types in the lung where its overstimulation and overactivity has been linked to alveolar inflammation, lung injury and fibrosis [46,47].

**[C]. Cardiovascular effects**

Tachycardia and hypertension are the most commonly reported symptoms from SC's intoxication. Repeated use of synthetic cannabinoids has been associated with adverse cardiovascular effects including arrhythmias, cardiomyopathy, stroke, myocardial infarction and heart failure [48] .

**[D]. Gastrointestinal and Nephrotoxic effects**

Chronic synthetic marijuana use can lead to a syndrome called Cannabinoid hyperemesis or CHS, characterized by cyclical vomiting, nausea, abdominal pain and cramps [45,49]. An unusual sign of this condition is the urge to take hot baths to bring temporary relief to the symptoms caused. Bouts of vomiting may result in dehydration which can further lead to a type of kidney failure called as Cannabinoid hyperemesis acute renal failure (CHARF) [49].

**[E]. CNS effects**

Commonly reported mild primary effects include dilated pupils, drowsiness, droopy eyelids, nystagmus, slow and slurred speech, diaphoresis [50]. Some neurologic effects of SC's include psychosis, memory loss, anxiety symptoms, hemorrhagic and ischemic stroke and emboli. There has been incidents of seizures and cognitive deficits in case of prolonged use. Although these symptoms may appear minor but they can have significant impact on an individual's ability to perform daily activities that requires attention and motor coordination, especially driving [45,51].

**[F]. Psychosis**

A mental health condition where an individual loses contact with reality, unable to differentiate between what is real and what isn't (delusions). This may manifest in the form of visual or auditory hallucinations, paranoia, disorganized behavior and suicidal thoughts. Clinical Studies show that its long-term use is associated with structural and functional changes in the CNS. These alterations were predominantly observed in pre-frontal and limbic regions which are involved in cognitive functions, resulting in a condition called Psychosis. Toxicological studies have shown that hallucinations were commonly reported in patients who had consumed SC products. These individuals also showed symptoms of delirium, confusion and agitation [13,45].

**[G]. Seizures**

Seizures are uncommon after marijuana use but as per findings, SC's can potentially produce seizures and hypothermia in individuals [52]. However, mechanism behind the proconvulsant effect of synthetic cannabinoids is mostly unknown, but it might be due to the potent full agonist effects of the cannabinoid receptor $CB_1$.Moreover, it has been reported that sudden or abrupt discontinuation of SC's is linked with severe withdrawal symptoms such as recurring seizures with cardiovascular and respiratory risks like tachycardia, chest pain, palpitations, dyspnea, etc. [53,54]. Other common adverse effects include headache, anxiety, insomnia, nausea, loss of appetite and mood swings. Synthetic cannabinoid products are composed of a variety of chemicals and additives that may culminate in wide-ranging signs and symptoms, which makes it difficult to deduce the specific etiological compound responsible for clinical symptoms on the users [54-56].

## Conclusion

Synthetic cannabinoid products have rapidly

evolved since first appearing in the market. Cannabinoid based products have become progressively popular despite the unpredictable risks associated with their use. Since, SC's contain a wide range of highly potent full agonists of the cannabinoid receptors that produce similar effects to THC, the outcomes are more severe and fatal. Plant based cannabinoids (phytocannabinoids) are usually associated with psychotropic effects such as euphoria, relaxation, feeling of well-being but synthetic marijuana is linked to some serious undesired effects including hallucinations, delusion, psychosis and maybe death. Chronic use of cannabinoids is also associated with functional and structural neuronal alterations depending on the type of cannabinoids. There has been evidences of severe physical effects such as kidney damage, lung injury, gastrointestinal and cardiovascular effects. The pace at which manufacturers are creating new combinations continues to be a problem to the forensic toxicologists. Clinicians are facing an ongoing challenge of identifying and treating patients who have had intoxicated drugs of unknown composition and origin. The toxicity and pharmakinetics of SC's intoxication is difficult to estimate due to its complex profile and the rapidly changing forms of these compounds. In the light of ever-growing popularity of commercial SC products, it is the need of the hour to educate the public about the hazards associated with these drugs with international collaboration and communication. The necessity for forensic toxicologists and researchers to identify the target metabolites for the novel SC's and to inspect new metabolic patterns is likely to continue in a long term because of its changing forms.

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# A Study of Metacognition in Adults with Type II Diabetes Mellitus – A Cross Sectional Study

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## Abstract

**Introduction:** Elderly individuals often interpret their memory ability and awareness through perceptions and knowledge of general and specific incidents of forgetting otherwise known as metamemory. Type II diabetes is associated with cognitive decrements, accelerated cognitive decline, and increased risk for dementia. Hence, it is important to focus into the link between Type II diabetes and metacognition. **Aim:** The aim is to assess the level of metacognition in older diabetic adults. **Materials & Methods:** The present study was a questionnaire based cross-sectional study. Hundred and twenty Type II diabetes mellitus patients were recruited and they filled the Multifactorial memory questionnaire (MMQ) along with the details of diabetes mellitus. The questionnaire evaluated metacognition, its components are satisfaction, ability and strategy. Pearson correlation was done to analyse the association between metacognition and blood glucose values. Cronbach's alpha test was done to check the internal consistency of MMQ scores. **Results:** Association between corresponding subscale scores and blood glucose which indicates a weak correlation between blood glucose, satisfaction ($r$ = 0.07) and strategy ($r$ = 0.05). In addition, the MMQ subscales and the total score showed good internal consistency (αs = 0.83-0.89).**Conclusion:** Diabetic individuals had poor metamemory scores eventhough they were on insulin therapy. The study would help the diabetic individuals to identify any early stage of cognitive impairment and create an awareness to delay or prevent any further metacognitive dysfunction.

***Keyword:*** *Insulin, Cognitive disorder, dementia, Hippocampus*

## Introduction

Metamemory is a multidimensional construct that includes what people believe about their own memory and how they control and monitor their memory processes [(1)]. People tend to fear a lot since these symptoms can interfere with or prevent their day-to-day activities. Also one of the most common complaints of a diabetic patient is their forgetfulness and their decreases in cognition [(2)]. Diabetes is a known risk factor for cognitive impairment, owing to mechanisms such as chronic hyperglycemia or hypoglycemia, insulin resistance or hyperinsulinemia, oxidative stress, and build-up of beta amyloid protein in the brain. It also affects memory related knowledge, perceptions, appraisals, emotions, self-regulation,

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beliefs about the functioning, development, and capacities of one's own memory and the human memory system [3,4]. Diabetes mellitus increases the risk of cognitive impairment and dementia. Impairment of insulin signaling is a critically important factor and may be the cornerstone of the development of these cognitive sequences regardless of diabetic status [5-8].

Dementia is a term used for forgetfulness and decrease in cognition. It is a common complaint of a diabetic patient and they fear that their forgetfulness will lead to greater problems and higher health risks. Hence, if these people interpret their metamemory, it will be useful to some extent to identify what mistake they tend to make and how they can prevent it from happening again [9]. Cognitive dysfunction with its wide range, from mild cognitive impairment through dementia, is one of the chronic complications of diabetes mellitus [10]. This will not allow them to bring back the memory to full extent but it will at least be useful for them to look for alternatives to make sure they don't forget events happening every day. So, the state of metamemory in a diabetic patient will surely result in better understanding of the cognitive condition by the physicians. Therefore, the purpose of this study is to investigate and understand the relation between the decrease or increase in metamemory in a diabetic patient.

## Materials and Method

### Ethical Consideration:

The study proposal was approved by the board of the Saveetha medical college and hospitals (IRB No. SMC/IEC/2020/03/026). The purpose and objective of the study was clearly explained to the participants through an information sheet. It was emphasized that their participation was optional and the confidentiality of data was assured. The participants were requested to sign a consent form attached with the questionnaire, to ensure their willingness to participate in the study.

### Study setting and design:

This was a cross-sectional, descriptive correlational study. This standard questionnaire was done by 120 diabetic patients who volunteered to participate in this study. A convenience sample of participants from 45 to 72 years old with T2DM was recruited from Medicine OP, Saveetha Hospitals. The questionnaire was administered through face-to-face contact by the investigator with potential participants. Potential participants who expressed interest in the study were screened for eligibility based on the inclusion/ exclusion criteria. Inclusion criteria were as follows: ages from 40 to 75; living with diabetes mellitus for at least 2 year on insulin therapy; ability to read, speak, and understand english. Patients with hypertension and hyperlipidemia were included. Those with type 1 diabetes or who did not know what type they had and cognitive impairment (Alzheimer's disease) were also excluded. After the inclusion/exclusion criteria were applied, 120 participants have received paper copies of the study's survey instrument.

### Procedure:

### Perceived memory:

The multifactorial memory questionnaire is a standard metamemory questionnaire (MMQ) which helps to assess a Metamemory of a person. It consists of three scales measuring separate aspects of metamemory. Items are rated on a 5-point Likert scale (0 = strongly agree, 1 = agree, 2

= undecided, 3 = disagree, 4 = strongly disagree) based on the test's takers experiences. The three MMQ scales and their respective metamemory domains include: MMQ-Satisfaction (formerly called MMQ-Contentment). This scale measures satisfaction, concern, and overall appraisal of one's own memory. Each of 18 statements is rated based on degree of agreement. The score range is 0 to 72, with higher scores indicating a higher degree of satisfaction. MMQ-Ability. This scale measures self- perception of everyday memory ability. Respondents Rate how often they experienced each of 20 common memory mistakes over the previous two weeks. The score range is 0 to 80, with higher scores indicating better

self-reported memory ability. MMQ-Strategy. This scale measures the use of practical memory strategies and aids in day-to-day life. Respondents rate how often they used each of 19 memory strategies over the previous two weeks. The score range is 0 to 76, with higher scores indicating greater use of memory strategies.

## Data Analysis

Statistical analysis was done using SPSS Version 25.0. Descriptive variables were reported (Mean with standard deviation, Percentage) for all demographic variables. Pearson's correlation analysis was used to assess correlations between blood glucose and the survey scores (Satisfaction, ability and strategy) and Cronbach's alpha was calculated to measure internal consistency among the individual scores [(11)]. The significance level was set at 0.05.

## Results

Among the 120 participants, the mean and SD for age, height & weight and gender difference were calculated (Table 1). Mean and SD for blood glucose levels and MMQ Subscale scores (Satisfaction, ability and strategy) are given in Table 2. Age and blood glucose levels are correlated with MMQ subcomponents (Table 3). The internal consistency of subscale scores are measured by cronbach's alpha to check the reliability. There was a significant negative relationship between age and MMQ subscales (Satisfaction, ability and strategy). This relationship suggests that in diabetic individuals, increasing age is associated with decreased satisfaction, ability and strategy. Blood glucose levels had a weak positive correlation with MMQ-contentment ($r$ = .07, $p$ = .57) or MMQ-ability ($r$ = .19, $p$ = .14) or MMQ-strategy ($r$ = .05, $p$ = .23). Based on the MMQ subcomponent scores the study participants were found to have more worries about their memory (MMQ-contentment), report significantly more instances of forgetfulness (MMQ-ability), and use less memory aid strategies in their day-to-day activities (MMQ-strategy) (Table 2 & 3). In our evaluation with a sample of 120 middle-aged and older adults analyses using Cronbach's alpha indicated good internal consistency for the Satisfaction ($\alpha$ = .89), Ability ($\alpha$ = .83), and Strategy ($\alpha$ = .87) scales (Table 4).

**Table 1: Demographic characteristics of the participants**

| Parameter | Mean | SD | % |
|---|---|---|---|
| Age | 58.44 | 13.2 | |
| Height | 155 | 10.3 | |
| Weight | 68.55 | 14.55 | |
| Female | | | 29 |
| Male | | | 26 |
| Data are represented as mean ± SD and percentage. | | | |

**Table 2: Summary statistics for blood glucose levels and MMQ raw scores:**

| Scale | Mean | SD | SEM |
|---|---|---|---|
| Blood glucose levels | 150.22 | 17.02 | 2.30 |
| Satisfaction | 39.22 | 12.81 | 1.73 |
| Ability | 40.45 | 12.90 | 1.74 |
| Strategy | 38.41 | 11.91 | 1.72 |
| Metacognitive subscale scores in Mean ± SD | | | |

**Table 3: Showing the correlations between age, blood glucose and cognitive variables**

| Scale | Age | Blood glucose | |
|---|---|---|---|
| Satisfaction | r = - 0.40 | r = .07 | p = 0.57 |
| Ability | r = - 0.34 | r = .19 | p = 0.14 |
| Strategy | r = - 0.42 | r = .05 | p = 0.23 |
| Age and blood glucose levels are correlated with MMQ subcomponents | | | |

**Table 4: Showing the Internal consistency of MMQ subscales:**

| MMQ Subscales | Cronbach's α |
|---|---|
| Satisfaction | .89 |
| Ability | .83 |
| Strategy | .87 |

## Discussion

In the present study, presence of diabetes in elderly adults showed a negative correlation on all the metacognitive components. When the blood glucose values were correlated with metamemory components it showed a weak positive correlation. Although the individuals were on insulin therapy and maintained their blood glucose levels were on almost normal range. Simply, the study indicates that people with diabetes report more worries about their memory, more forgetful-ness, and more use of strategies to ameliorate memory difficulties. Though the underlying mechanism is not explored extensively, the following mechanisms could be speculated. In one study, the relationship between T2D and cognitive impairment had been evaluated and the subjects with diabetes had lower Mini mental state examination score than those without diabetes [12]. Elderly-associated mild memory and cognitive impairment and alzheimer's disease are accompanied by atrophy of hippocampal formation in diabetes mellitus[13-16].The hippocampus

and, to a lesser degree, the thalamus are responsible for deciding which thoughts are important enough to be saved as memories[17].

The prevalence of neurodegenerative disorders leading to dementia is raising worldwide due to aging of the population (increased lifespan), a situation that represents an enormous social burden [18]. BDNF is one of the essential proteins for the maintenance of neuronal functions including synapse function and neuronal transmissions. In the diabetic brains, both protein and mRNA levels of BDNF were severely reduced. These results suggest that, in diabetes, synapse dysfunction is, at least in part, caused by a failure of BDNF synthesis in the brain [19]. In type 2 diabetes, however, psychomotor speed and executive function, as well as memory, are greatly affected [20,21]. Therefore, walking speed is reduced, balance is impaired, risk of falls is increased and fractures are more frequent in elderly diabetic patients, reducing quality of life. Although it has still to be adequately explained how it is associated with changes in the brain, executive dysfunction has been reported to be associated with inability to carry out lower-extremity tasks [22,23]. The incidence of diabetes has been increasing because of dramatic changes in lifestyles, and combined with longer lifespans as a result of advances in medical technology, this has brought about an increase in the number of elderly diabetic patients. Together, aging and diabetes have contributed to dementia becoming a serious problem worldwide.

## Conclusion

Diabetic individuals had poor metamemory scores eventhough they were on insulin therapy. The study would help the diabetic individuals to identify any early stage of cognitive impairment and create an awareness to delay or prevent any further metacognitive dysfunction. The awareness can reduce metacognitive dysfunction in early stage of diabetes. Progression to dementia reduces quality of life, and imposes a burden on both patients themselves and the families supporting them. Therefore, preventing the complication of dementia will become more and more important in the future.

## Limitations:

The small sample size from a single area of the country also limits generalizability. The current study used only the previous reports of blood glucose values which were measured in past 6 months. History of insulin administration of individual person was not taken into account. Future research is needed to investigate relationships between these metacognition variables, objective neuropsychological tests, and functional MRI imaging.

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# Anti-Smoking Campaign – An Innovative Initiative against Smoking by Tertiary Care Teaching Institute of North India

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## Abstract

**Background:** Smoking as a practice has been in existence since 5000 B.C. Smoking in public places was banned in India from 2nd October 2008. According to reports by WHO Tobacco kills more than 7 million people each year. Studies show that few people understand the specific health risks of tobacco use. The present study was done to analyze the impact of the Anti-smoking Campaign in the Institutes' Campus and also to make people aware of the harmful effects of smoking and motivate them to quit this habit.

**Methodology:** The campaign constituted of various interventions as screening & collection, monitoring through CCTV Surveillance, imposing penalty as per the Act, Destruction of collected material, Behavioral change communication through Anti-smoking March, Public Lectures, Display of Pamphlets/Signages at various places in the institute & No smoking Pledge and involvement of top management.

**Results:** Total about 2613 Kgs of Tobacco and Tobacco products has been collected and destroyed from March 2014 till April 2020. On an average 40 Kilograms of Tobacco material is collected and destroyed per month. Till April 2020, 108 offenders who were caught smoking in the institute were penalized. 'No smoking Pledge' was taken by all the participants.

**Conclusion:** The success achieved by the anti-smoking campaign in the Institute suggests that there is a dire need and challenge before us to launch such type of campaigns in all the institutes worldwide to curb this menace from our society.

***Keywords:*** *Anti-smoking campaign, Tobacco Products, Tertiary Care Institute, Innovative Initiative*

## Introduction

Smoking as a practice has been in existence since 5000 B.C. Smoking was first implicated in the 1950s as a risk factor for lung cancer.(1) Since then it has been linked with cardiovascular diseases and cancers of the mouth, trachea, oesophagus, and digestive tract.(2) As a result a wide range of health education programmes aimed at reducing smoking have arisen.(3) The first legislation regarding tobacco in India was the Cigarette (Regulation of Production, Supply and Distribution) Act, 1975, which mandated specific statutory health warnings on Cigarette packs in 1975. The Cigarettes and Other Tobacco Products (Prohibition of Advertisement and Regulation of Trade and Commerce, Production, Supply and Distribution)

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Act, 2003 (COTPA) came into force on 1st May 2004.[4,5] The Act extends to the whole of India and is applicable to cigarettes, cigars, bidis, gutka, pan masala (containing tobacco), mavva, khaini, snuff and all products containing tobacco in any form.[6] Smoking in public places was banned nationwide from 2nd October 2008. Even after knowing this, there are billions who smoke, as they believe it will bust their stress or just because their peers do.

According to reports by WHO Tobacco kills more than 7 million people each year.[7] More than 6 million of those deaths are the result of direct tobacco use while around 890,000 are the result of non-smokers being exposed to second-hand smoke. Nearly 80% of the world's more than one billion smokers live in low- and middle-income countries, where the burden of tobacco-related illness and death is heaviest.[7] Tobacco users who die prematurely deprive their families of income, raise the cost of health care and hinder economic development. [8]

Second-hand smoke is the smoke that fills restaurants, offices, hospital vicinity or other enclosed spaces when people burn tobacco products such as cigarettes, bidis and others. There are more than 4000 chemicals in tobacco smoke, of which at least 250 are known to be harmful and more than 50 are known to cause cancer.[9]

There is no safe level of exposure to second-hand tobacco smoke i.e passive smoking.

Since the publication of the Surgeon General's Report on Smoking and Health in 1964, numerous individuals and organizations interested in health have engaged in a variety of activities designed to encourage people to quit smoking or to adopt less hazardous smoking behaviours.[10]

Intervention in previous large scale health promotion media campaigns has generally utilized the communication-behavior change framework. This entails using the media to deliver information on which the receiver can rationally act or not act.[11]

**Tobacco users need help to quit**

Studies show that few people understand the specific health risks of tobacco use. For example, a 2009 survey in China revealed that only 38% of smokers knew that smoking causes coronary heart disease and only 27% knew that it causes stroke [9].

So to make people aware of harmful effects of smoking and to prevent the use of Tobacco and Tobacco products in the Institute, Department of Hospital Administration along-with Security wing of the institute has initiated an anti-smoking campaign in March, 2014. This Innovative campaign was started with the goal to make the Institute smoke free.

The purpose of this paper is to contribute to an improved understanding by examining the effects of an Anti-smoking campaign at Institute level as there are many studies highlighting the effects of Anti-smoking media campaigns on the youth and general public, but the studies regarding effects of Anti-smoking campaign at the level of patient care institutes such as Hospitals is limited. This study evaluates the impact of the Anti-smoking Campaign in the Institutes' Campus and also to make people aware of the harmful effects of smoking and to motivate them to quit this habit.

## Methodology

The present study was conducted to assess the impact of various intervention techniques as a part of Anti-smoking campaign on the attitude of employees and people visiting the Institute. Necessary permission was taken from Institute's ethics committee.

**Intervention Techniques**

*SCREENING & COLLECTION*

People visiting the Institute were made aware about the harmful effects of smoking and also that hospital premises are No Smoking Zone. Security guards posted at all entry points in all the shifts were provided with boxes in which tobacco products voluntarily surrendered by public were collected.

A security guard was also deputed with florescent uniform to motivate public who are sitting in the lawn/grounds of the institute & other isolated places to surrender any kind of tobacco products with them in the box (Figure 1).



**Figure-1 Collection of tobacco products by Security Guards at entry/exit gates**

*MONITORING*

CCTVs are installed at various places in the institute. Apart from CCTVs stated objectives, these were also used to keep a check on smoking and use of Tobacco products by public and the staff.

*DISPOSAL*

The collected tobacco products were incinerated at regular intervals and the proper record of disposal is maintained.

*PENALTY*

As per section 21 and section 24 of the COTPA Act 2003, the offenders who were caught smoking in the campus could be penalized with an amount of Rs.200/-. As per the provisions of the act, the authority to penalize has been delegated to institutes Security Officers by the UT Administration, Chandigarh.

*BEHAVIOUR CHANGE COMMUNICATION*

People were made aware of the harmful effects of smoking and chewing Tobacco through Anti-smoking March, Public Lectures, Display of Pamphlets/ Signages at various places in the institute & No smoking Pledge by the volunteers on "World No smoking Day" i.e on 31$^{st}$ May every year since 2014. Both carrot & stick was used as penalty was imposed on those who were caught smoking in the premises.

*INVOLVEMENT OF TOP MANAGEMENT*

Efforts are being done to sensitize the Top management to involve in this noble cause with the aim to make Institute campus Tobacco free. The issue was discussed as an agenda in the Hospital Management Board. The efforts to make the Institute campus smoke free was also appreciated during various meetings of Hospital officers being held under the chairmanship of Medical Superintendent and it was regularly reiterated that smoking is harmful and also banned in the public places and Hospitals as per act.

## Results

The present study was aimed to assess the impact of anti-smoking campaign in the institute.

*SCREENING & COLLECTION, MONITORING and DISPOSAL*

Total about 2613 Kgs of Tobacco and Tobacco products has been collected in the boxes at all entry and exit gates of various buildings in the Institute and destroyed from March 2014 till April 2020. On an average 40 Kilograms of Tobacco material is collected and destroyed per month. Since March 2014 till April 2020 with various interventions the amount of tobacco & tobacco products collected are 2613 Kilograms. The Tobacco material collected was segregated for different products like Cigarette, Bidi, Tobacco and others (like Zardaa, Khaini etc.). On an average 1.3 Kg of smoking material is collected daily which includes about 25% as Cigarettes, 5% as chewable tobacco and 30% other products. On an average 40 Kilograms of tobacco material is collected & incinerated per month. The data of yearly is shown in figure-2.

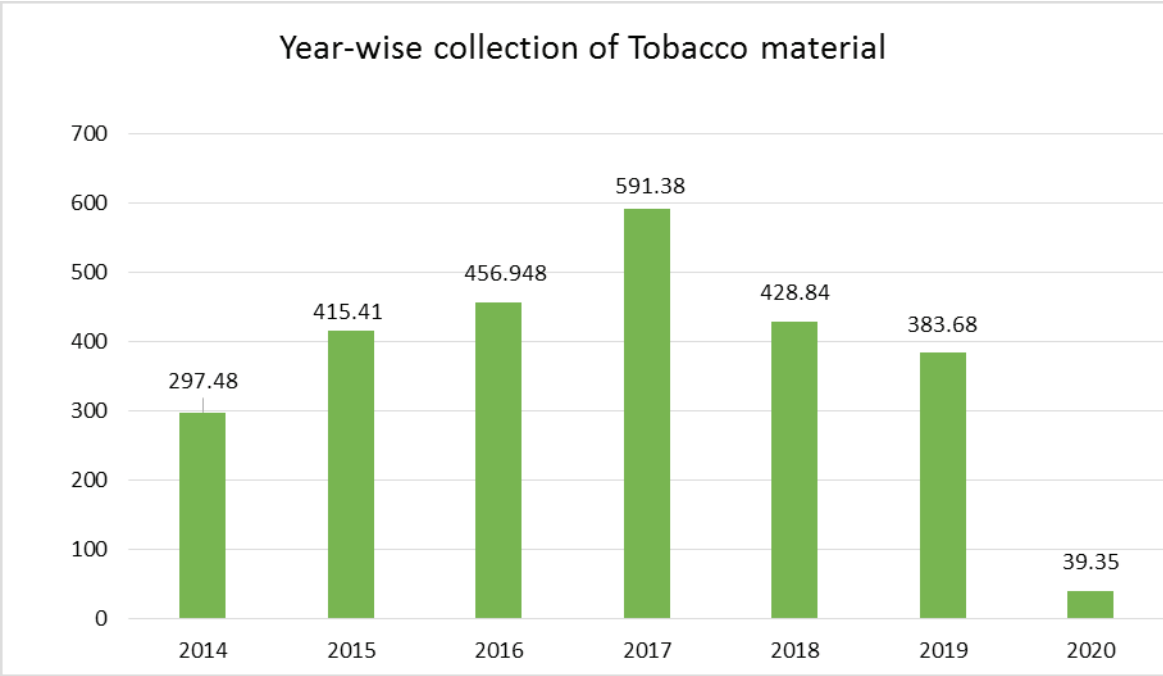


**Figure-2 Year-wise collection of Tobacco material**

*PENALTY*

Till April 2020, 108 offenders who were caught smoking in the institute were penalized and amount of Rs 21,600/- was collected. The last penalty was imposed in August 2019. The reduction in number of offenders in the recent months suggests that Anti-smoking campaign has a huge impact on the mindset of people visiting Institute.

*BEHAVIOUR CHANGE COMMUNICATION*

Anti-smoking March and Public lectures were under-taken every year since 2014 in institute on "World No tobacco day" to sensitize people about harmful effects of smoking and chewing tobacco, so as to induce the behavioral change among people regarding smoking. 'No smoking Pledge' was taken by all the participants (which includes Security Guards, Sanitation and Housekeeping staff, Volunteers of NGOs, Doctors and other support staff) agreeing not to smoke and to help others to try to quit. Various organizations also contributed in this endeavor for eg; No Smoking March conducted on 31$^{st}$ May 2017 in the Institute Campus in Collaboration with the State Bank of India received immense positive response from the staff (including Doctors, Nurses, Security and Housekeeping staff) and Patient's and Patient's attendants visiting the Institute.

*INVOLVEMENT OF TOP MANAGEMENT*

In August 2015, Hospital Management Board was apprised about the Anti-smoking Campaign in the Institute & was introduced as an Agenda to get the support of Top Management in this noble initiative and to fight against Tobacco use. The agenda showcasing the efforts of the Department of Hospital Administration and Security wing was duly appreciated and necessary permissions were accorded to send communications in the form of circulars and SMS to all section Heads and all Head of the Departments of the Institute at regular intervals to increase awareness among staff members working under them on the issue. The efforts to make the Institute campus smoke free was also applauded in various administrative meetings of Hospital officers being held under the chairmanship of Medical Superintendent. Simultaneously, it was and is reiterated regularly that smoking is banned in the public places and Hospitals and penalty could be levied if caught smoking in the premises of the Institute.

## Discussion

Among smokers who are aware of the dangers of tobacco, most want to quit. Medication and counseling can more than double the chance of success for a smoker who tries to quit.

National comprehensive cessation services with full or partial cost-coverage are available to assist tobacco users to quit in only 24 countries, representing 15% of the world's population.

A study conducted on Anti-smoking campaign in California suggests that the anti-smoking media campaign not only reduces the prevalence of smoking among adults and adolescents, but also brings significant long term benefits in smoking reduction, by inducing more future attempts to quit among adult smokers and deterring more initiating intentions among adolescents.[12]

The present study was done to prevent the smoking and use of Tobacco products in the institute. The decrease in number of offenders in the Institute campus (as reflected by almost zero penalty) suggests that the efforts of Department of Hospital Administration and the security wing of the institute had a great impact in making the Institute campus smoke free.

The study also suggests involvement of Top management is required for the success of such campaigns.

The success achieved by the anti-smoking campaign must be credited to a number of factors including- involvement of doctors, nurses and other staff, Top management of the institute through Hospital Management Board, sensitization classes of security, messages on CUG mobiles to the staff to disregard smoking, support of UT administration, use of various surveillance methods to catch the offenders and above all the involvement of Public to support the Noble cause.

The study has limitation of being a unique campaign of such kind at the institute level, though several studies are done about the effectiveness of mass media campaigns at macro levels.

## Conclusion

Present study suggests that there is a dire need and challenge before us to launch such type of campaigns in similar institutions/organizations so as to make people aware about the harmful effects of smoking or of using Tobacco products.

**Ethical Approval:** The ethical approval for this study was obtained by the Institutional Ethics Committee (vide No. INT/IEC/2019/001422 dated 17.07.2019)

**Sources of Funding**: Nil

**Conflict of Interest**: Nil

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# The Economic Impact of Government Sponsored Health Insurance Schemes on a Tertiary Care Multispeciality Hospital in South India

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## Abstract

The number of people registered with health insurance schemes has increased from 161.2 million(2011-12) to 335 million(2016-17), which is two-fold in just 5 years.[1] As per the forecasts regarding the increase in healthcare insurance market in India, it is evident that the hospitals have to face the management of patient costs through the insurance schemes in large numbers as the number of claims will increase eventually. With the present condition of schemes reimbursing the claims with a huge time lag of about 3-4 months, the hospital have to carry the burden of opportunity costs lost on these claims.[2] This indicates the responsibility hospital administration should have to face the ever increasing claims and manage them without incurring loss. This study was intended to do a cost analysis between surgical procedures performed under two categories, Aarogyasri Health Insurance Scheme, A Government Sponsored Health Insurance Scheme in the state of Andhra Pradesh and Cash category, where patients do not hold any insurance policy and pay by cash all the hospital expenses. On the basis of revenue generated the top 15 procedures under Insurance scheme are determined and costing is done using Activity Based Micro Costing method. Costing is done for the determined 15 procedures under both the categories of payment modes. The results are compared to evaluate the economic impact of the health insurance scheme on hospital.

***Key words:*** *Health insurance, ABC microcosting, surgical procedures, hospital economy, costing.*

## Introduction

The healthcare industry, consuming around 9.1% of global GDP, is one of the fast growing industries around the world. In India healthcare industry achieved to be 3rd largest growth sector in the country with 3.9% of GDP.[3]

With 1/6th of the world population, India carries the burden of expectations to deliver the basic needs of its citizens. Healthcare being one of the foremost needs among others, the scenario of the health of Indian population is still far from achievable by its

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own standards. With a population of 111 crores people and rising, it is surely a herculean task to provide and satisfy the healthcare needs of the nation by the government. The demographics of the country when studied shows that there is an increase in the life expectancy and growth in socio-economic status, which means that Indian's despite living longer also will have a remarkable increase in group/segment of Indians over the age 65. It has been estimated by the Oxford Economics that 95 million Indian citizens will be above the age of 65 years by 2021.[4] On the contrary, The government spending of 1.41% of GDP, ranking India at 187th position among 194 countries reflects the healthcare scenario in the country.

The income of the middle class population of the country which is estimated to be at 7% CAGR for five year period from 2016-2021 indicates their expectations for high quality healthcare. With respect to this the government spending on healthcare is also expected to increase from US$287 Billion (2016) to US$407 Billion (2021), Whereas household spending is projected to increase from US$1.3 Trillion (2016) to US$2.[2] Trillion. Spending on healthcare, both by the government and household is expected to increase two-fold.

Globally, medical insurance sector is considered in high regard due to its feasibility, size of the market and potential to penetrate into the market with a continuous upward growth. The universal health coverage adopted by UN recognizes the responsibility of Governments to urgently and significantly scale up efforts to accelerate the transition towards universal access to affordable and quality health-care services. The adaptation of Universal Health Coverage by UN recognizes the responsibilities of Governments to immediately increase the efforts to speed up the process of transformation towards global access of affordable and quality healthcare services.

To achieve Universal coverage, all the nations started to develop and initiate financing models so as to provide affordable healthcare to everyone without incurring any losses to the providers and the governments. The most chosen model was health insurance through Government sponsorship or Private payers.

Indian Government too initiated many health insurance schemes to benefit the citizens of the country to avail best of the healthcare at affordable rates

It is very evident that there have been many government health insurance schemes in India that are designed to assist public. Various schemes are made flexible enough to incorporate ever-changing need of families and changing scenarios of the healthcare in the country. The governments have tried to add new schemes and enhance the benefits of existing schemes to ensure the public is benefitted as and when the need arises.

Apart from the Central Government schemes formulated and introduced by the Government of India, each state of the country has its own health insurance schemes formulated to benefit the people within their circles of states. Andhra Pradesh is one such states of India which is promoting Healthcare of the people through state sponsored health insurance schemes and trying to benefitting as many people as possible and reduce the out of pocket expenditure. The major objective of these state sponsored health insurance scheme is to make the healthcare affordable and equitable to every individual of the state and country.

World Health Report, World Health Organization, 2010 states that Every country can maintain what it has achieved or do something to move closer to universal health coverage. Many countries have gone through struggles to establish a system of universal health coverage and all the struggles are well documented which can be taken as templates by the policy makers in the country. There are lessons to be learnt. One of which concerns the importance of social willingness expressed through political engagement to achieve the desired objective. Reform has always resulted where

there is grass roots demand and the active involvement of civil society.[1] This conclusive statement and the recommendations by the WHO in World Health Report-2010 have led to making the UN resolution on Global Health and Foreign Policy, which directed all the nations to adopt and implement universal health coverage.[5] Owing to the UN resolution, India, along with other Nations, initiated Universal Health Coverage to provide Health care to every individual under various health insurance schemes.[1]

The Health insurance market in India has been seeing a consistent incline with the forecasted growth rate from 7.8% during 2011-2016 to 12.6% for the period between 2017-2021 in Government sector and from -3.2%(2011-16) to 12.6%(2017-2021) in private health insurance sector. Govt. health spending is forecasted to increase almost two-fold from US$31.0 billion(2016) to US$56.2 billion(2021), whereas the private insurance is also increasing almost two-fold from US$1.9 billion(2016) to US$3.5 billion(2021).[4]

With the increase in the health insurance market, from the hospital administration perspective keeping in view the irregular reimbursement periods, which is as long as 3-4 months, particularly in Public Health insurance schemes, it is vital to study the loss through opportunity costs associated with the package costs of the schemes and analyze the feasibility of the scheme.[5]

The statistical data taken from Statista-the statistics portal indicates that the number of people registered with health insurance schemes has increased from 161.2 million(2011-12) to 335 million(2016-17), which is two-fold in just 5 years.[1] As per the forecasts regarding the increase in healthcare insurance market in India, it is evident that the hospitals have to face the management of patient costs through the insurance schemes in large numbers as the number of claims will increase eventually. With the present condition of schemes reimbursing the claims with a huge time lag of about 3-4 months, the hospital have to carry the burden of opportunity costs lost on these claims.[2] There is a need for the hospitals, on the basis of forecasts of healthcare insurance market growth, to do a cost analysis to evaluate the feasibility of the schemes based on the profit or loss the hospital is incurring through the opportunity costs loss.

This study is intended to do a cost analysis of the surgical procedures in hospitals and compare them with the package costs of the Govt. Sponsored Health Insurance Schemes and report on the feasibility of the schemes in a tertiary care multispecialty hospital. The costing of the surgical procedures identified under Govt. sponsored health insurance schemes is done through bottom-up approach using Activity Based Micro costing.

Acitivity based microcosting is a very efficient tool to be used in healthcare facilities.[6]

Activity based micro costing- the bottom up approach can be done following three standard steps

- Mapping activities of the procedure
- Computing the cost of each activity
- Computing the unit cost of each procedure

Secondary activities can also be taken into account which are those activities performed by the surgeons, nurses and administrative staff and are not directly linked to patient.[7]

A detailed information relating to all expenditures based on material used, drugs, pre & post-operative examinations, professionals involved, equipment deployed, time spent in OT, days spent in ICU and wards can be carried out using ABC micro costing.[8]

Various cost heads to be considered under ABC micro costing technique are[9]

- Production cost
- Fixed cost
- Variable cost
- Total cost

- Direct cost
- Indirect cost

The labor costs can be taken on the basis of standard costs per minute of per day. This equals the normal salary divided by number of working days or minutes per year.[10]

One Govt. sponsored health insurance schemes are taken for the study. The scheme is AarogyaSri sponsored by the Government of Andhra Pradesh. Policies and procedures of the procedures under these schemes are obtained from the online portals dedicated for the schemes by the Government of AP.

## Methods

Study setting and target department

Study setting was a 350 bedded tertiary care multispecialty hospital in South India. The study was conducted in the operation theatre complex of a the hospital. The OT complex of the hospital has a total of 8 operation theatres. Total number of surgeries performed between the time period July 2018 & December 2018 are 5956. On an average 993 surgeries are performed per month in all the categories. This study was intended to do a comparative analysis between the surgical procedures that are performed under the categories of cash and Aarogyasri health insurance scheme. Total number of surgeries performed under Aarogyasri and cash category between the study period are 2353 & 1729 respectively. Average number of surgeries performed under these two categories i.e., Aarogyasri health insurance scheme and cash, per month are 392 & 288 respectively.

Study period

Surgical procedures done from July 2018 to December 2018 are reviewed.

Study Design

The study was done in retrospective approach using the cross sectional data obtained from the operation theatres. The initial data collected was of all the surgeries performed under Aarogyasri Scheme for the time period between July 2018 & December 2018. Total number of all surgical procedures and the revenues generated by them were analysed. This data was used to identify the top 15 surgical procedures performed under Aarogyasri scheme.

Costing of all the identified 15 surgical procedures is done using Activity Based Micro Costing. Average length of patient stay is calculated analysing the length of stay of all the patients in the identified procedures. The stay of patient in the hospital i.e., from the moment patient enters the hospital until the discharge is divided into 5 phases.

Ø Pre-admission consultation & diagnostics

Ø Pre-surgery admission

Ø Surgery

Ø Post-surgery ICU

Ø Post-surgery ward

At each phase various costs are calculated which are categorised as direct & indirect costs levied upon the patient by the hospital. Various cost heads taken into consideration are

Ø Human resource

Ø Equipment

Ø Electricity

Ø Pharmaceuticals

Ø Consumables

Ø Utility

Ø Administration

Human resource

The human resource cost is calculated at each phase of the patient stay in the hospital. In the pre-

admission consultation phase, human resource personnel involved are Aarogyasri desk personnel one in number, physician consultant and assisting nurse for consultation services, lab & radiology technician if any investigations were prescribed for pre-assessment. Consultation time per patient was recorded by time & motion study and average time for patient is calculated. Based on the consultation time the physician consultant & assisting nurse cost were scaled down from their respective salaries per month. Lab and radiology technician costs were calculated based on the average time taken for prescribed test for a particular procedure and the salaries were scaled down to that time obtained.

**Equipment**

The policy of the hospital regarding equipment is that, depreciation value is set on the item so as to attain return on investment within 7 years from the procurement of that item. All the equipment that is deployed to serve the patient for whole length of stay is taken into account. This equipment includes diagnostics, radiology, surgical procedure, ventilators & other equipment in ICU. Cost for that equipment that exceeded 7 years of procurement is taken as zero. For all the equipment falling within 7 years, depreciation is calculated which is scaled down to the procedure time or up time for the length of patient stay.

**Electricity**

Electricity department of the hospital maintains segregated consumption reading meters for every department, ward and block of the hospital. Consumption by diagnostic lab and radiology is calculated based on the consumption power of the equipment used. Whereas in wards, ICU and Operation theatre consumption is divided and attributed on per bed basis and calculated with the unit price of electricity.

**Pharmaceuticals**

This cost is calculated based on the standard drugs prescribed to patient for the determined procedures during different phases of the patient stay.

**Consumables**

Consumables include gloves, mouth masks, CSSD material, linen material, etc. used during the consultations and surgical procedure.

**Utility**

It is the cost attributed to the area of space used by the patient at different phases of stay in hospital. That being said, the space occupied by the bed in wards, ICU and operation theatre is determined and calculated with rent of the hospital per square foot.

**Administration**

All the administration charges levied upon the patient are indirect costs. This is taken as 10% of the package cost per procedure. Aarogyasri desk personnel, billing staff, back office personnel for insurance schemes and other administrative personnel are included in this.

## Results & Discussions

Top 15 surgical procedures performed under the Scheme are determined based on the revenues generated by them. Table 1 represents the details about them.

Table 2 is a comparison between package costs given by the scheme and cost incurred by the hospital.

Costing of the procedures is done on par with the specifications provided in a study which was conducted to compare the effectiveness of different costing methods.[10]

It is evident from the findings that except 3 procedures all other surgical procedures under scheme are generating positive margin. The 3 procedures

giving negative margins are, Whipples, Mastectomy and Arthroscopy.

Table 3 is a comparison between unit cost of procedure levied upon patient and cost to hospital in Cost category. The margins yielded are almost 50% of package cost of the procedures.

Comparison is done between the net margins yielded by the procedures performed under both categories.

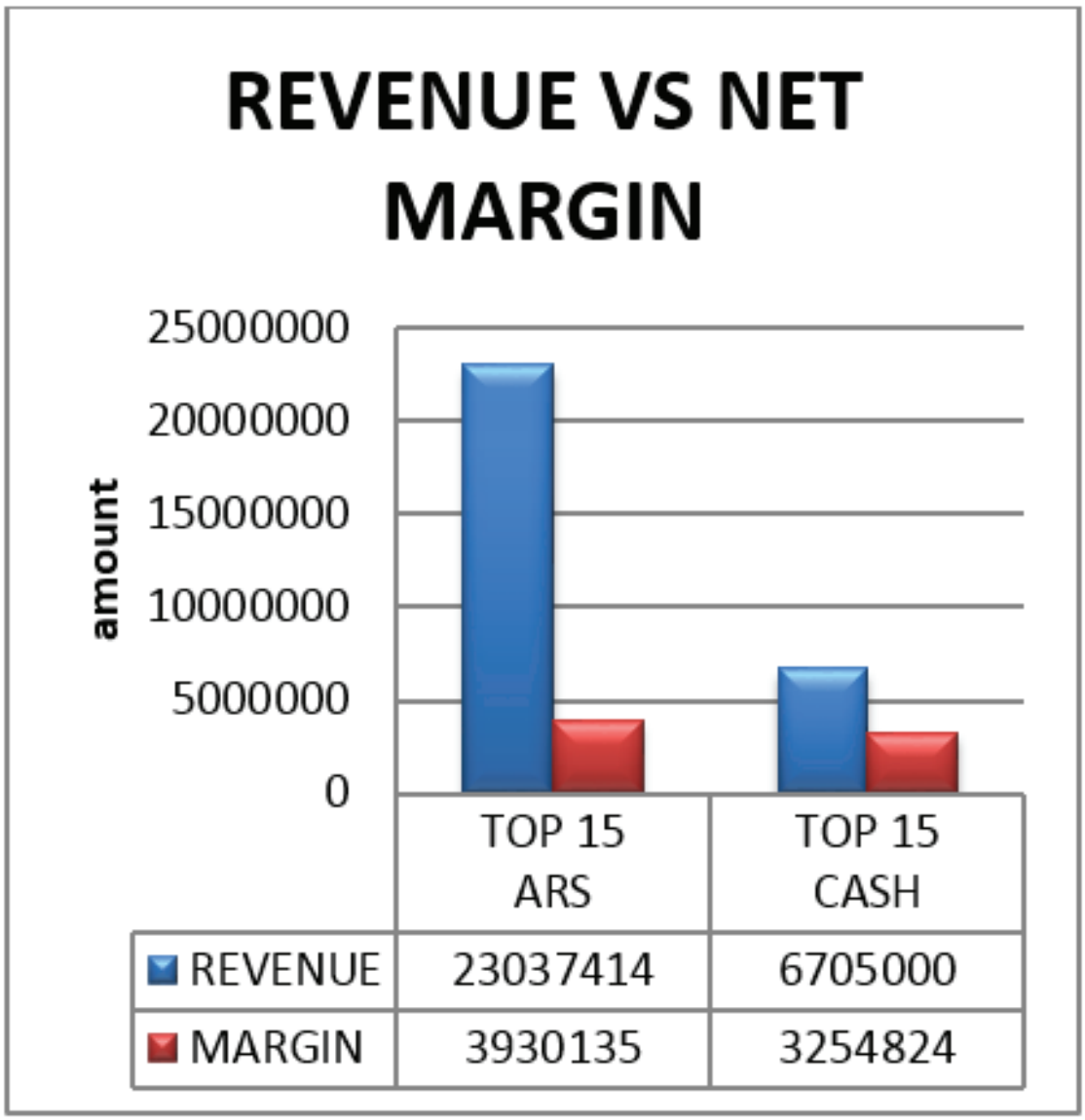


**Chart 1 depicts the difference between revenue generated and margins yielded by all 15 procedures under both the categories.**

**Table 1. Total revenue from surgeries**

| PROCEDURE | NUMBER OF SURGERIES | TOTAL AMOUNT |
|---|---|---|
| CORONARY BALLOON ANGIOPLASTY | 62 | 4461145 |
| CORONARY BYPASS SURGERY | 36 | 4289023 |
| SPINAL FUSION | 59 | 3011319 |
| PCNL | 70 | 2621483 |
| SURGICAL CORRECTION OF LONGBONE | 50 | 1664743 |
| MITRAL VALVE REPLACEMENT | 10 | 1545000 |
| LATERAL PANCREATICOJEJUNOSTOMY | 12 | 1226671 |
| URSL | 45 | 1140168 |
| WHIPPLES | 8 | 877200 |
| LAP SURGICAL TREATMENT FOR HERNIA WITH MESH | 18 | 838805 |
| MASTECTOMY | 23 | 770164 |
| MYRINGOPLASTY | 49 | 753715 |
| EXCISION OF BASAL BRAIN TUMOR | 9 | 643070 |
| ARTHROSCOPY | 18 | 627773 |
| CORPECTOMY FOR SPINAL FIXATION | 6 | 433500 |

**Table 2. Package and Cost to Hospital**

| PROCEDURE | PACKAGE (In rupees) | COST TO HOSPITAL |
|---|---|---|
| CORONARY BALLOON ANGIOPLASTY | 66310 | 59757.73 |
| CORONARY BYPASS SURGERY | 118163 | 108127.8 |
| SPINAL FUSION | 51000 | 45253.52 |
| PCNL | 34966 | 27055.82 |
| SURGICAL CORRECTION OF LONGBONE | 30600 | 19794.12 |
| MITRAL VALVE REPLACEMENT | 147900 | 90508.58 |
| LATERAL PANCREATICOJEJUNOSTOMY | 102000 | 58962.53 |
| URSL | 26168 | 20237.8 |
| WHIPPLES | 102000 | 112152.1 |
| LAP SURGICAL TREATMENT FOR HERNIA WITH MESH | 51000 | 30732.98 |
| MASTECTOMY | 30600 | 32819.47 |
| MYRINGOPLASTY | 15382 | 13800.87 |
| EXCISION OF BASAL BRAIN TUMOR | 71400 | 68981.29 |
| ARTHROSCOPY | 25500 | 32059.34 |
| CORPECTOMY FOR SPINAL FIXATION | 76500 | 50060.5 |

**Table 3. Cost to hospital and margin**

| PROCEDURE | UNIT PROCEDURE | COST TO HOSPITAL | MARGIN |
|---|---|---|---|
| CORONARY BALLOON ANGIOPLASTY | 125000 | 70763.42 | 54236.58 |
| CORONARY BYPASS SURGERY | 200000 | 121808.65 | 78191.35 |
| SPINAL FUSION | 95000 | 51504.42 | 43495.58 |
| PCNL | 75000 | 31017.74 | 43982.26 |
| SURGICAL CORRECTION OF LONGBONE | 55000 | 22513.89 | 32486.11 |
| MITRAL VALVE REPLACEMENT | 300000 | 121734.76 | 178265.2 |
| LATERAL PANCREATICOJEJUNOSTOMY | 160000 | 65576.6 | 94423.4 |
| URSL | 50000 | 19549.89 | 30450.11 |
| WHIPPLES | 200000 | 125333.76 | 74666.24 |
| LAP SURGICAL TREATMENT FOR HERNIA WITH MESH | 80000 | 35863.14 | 44136.86 |
| MASTECTOMY | 80000 | 42332.23 | 37667.77 |
| MYRINGOPLASTY | 40000 | 20200.67 | 19799.33 |
| EXCISION OF BASAL BRAIN TUMOR | 140000 | 81690.71 | 58309.29 |
| ARTHROSCOPY | 60000 | 36969.82 | 23030.18 |
| CORPECTOMY FOR SPINAL FIXATION | 95000 | 50156.72 | 44843.28 |

## Conclusion

This study was conducted to assess the economic impact Government Sponsored Health Insurance Schemes create on the hospital. Top 15 procedures under ARS scheme based on their revenues were taken to do Activity based micro cost under both scheme and cash category. The results were conclusive. The profits generated by ARS scheme and cash category are 17% & 48.5% respectively of their revenues.

The conclusion that can be taken from the results is that same amount of resources are being pooled in under both the categories to produce two different

margins. For the same amount of resources pooled into procedures under scheme as that of cash category, the profit generated is 33% less, which means if all the procedures under scheme are performed under cash there would be 33% more profit to the hospital. This is a loss of opportunity cost.

Hospital resources are being directed towards procedures under ARS scheme only to produce 33% less margin than those performed under cash.

**Ethical Clearance**- Taken from IEC committee of hospital

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**Conflict of Interest** - None

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# Hate Speech Against Disabled Persons: A Forensic Analysis of Legal Framework in India and Beyond

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## Abstract

Hate Speech is a social menace. Disability hate speech is nothing but hatred perpetuated against any disabled person. It is prevalent offline as well as on the virtual platform. Despite the legal provisions against hate speech, instances of hate speech are increasing rapidly.

There are many developed countries such as the US, UK or Australia that have specialized laws and machinery at place to regulate speech towards the persons with disabilities. However, India lags behind in framing policies or enacting laws, be it protecting the interest of disabled persons against hate speech or hate crime perpetuated against them.

Amidst pandemic, discrimination and untold miseries faced by disabled individuals mount day by day, in real world as well as virtual world. In these challenging times, there is a need to check as to what are the laws in India that protect people with disabilities against hate speech. There is a need to analyze in a forensic perspective the remedies available for the victimized individuals with disability. This research paper further put forth various suggestions and means to improve protection of such persons with disabilities.

***Keywords:*** *Disability, Disabled Persons, Discrimination, Hate Crime, Hate Speech*

## Introduction

"My advice to other disabled people would be, concentrate on things your disability doesn't prevent you doing well, and don't regret the things it interferes with. Don't be disabled in spirit as well as physically."

– Stephen Hawking

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Any living species across the globe that we live suffers from mental or physical disabilities, in form or the other. However, intellect of human beings is capable enough to identify and protect the concerns of persons with disabilities. Apart from providing those devices, gadgets, instrumental supports and mechanical aid to cover their day-to-day activities, ambitions and passions, their disability often poses bullying, hatred and discrimination in the society.

Hatred is a universal phenomenon. Countries like US or the UK have already enacted comprehensive laws to protect persons with disabilities against hatred, bullying or discrimination. There are notable international frameworks to protect disability hate

speech. However, India being a developing country, it lacks concrete statutory provisions to counter the menace of hate speech against disabled persons.

## Materials and Method

This research paper is based on doctrinal study. The authors relied on several books and online sources, analyzing and interpreting them.

## Discussion

### What is Disability Hate Crime?

Article 2 of the United Nations Convention on Rights of Persons with Disabilities [1] (CRPD) defines the term 'discrimination on the basis of disability' as "any distinction, exclusion or restriction on the basis of disability which has the purpose or effect of impairing or nullifying the recognition, enjoyment or exercise, or an equal basis with others, of all human rights and fundamental freedoms in the political, economic, social, cultural, civil or any other field. It includes all forms of discrimination, including denial of reasonable accommodation".

Hate crime is defined as an offence in which the victim is targeted because of the actual or perceived race, color, religion, disability, sexual orientation, or national origin of that victim.[2] According to Hate Crime Statistics Act 1990, hate crime is an offence that manifests evidence of prejudice based on race, religion, disability, sexual orientation or ethnicity. The European Network on Independent Living recommends Disability Hate Crime as someone commits a crime that is motivated by hostility or prejudice, because the victim is a disabled person or is perceived to be disabled.[3]

In India, there is no legal definition for disability hate crime but it is considered as an offence in the Indian Penal Code (IPC). In a nut shell, disability hate crime is a form of hate crime involving the use of violence against people with disabilities.

### Defining Hate Speech

Broadly speaking, hate speech is an offshoot of hate crimes. Hate speech, while not universally accepted in definition, can be understood as the 'promotion, endorsement and encouragement of a vilification of others based on innate differences'. What is derogatory or not is an ongoing debate not only in India, but also across the globe.

The right to freedom of speech and expression doesn't include the freedom to insult someone or disrespect a community on the basis of caste, religion, race, region, gender, disability, place of birth or language. Although there is no uniform definition of hate speech across the globe, hate speech creates a widening divide among the public.

### International Legal Framework

The United Nations Convention on the Rights of Persons with Disabilities, 2006[4] is the predominant initiative for protecting persons with disabilities from hate crimes. Article 15 of the Convention deals with freedom from torture or cruel, inhuman or degrading treatment or punishment. It gives state parties to enact legislations or administrative and judicial measures to ensure equal rights of disabled persons and protection from hatred. Further Article 16 deals with freedom from exploitation, violence and abuse. Through this Article, the convention provides for rehabilitation programmes, psychological recovery and social reintegration. It also focuses to craft child – focused and women-focused laws and policies.

Optional Protocol to the Convention on the Rights of Persons with Disabilities, 2007[4] is the document running on parallel to the Convention. The protocol recognizes the competence of the Committee on Rights of the Persons with Disabilities to receive and consider communications from and on behalf of individuals or groups subject to its jurisdiction who are the claimants as victims of a violation by that state party of the provisions of this Convention [5]. The Committee shall not receive if the party is

not a signatory to the protocol. Further, Article 2 deals with admissibility of the communication and identifies anonymous communication; incompatible communication, non-exhaustion of domestic remedies, ill-founded and communication prior to the protocol are inadmissible.

The Charter of Fundamental Rights of the European Union, 2000 prohibits discrimination based on racism, xenophobia, religious intolerance or person's disability, sexual orientation or gender identity. Article 21 confirms the same. Article 26 deals with integration of persons with disabilities wherein it is stated that the European Union recognizes and respects the right of the persons with disabilities to benefits from measures designed to ensure their independence, social and occupational integration and participation in the life of the community.[6]

The United Kingdom the Public Order Act, 1986 [7] prohibit discrimination of class and spread of hatred based on color, sex, nationality, citizenship, disability etc. The liability for the same was inserted under Section 4A which says summary conviction to imprisonment for term not exceeding six months or fine not exceeding level five standard scale or to both.

Unfortunately, United States don't have a specific legislation regulating hate speeches. But Justice Samuel in the case of United States v. Schwimmer [279 U.S. 644 (1929)] held that: "Speech that demeans on the basis of ethnicity, gender, religion, age, disability is hate speech. Or any other similar ground is hateful; but the proudest boast of our hate speech jurisprudence is that we protect freedom of press "the thought that we hate"". Further, in the case of Matal v. Tam [582 U.S. (2017)], Justice Anthony Kennedy observed: "A law that can be directed against speech found offensive to some portion of the public can be turned against minority and dissenting views to the detriment of all. The First Amendment does not entrust that power to the government's benevolence. Instead, our reliance must be on the substantial safeguards of free and open discussion in a democratic society".

When it comes to Australia, it has enacted the Tasmania's Anti-Discrimination Act, 1998[8] which prohibits anyone from inciting hatred. Article 19 states that: "A person, by a public act, must not incite hatred towards, serious contempt for, or severe ridicule of, a person or a group of persons on the ground of – (a) the race of the person or any member of the group; or (b) any disability of the person or any member of the group; or (c) the sexual orientation or lawful sexual activity of the person or any member of the group; or (d) the religious belief or affiliation or religious activity of the person or any member of the group." Besides, Victoria of Australia enacted Racial and Religious Tolerance Act, 2001 wherein Section 8 (1) states: "A person must not, on the ground of the religious belief or activity of another person or class of persons, engage in conduct that incites hatred against, serious contempt for, or revulsion or severe ridicule of, that other person or class of persons.

In South Africa, a similar provision has been incorporated in its penal books. Section 10 (1) of the Promotion of Equality and Prevention of Unfair Discrimination Act, 2000[9] reads: "No person may publish, propagate, advocate or communicate words based on one or more of the prohibited grounds, against any person, that could reasonably be construed to demonstrate a clear intention to be hurtful, be harmful or to incite harm, promote or propagate hatred. The "prohibited grounds" include race, gender, sex, pregnancy, marital status, ethnic or social origin, color, sexual orientation, age, disability, religion, conscience, belief, culture, language and birth."

**Indian Legal Framework**

As per Census 2011, out of 121 crores total population, 2.68 crores persons are physically disabled; which means 2.21 percent of Indian Population is physically disabled.[10] As per United Nations Disability and Development Report 2018, India is the third last country that spends on social programmes for persons with disabilities as a percentage of GDP out of 56 countries.[10] The data

was collected in 2014 for the purpose. The percentage population of disabled persons shows the need of protection against hate speech.

One of the comprehensive legislations in India for protecting the rights and interests of disabled persons is The Rights of Persons with Disabilities Act, 2016.[11] Section 3 of the said Act deals with equality and non-discrimination of disabled persons. Section 4 deals with the women and children with disabilities. Further, Section 6 provides for protection from cruelty and inhuman treatment to the disabled persons. In addition to it, protection from abuse, violence and exploitation is taken care in Section 7 of the said Act. It is pertinent to note that, unfortunately, the entire statute is silent about the hate speech against the disabled persons.

Hate speech is spreading of hatred against a particular individual or a class thereby causing defamation. Article 19 (2) of the Constitution [12] restricts the freedom of speech and expression. By virtue of the said provision, freedom is restricted where there is involvement of defamation or incitement to an offence. In addition, this Article shall be read with Article 15 of the Constitution of India that prohibits discrimination on the grounds of religion, race, caste, sex, or place of birth.[12] The provision states that disabled persons shall not be restricted from entering places and enjoying.

Extending the protection granted by the existing legal framework in India, the Law Commission of India in its report [13] titled 'Hate Speech' recommended certain changes in Section 153 of Indian Penal Code (IPC). It suggested the Criminal Law (Amendment) Act, 2017. It proposed an amendment in Section 153C which deals with prohibiting incitement to hatred on grounds of religion, race, caste or community, sex, gender identity, sexual orientation, place of birth, residence, language, disability or tribe. The said Section protects disabled persons from hate speech and imposes punishment for committing hatred. The punishment involves imprisonment of either description for a term which may extend to two years and fine up to ₹5000 or with both. It is recognized as cognizable and non-bailable offence and is tried before the Judicial Magistrate of First Class.

Although Section 66A of the Information Technology Act, 2000[14] was enacted to regulate spreading offensive messages having nature of enmity, hatred, ill-will etc., this Section was held unconstitutional in the case of Shreya Singhal v. Union of India [(2013) 12 SCC 73] by the Hon'ble Supreme Court. Thus, in succinct, there is a legal hiatus prevailing when it comes to laws to protect disabled persons against hate speech in the cyberspace.

Suggestions

In comparison with the legal framework and the judicial interpretation of other countries, the legal framework in India is weak to protect the persons with disabilities against hate speeches. Following suggestions are drawn to protect persons with disabilities against hate speech:

a. An exclusive legislation shall be enacted dealing the entire subject related to hate speeches against persons with disabilities.

b. There is a great requirement of organizing awareness programmes right from school level to post graduation level, from village to metro cities, and from small work places to large corporate offices.

c. Heavy penal sanction for propagating hatred against handicapped shall act as a reformative measure, so that society will reform.

## Conclusion

Passion and ambition never suffer from disabilities, so do the persons with disabilities. The only challenging part is their acceptance by the society, at work place or at their own house. Disability poses a hindrance only when others spread hatred and non welcoming to the person with disability and hence need comprehensive law to able others to

accept and stop hatred to the persons with disabilities. While at the international sphere, there are several laws to regulate member countries. Many countries have strong legislative framework whereas others have strong judicial interpretation. However, when it comes to India, the legal framework lacks appropriate legislation and interpretation.

As already 2.21 percent of India's total population consists of persons with disabilities and there is no proper legal framework to protect and integrate them in the real and virtual platform. It is high time to realize that the country has no specific legislation that covers entire aspect of persons with disabilities.

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**Statement of Informed Consent:** This research paper being a review of doctrinal sources mainly perusing various online contents, no consent was required.

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# Assessment and Evaluation of Implantology Courses for Post Graduate Students

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## Abstract

**Introduction-** In dentistry, dental implants have become an indispensable established therapy to replace missing teeth in different clinical situations. Henceforth, implant education has become an integral part of postgraduate prosthodontic curriculum.

**Materials and Methods-** This study was conducted in the Department of Prosthodontics, Crown and Bridge at S. Nijalingappa Institute of Dental Science & Research, Gulbarga, Karnataka. A questionnaire of 22 questions was mailed to the postgraduate students of prosthodontics. A total of 83 students participated in the study. The forms were analyzed by using suitable descriptive statistics.

**Results-** A variety of responses were received from the participants. A total of 82 (98.8%) participants were included in the study. Most of the participants (around 56 of them) start to place implants from the second year and remaining. 69 of the participants responded in affirmation with the use of CBCT for routine implant cases. 54 participants reported that overall, in a month, more than 10 implants were placed in their department. In culmination, out of the 83 participants 69 of them want to learn more as implantology is evolving as a separate branch in dentistry.

**Conclusion-** Implantology treatment is constantly evolving as the prime line of treatment in different edentulous situations. Within the limitation of this study, it is concluded that dental colleges of India having active postgraduate program are moderately equipped with Implantology syllabus and courses. Although, there are variations in responses, but it has made necessary to review and standardize dental implant curricula among institutions.

***Keywords*** *– Dental implants, Implantology course, CBCT, Edentulous.*

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## Introduction

In dentistry, dental implants have become an indispensable established therapy to replace missing teeth in different clinical situations.[1] A dental implant

is a prosthetic device made of alloplastic material implanted in the oral tissues beneath the mucosal or/and periosteal layer, and on/or within the bone to provide retention and support for a fixed or removable dental prosthesis. [2] The use of dental implants in the treatment of missing tooth has proved effective in the long term. Success rates of 82.9% after 16 yrs. of follow-up have been reported. Since the introduction of the concept of osseointegration in dentistry, the dental implants treatment has become quite popular for the replacement of the lost or missing teeth.

Implant dentistry is currently being practiced in an atmosphere of enthusiasm and optimism.[3] Therefore, for a successful implant therapy for patients it is important that students graduating from dental schools should have solid knowledge and extensive understanding of the implant treatment.[1] Henceforth, implant education has become an integral part of postgraduate prosthodontic curriculum.

Although, several workshops and courses are held in the institutes regarding implantology, it is important that the quality of the programmes be assessed with thorough evaluation of knowledge of the postgraduate students. Even though, several surveys have been conducted to assess the status of the implant curricula as a part of the undergraduate programme, but seldom one finds a survey assessing the current status of oral implantology for the postgraduate curricula. Hence, this study was aimed to know the knowledge of the current dental postgraduate students with respect to implantology in general, the idea of placement of implants, treatment planning, treatment charges and the level of satisfaction they have with their present implantology course.

## Materials and Methods

*This study was conducted in the Department of Prosthodontics, Crown and Bridge at S. Nijalingappa Institute of Dental Science & Research, Gulbarga, Karnataka. A questionnaire of 22 questions was mailed to the postgraduate students of prosthodontics. Those who were not willing to be a part of the study were excluded. The participants were sent an email enclosing both the participant information sheet and a link created using Google Docs for survey questionnaire. Responses received were stored in Google Spreadsheet. A total of 83 students participated in the study .The forms were analyzed by using suitable descriptive statistics.*

## Results

A total of 83 students participated in the study. The distribution of participants per year constituted as 28 students (33.73%) from first year, 37 students (44.58 %) from second year and 18 students (21.69 %) from third year. Numerous students from various states participated in the study. Out of the 83 participants, 30 participants were male (36.14%) and 53 (63.86%) were female. There were 79 participants who aged less than 30 and only 4 of them were above 30 years old (table 1).

Out of the 83 participants, it was observed that 57 of them used conventional implants, only a single participant out of the 83 respondents had used basal implants and 4 of them were unaware of the type of implant used in their department/ college.

Out of 83 participants, 5 participants responded that from the 1st year itself they are allowed to place implants whereas most participants (around 56 of them) start to place implants from the second year and remaining 21 participants were allowed from third year.

Out of the 83 participants, currently only 35 of them had placed implants, out of which 30 of them placed more than 10 implants and 48 participants had not placed implants.

Out of all participants, 69 of them responded in affirmation with the use of CBCT for routine implant cases, whereas 13 of them were unaware about the imaging used. Out of the 69 participants only 25 of them had the CBCT facility available at their college premises .It also gives the numbers for, if the prosthesis placement is done by the participants or not.

54 participants reported that overall, in a month, more than 10 implants were placed in their department, 10 of them said that greater than 10 implants were placed whereas 19 of them were not aware of the logistics.

It was also asked if the participants performed full mouth implant placements. On asking specifically, if they performed full mouth prosthesis placement from start to finish, 44 of the participants said yes whereas 21 of them said no. 16 of the participants did it with assistance of prosthodontists whereas 2 of them reported that such cases were directly sent to the Dept of prosthodontics. It also describes the charges for the placement of single and full mouth implants respectively. Out of the 83 participants, 30 of them had performed or assisted in sinus lift surgeries and 53 of them had never done the same.

When asked about the management 21 of them responded with removal and replacement of the same. In culmination, out of the 83 participants 69 of them were not satisfied with the implant education provided by their Institute.

**Table 1: Distribution of responses according to Year of Post-Graduation, States, Gender and Age**

| | Number of responses | Percentage |
|---|---|---|
| **Year of Post-Graduation** | | |
| First year | 28 | 33.73 |
| Second year | 37 | 44.58 |
| Third year | 18 | 21.69 |
| Total | 83 | 100 |
| **States** | | |
| Karnataka | 39 | 46.99 |
| Maharashtra | 20 | 24.09 |
| Kerala | 10 | 12.05 |
| Andre Pradesh | 08 | 9.64 |
| Uttar Pradesh | 03 | 3.61 |
| Gujarat | 02 | 2.41 |
| Goa | 01 | 1.21 |
| Total | 83 | 100 |
| **Gender** | | |
| Male | 30 | 36.14 |
| Female | 53 | 63.86 |
| Total | 83 | 100 |
| **Age** | | |
| ≤ 30 | 79 | 95.18 |
| >30 | 04 | 4.82 |
| Total | 83 | 100 |

## Discussion

It is not uncommon to find that a conventional partial and/or complete denture may fail to provide psychological comfort, satisfactory function, esthetics, or speech. With an increase in awareness regarding dental implant therapy amongst the patients and newer advancements in the field of implant design, materials, diagnostic and surgical techniques has made it one the most successful treatment modality in the current dental scenario to achieve the above goals. Implant training is an integral part of the post graduate programme .[4]As no such evidences are available which determines the current trends of dental implantology amongst the post graduate prosthodontic programs of dental colleges of India, therefore this questionnaire study was employed to identify the status of Implantology courses and the learning experience the students are having.

A total of 83 students participated in the study, mostly belonging to the department of prosthodontics, crown and bridge. Out of the 83 participants, 30 were male and 53 were female participants. 79 of the participants belonged to the age category of less than 30 and just 4 of them were aged above 30. The responses were acquired from different states including Karnataka, Maharashtra, Kerala, Andhra Pradesh, Uttar Pradesh, Gujarat and Goa, out of which participants from Karnataka were the maximum, i.e. 46 per cent. Most of the students belonged to second year MDS, i.e. 37, followed by participants from first year MDS i.e. 28 and third year MDS i.e. 18. (Table 1)

Around 98 percent of the participants i.e. 82 of them, were aware and had knowledge about dental implant treatment. The result of the study was in accordance with a study done Nagpal D et al where they concluded that knowledge about dental implants was widespread among postgraduates and dental practitioners of their area.[5] A fair knowledge about implant kits is also an important attribute towards implant treatment. According this study, 81 of the participants agreed with the presence of implant kit in their department while 1 participant disagreed with the presence of kit in their department and 1 participant was not aware of it. Out of the 81 respondents to the previous question, 5 participants reported with single kit, 42 of them had 2 kits, 24 of them had 3 kits and 10 had more than 3 kits. According to a study by Bhatt NN 92.8 percent favored the standardization of implant surgical kits across all brands as it lessens the hassle of buying different implant systems and makes the practice of implantology simpler , which is a number similar to this study.[4]

More than 90% of the available Implant system all around the world follows system of crestal Implants. Advocates of basal Implant systems call it to be a better alternative to Crestal Implants in terms of ability to restore almost any type of case, shortened treatment time, less chances of failure. However, the long term results are yet to be proven.[6] Regarding the usage of type of implants 57 of the participants used conventional implants, single participant used basal implants and 21 of them used a combination of both of the above.

In response to the question, that if any quota is present for the completion of their post graduate programme, in terms of implant placement, 40 students responded in affirmation whereas 16 of them did not have any such quota and rest 16 of them were unaware. In numbers, 7 of them responded with over 10 cases of implant placement as their quota and 33 of them with less than 10 cases as quota which holds in accordance with the guidelines of dental council of India. In order to be acquainted to dental implant surgeries, the postgraduate students are required to assist their seniors or faculty. Out of the 83 respondents placing implants, 11 responded with 2 surgeries to be assisted, 28 responded with 3 surgeries to be assisted and 40 responded with greater than 3 assistances and 4 participants after 1 assistance.

Although, certain studies mention the need of a different course for Implantology, but there are

studies which also emphasis the need of conducting implants in post-graduation itself. Most of the dental practioners were not practicing implant dentistry due to lack of skills. This indicates a need to revise dental curriculum at various dental schools to improve the knowledge and thus practice of implant dentistry.[7] According to the responses from this study, only 5 participants from I MDS were allowed to place implants, 56 from II MDS and 21 from III MDS. Out of all the participants 30 of them had placed ≤10 implants, 5 of them had placed more than 10 and 48 of them had not placed any implants till date.

Implant courses, are either company sponsored or are affiliated to various organizations.[8] 51 of the participants responded in affirmation that the Implantology workshops are being held in their college, while 17 of them had no such workshops and 15 of them were unaware. The workshops varied in duration of 2-4 days, to weeks and months and only 3 responses were received where the course was more than a year. Specifically asking if the dental implant workshops recognized by the affiliated university/DCI/ Dental implant company, 44 responded in affirmation, 6 of the respondents disagree and 33 of them were not aware 2 of the respondents had affiliation with the university, 13 of them with the Dental council of India,36 of them with dental implant company and 32 were not aware.

Dental implant placement often requires advanced imaging for its accurate placement. 69 of the participants used the same, whereas 13 participants were unaware. According to a study by Bhatt et almost participants had an opinion that a combination of the existing diagnostic aids (Mounted cast, IOPA, OPG, CT scan) must be utilized to plan treatment for implant-supported prosthesis. IOPA, OPG and CT scan are important to provide an insight into bone quality and quantity as well as determine the relation of implant to surrounding vital structures.[4] In this study, out of the 69 respondents, 25 had imaging modality in their premises and 44 did not have. Beas et al in their study recommended to use 3D imaging for all implant planning, with CBCT as the imaging modality of choice.[9] Apart from this, out of the 83 participants, 34-used digital software for planning whereas 31 of them were not aware and 18 did not use any.

With the increasing interest in implant dentistry, most of the practitioners who do implants prefer doing both the surgical and prosthetic phases themselves. [8] 54 respondents gave answers in affirmation to placement of prosthesis from start to finish, whereas 25 of them did it with the help of prosthodontists and 2 of them directly sent it to department of prosthodontics. For the question “Do you perform full mouth implant placement?, 47 answered in affirmation whereas 24 of them did not, while 11 of them did it with the assistance of prosthodontists and 1 of the response was to directly send it to the department of prosthodontics. In a study conducted by Gibson et al, in UK, majority of the practioners always want to provide both simple surgical and restorative aspects which may reflect the desire of GDPs to provide all aspects of treatment for their patients. Similarly in our study, 44 respondents performed full mouth prosthesis placement from start to finish, 21 of them disagreed, 16 did it with the assistance of prosthodontists and 2 directly sent it to Dept of prosthodontics. In total 54 respondents placed less than 10 implants per month in their department, 10 of them placed greater than 10 implants per month, 19 of them were not aware.

Cost of implants is a major key factor in this type of treating modality. For treatment charges, 30 participants responded less than 10,000 and 53 of them responded saying more than 10,000 which was similar to study by MP Sakshi et al where majority of their participants agreed to the treatment charges of placing single implants 10,000 or more than that.[10] When asked about full mouth treatment, 53 participants responded with more than 20,000 and 21 of them said less than 20,000. Only 9 of the respondents were not aware of the same.

In this study, 30 of the participants had performed or assisted sinus lift procedures during implant placement while 53 had not. This is in contrary to a study conducted by Bhatt NN et al where maximum participants preferred direct sinus floor elevation. Some participants did not prefer to perform sinus floor elevation to avoid invasive procedure.

One-stage implant is an endosseous dental implant designed to be placed following a one-stage surgery protocol.[11] For the question, "How many single stage implants have you placed till date?" 7 participants had placed 1 implant, 4 had placed 2, 9 participants had placed 3 implants, 4 of them had placed more than 3, rest 59 had not placed any single stage implants. For second stage implants, 5 participants had placed a single implant, 11 of them had placed 2 implants, 5 of them had placed 3 implants, 14 of them had placed greater than 3 implants and 48 of them did not place any implants.

Possible occurrence of implant failure is a major concern for implantologists and knowledge in such unavoidable fact is clinically essential. [12] Out of the 83 participants, 60 had zero failures, 15 faced single implant failure, 5 of them faced two implant failures and 2 of the participants faced 6 implant failures. Out of these participants 21 of them removed and replaced it. The results of the study are contrary to a study done by Bali A et al where they concluded that there is a deficit in the level of information regarding peri-implant diseases, so further education is required.[13] Similarly , in a study conducted by Al-Dwairi ZN, they stated that there is a need to increase the knowledge and awareness of dental practitioners who are practicing dental implantology regarding the potential risk factors that could potentially impact upon implant failures through continuous dental educational programs and workshops.[14]

In culmination, 69 of the participants, of this study, were not satisfied with the implant education in their institute and only 14 of them were satisfied. After thorough evaluation, it can be concluded that with growing popularity of implants, there is a need to introduce basic Implantology at undergraduate level and training programs in implants should be provided or undertaken at postdoctoral level to improve their skills and knowledge and gain confidence to perform the treatment.[1]

## Conclusion

Dental implants are the most preferred treatment modality for the replacement of missing teeth. Within the limitations of this study, it is concluded that dental colleges of India having active postgraduate programs are moderately equipped with Implantology syllabus and courses. The quality of awareness and education amongst the postgraduate students is not up to a level where they can treat patients single handedly. Although, there are variations in responses, it has made necessary to review and standardize dental implant curricula among institutions. Implantology curriculum in the postgraduate courses should be given more emphasis to improve the clinical skills and knowledge of the students. Postgraduate students should be encouraged to attend implantology clinics from the first year of their post graduation.

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# Effectiveness of Learning Software for Teaching Curricula and their Influence on Developing Thinking Skills and Achievement among Senior Students

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## Abstract

The developments taking place in the world in terms of informational modernity, called the knowledge-technology revolution. Such developments have relied on the use of computers, which have become the main sphere of an individual's life in various educational, sports, and other disciplines, in the service of the learners. Hence, the importance of research in keeping pace with the educational process of what has happened in the world in terms of inventions and developments, which accommodate technology education and becomes an integral part of its curriculum and teaching methods in line with the characteristics of society.

The research problem lies in that many of the teachers adopt the traditional method in teaching the curricula without updating the teaching method by incorporating the educational software for this course lesson, and the extent of its influence on developing thinking skills and achievement for fourth-stage students in the Department of Physical Education and Sports Sciences - College of Basic Education at Mustansiriya University that teaches this course in the curriculum of this stage.

The researchers concluded that these students are equal in their acquiring of thinking skills and obtaining information, concepts, and subjects of the curricula, and there is an association in the achievement test for the study group.

***Keywords:*** *Achievement, Learning Software, Thinking Skills*

## Background

Recently, the world is witnessing amazing developments, as the individual faces high-speed rhythms of an explosion of knowledge which are characterized by permanence and continuity of information bursts which is called (the technological knowledge revolution)[(1)] where the computer is considered its main gateway and invades all areas of life, especially in terms of education and sports. The computer, with its software, has many characteristics and advantages that make its use in educational fields and various situations because it is the language of communication in this era. The development that occurred in educational software placed learners among many options, including the difference in time and place, the type of training and education, improving opinions and improving the educational experiences of students through interactive science experience. The content of any academic curriculum occupies the

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information and activities related to skills, attitudes, problems, and exercises and mental activities, and the aim of these content is to develop methods of thinking of all kinds. It is the path to theoretical and applied creativity in various fields of science, knowledge and art. In spite of the increased risk and importance of the educational process, especially in this era, which is called the computer era or the age of technology and the information revolution, which plagues people from every side, encircles and encompasses him, as it becomes worthless if he does not know it or deal with him. Since the person is civil and social by nature, he cannot keep himself abreast from it or remain isolated from the world and the developments, inventions, and events taking place around it that make him obliged to keep pace with development and look forward to what is happening in it.

As such, the importance of this study lies in keeping pace with the educational process with what is happening in the world in terms of information revolution, inventions, and developments that assimilate educational and technological process and become part of its curriculum, methods, and teaching methods in line with the characteristics of society.

**The research problem**

Many teachers resort to filling the learners' minds with information and facts about the traditional method that makes them receptors of what is thrown at it without thinking, which led to a decline in the efficiency of educational process. While, the curriculum subject, like other subjects, is not limited to the cognitive aspect alone, but there are other aspects that are higher than that, foremost of which is thinking. The researchers decided to study the effectiveness of educational software for the academic curricula and its influence in developing thinking skills and academic achievement for fourth-stage students.

This study aims to (1) identify the effectiveness of educational software in the curricula and their influence in the development of thinking among fourth-stage students, and (2) identify the effectiveness of educational software in the curriculum course and their influence in the development of achievement among fourth-stage students.

**Research Hypotheses**

1. There are statistically significant differences in the thinking skill for the curricula course for the fourth stage between the study and control groups.

2. There are statistically significant differences in the achievement for the curricula course for the fourth stage between the study and control groups.

## Materials and Methods

An experimental design was used to guide this study. The study included a sample of (95) seniors who were recruited from Department of Physical Education and Sport Science for the period from October 7$^{th}$, 2018 to December 13$^{th}$, 2019. The study subjects were assigned into study group (n = 44) and control group (n = 44).

**Equipment and Devices**

Structured interview

Achievement test in curricula course

Thinking Scale which includes five skills (determining the problem, selecting the hypotheses, hypotheses testing, change, and design)

**Achievement Test**

To prepare the achievement test, the researchers selected some content of the curricula which include the old and new curriculum, the curriculum content, curriculum development, and curriculum evaluation. The researchers selected these contents owing to the plenty of new concepts for students besides the plenty of information presented for each item and their overlap with each other, in addition to the fact that these contents are suitable for preparing a group of units that can be formulated and help to develop thinking.

**Scientific Bases of Testing**

The content, concurrent, internal consistency,

criterion, and subjective validity tests were used as follow:

1. Formulating behavioral objectives for the test by Bloom's taxonomy of cognitive levels (remembering, comprehension, application, analysis, synthesis)[2].

2. Determining the percentage of content concentration/the percentage of each unit of content was determined.

$$Concentration\ Percent = \frac{Number\ of\ Lessons\ of\ Content\ PerSemester}{Number\ of\ Lessons\ Assigned\ for\ Study\ Application} * 100\%$$

1. Determining the number of questions: Four questions have been prepared that include the unit of the prescribed course, considering the convergence of cognitive levels for the objectives of the content of the course, and the total of questions of each lesson is proportional to the percentage of concentration. A questionnaire that demonstrates the appropriateness and validity of the scientific material and the behavioral goals was distributed explaining to a panel of experts to measure the objectives by items and the belonging of the item to the level of objectives and the appropriateness of alternatives for each item. After considering the experts' opinions, some questions were modified, and others were omitted.

2. Preparing a table of specifications for the test: Thirty questions were determined, considering the convergence of the distribution ratios of cognitive levels of the content objectives and the concentration ratio. Therefore, the researchers prepared a table of specifications in which the distribution proportions of cognitive levels of the content and concentration proportions were balanced to give a large goal.

Number of questions for each part = Percent of level of objective * concentration proportion * total number of questions

**Table 1. Specifications of achievement test**

| Level of objectives | | Remembering | Comprehension | Application | Analysis | Synthesis | Total |
|---|---|---|---|---|---|---|---|
| Lecture | Concentration proportion/ Objectives proportion | 25% | 35% | 20% | 10% | 10% | 10% |
| Old Curriculum | 10% | 1 | 0 | 0 | 0 | 2 | 3 |
| New Curriculum | 10% | 0 | 1 | 1 | 1 | 0 | 3 |
| Content | 10% | 0 | 1 | 2 | 0 | 0 | 3 |
| Curriculum Implementation | 20% | 1 | 2 | 1 | 1 | 1 | 6 |
| Factors affecting in Curriculum Implementation | 10% | 1 | 1 | 1 | 0 | 0 | 3 |
| Curriculum Development | 20% | 2 | 2 | 0 | 2 | 0 | 6 |
| Evaluation | 20% | 2 | 2 | 1 | 0 | 1 | 6 |
| Sum | 100.0% | 7 | 9 | 6 | 4 | 4 | 30 |

In table (1), the researchers have arranged the test items according to the content. That is, the sequence of items on the test paper is according to a logical sequence of the content of the subject, so that the items are related to the topic, consecutive, and within one form.

**2. Reliability**

The reliability coefficient was calculated by repeating the test on the same individuals twice under similar conditions, and the stability was also calculated using equivalent versions. The researchers applied two versions of the test on the same group members for an interval that usually ranges between two weeks, a correlation coefficient was found between the results in the two cases, and this method overcomes the bias of the recall effect.

**Final modification of the test items**

After the final modifications on study instrument which was composed of the question booklet which included the name of the test, the page of instructions and the test vocabulary of (30) paragraphs, distributed over the five cognitive levels, the remembering (7 items), comprehension (9 items), application (6 items), analysis (4 items), and synthesis (4 items).

**Thinking test instrument**

The researchers prepared the thinking test. The objective of the test was determined by identifying the problem and hypotheses and testing their validity. The content test was also formulated, as three main scales were used, which are the scale of Saleh, Al-Rawadi, and Al-Deghem. The scale deals with subjects of the school curriculum. The researchers considered some important criteria for setting up the thinking test, which include that the test is appropriate for fourth-stage students and it is intended to measure thinking. Then the test was presented in its preliminary form to a panel of experts in the field of curricula, teaching and learning methods in order to bring it out in its final form.

**Equivalence of the sample**

The achievement test and pre-thinking were applied to the experimental and control groups for the purpose of equivalence of the two groups (Table 3).

**Table 2. Achievement test and pre-thinking for the study and control groups for the purpose of groups equivalence**

| Variable | Groups | Number | Mean | Standard Deviation | t | Significance |
|---|---|---|---|---|---|---|
| Achievement test | Study | 44 | 12.5 | 2.88 | .18 | Non-significant |
| | Control | 44 | 11.95 | 2.75 | | |
| Thinking | Study | 44 | 16.35 | 2.19 | .65 | Non-significant |
| | Control | 44 | 16.77 | 2.85 | | |

Table (2) displays that the *t* value (0.18) at p = 0.05 indicates that there are no statistically significant differences in the achievement test between the study and control groups. For the thinking test, the *t* value (0.65) at p = 0.05 indicates that there are no statistically significant differences in the thinking test between the study and control groups.

**Pilot test**

The two tests were pilot-tested on a sample of 30 fourth stage students who were not included in the final study sample to know the success of the instructions in the test, the clarity of its items, and the determination of the test time.

**Preliminary Experiment**

The pretest was conducted on October 16, 2018 for the achievement test and on October 17th, the thinking test was conducted.

**Validity**

The constructive validity was calculated for the thinking test using the Pearson coefficient which was 0.89.

**Reliability**

The internal consistency was calculated using the split-half method which was 2.82 which is considered high consistency coefficient.

**Main Experiment**

After completing the final modification on the two tests, the researchers applied the content of the course material to the experimental group, unlike the control group that is studied in the conventional way. The duration of the exams was (5) weeks, at a rate of (2) hours per week, and an equal time between the two groups was considered. The researchers taught the subject without bias, and the thinking and achievement test was applied for the period from 7/10/2018 to 12/29/2019.

**Posttest**

The achievement test and the thinking test were applied to the fourth-stage students on two consecutive days under conditions like these of the pretest.

## Data Analyses/Statistics

Data were analyzed using the statistical package for social science (SPSS) for windows, version 26.

## Results and Discussion

**Table 3. Mean and standard deviation of the thinking test for the two group in the posttest**

| Variable | Group | Number | Mean | Standard Deviation | t | Significance |
|---|---|---|---|---|---|---|
| Thinking | Study | 44 | 18.04 | 2.87 | 0.09 | Non-significant |
| | Control | 44 | 17.13 | 2.88 | | |

Table (3) reveals that the means of students' grades for the study group are greater than these of the control group (18.04 vs 17.13 at p-value = 0.01) respectively which indicates non-significant differences between the study and control groups for the thinking test. As thinking is a complex process of the higher actual processes that require a long time and intensive and long teaching programs and cannot be developed quickly and to an acceptable degree within a relatively short period of time.[3]

**Table 4. Mean and standard deviation of the achievement test for the two group in the posttest**

| Variable | Group | Number | Mean | Standard Deviation | t | Significance |
|---|---|---|---|---|---|---|
| Thinking | Study | 44 | 22.05 | 2.68 | 3.05 | significant |
| | Control | 44 | 19.05 | 2.27 | | |

Table (4) displays that the means of students' achievement for the study group are greater than these of the control group (22.05 vs 19.05 at p-value = 0.01) respectively which indicates statistically significant differences between the study and control groups in favor of the study group. As thinking is a complex process of the higher actual processes that require a long time and intensive and long teaching programs and cannot be developed quickly and to an acceptable degree within a relatively short period of time. The researchers taught students in the experimental group using educational activities as the educational software increases the effectiveness and realism of students' learning and helps bring the topic closer to the lesson from the student's level of perception. It also improves the learning and teaching process both for the teacher and student.[4]

Also, educational software develops actual capabilities by providing more than one component of this programming, such as written and audio texts, animated images, and sound effects, which is reflected in their increased academic achievement, especially levels of understanding and application.[5]

## Conclusions and Recommendations

### Conclusions

1. It was found in the pretest for the thinking test that students of the study and control groups are equal in their acquisition of thinking skills, which indicates the absence of statistical significance between students of the two groups.

2. In the pretest for the achievement test, it was found that students of the study and control groups are equivalent in terms of information and concepts included in the subjects of the curriculum.

3. It is evident through the posttests for the achievement test that there is a statistically significant relationship in favor of the study group.

## Recommendations

1. Incorporating programs for developing thinking of all kinds into the curricula of the Faculties of Physical Education and Sports Sciences and teachers' preparation.

2. There is a need to provide special scales to measure thinking of all kinds and apply them to students of different stages.

3. Reconsidering the examination questions so that they measure all cognitive levels according to Bloom's taxonomy and do not focus on measuring lower levels only.

4. Conducting further similar studies in the rest of the other courses and at the different educational levels.

**Conflict of Interest**: The researchers confirm that there is no any conflict of interest.

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**Ethical Clearance**: The researchers obtained the ethical approval from the College of Basic Education, Mustansiriyah University

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# Health Satisfaction in Health Care between Patients of 3rd Tertiary Allergy Center in Kirkuk with Salam Primary Health Care Center Patients in Kirkuk

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## Abstract

**Background:** Healthcare capacity in Iraq has deteriorated due to the effects of wars and political instability. The Iraqi Ministry of Health expanded the provision of health services, by directing the public sector towards primary health care. This study aimed to assess patients satisfaction with the primary health care services provided in 3rd tertiary allergy center and Salam primary health care center patients in Kirkuk, and to determine the relationship between patient satisfaction and their demographic data.

**Method:** A descriptive study was carried out at the 3rd tertiary allergy center and Salam primary health care center in Kirkuk city, Iraq. Data were collected through the use of the constructed questionnaire from 100 patients who visit those centers for routine care, through the period of the 1st December 2020 to 31st January 2021. Data were analyzed through (Measures of central tendency and inferential statistics) using SPSS (V.22). Chi-square test for independence of two variables has been utilized to test the hypothesis, through the use of cross-classification table to examine the nature of the relationship between the variables. Ethical approval and permission to access the samples was obtained from Kirkuk Health Directorate in Iraq.

**Results:** Overall, out of 100 clients responded to our questionnaire, age showed neutral score (Mean = 2.93, SD= 1.47). and the age group (21 -30 years) were the highest represented (n=27; 27%). Mean of score was used for reference about the client satisfaction, if the mean of score up to more than 3 that means positive satisfaction with the available services otherwise it means dissatisfaction. Chi-square test for independence of two variables was applied, and the probaility of high level of satisfaction were reported as there were a significat relationship (P <0.005) in items related to (nursing care services, availability of medical services, quality of prescribed medication, cooperation of pharmacist, availability of laboratory services and. laboratory staff cooperation about diagnosis than other items.Also, there were a statistical significant difference (P <0.005) between respondents age more than other demographic characteristics.

**Conclusion:** The study highlighted the sources of patient satisfaction related to primary health care services. The medical and nursing staff have a crucial role in providing health services, so their job satisfaction can improve the quality of care for patients, and reduce health care costs. Additionally, these results can provide priorities for developing healthcare services that can improve patient satisfaction. The utilization of telemedicine services, patient-centered medical visits and eHealth applications can be adopted to increase patient satisfaction with primary health care services. The use of remote patient monitoring programs are also effective ways to treat the signs and symptoms of COVID-19 at home and protect health workers.

***Keywords:*** *patient satisfaction, primary health care services, healthcare utilization.*

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## Introduction

Health systems exist in complex and dynamic environments, and requires planning with the rapid development of technology and societal requirements [1]. Insufficient health education is related to health care, which may result in poor quality of care [2]. Healthcare capacity in Iraq has deteriorated due to the effects of wars and political instability. The Iraqi Ministry of Health expanded the provision of health services, by directing the public sector towards primary health care [3] The World Health Organization stated that the majority of patient interaction with the health service provider occurs in primary health care settings [4]

Measuring patient satisfaction in relation to the quality of health care service is important elements in the health care system, and help designing health care policies.[5] Patients evaluation of the healthcare services is extremely important, and the clients feedback on quality of healthcare is crucial to develop patient-centered approaches to providing health care [6]. Lack of access to self-care advice from a general practitioner can negatively affect patient satisfaction, given that the feeling of reassurance after a counseling is closely related to satisfaction, which increases the potential for benefit from a self-care behavior [7].

Patient-centered care can be associated with lower costs. Reduced medical care fees are an important outcome of patient-centered medical visits [8]. In traditional medical care, strong relationships between physicians and patients have a positive impact on patient satisfaction with healthcare services [9]. There is a link between patient experiences and healthcare and its use of health resources and expenditures [10].

**Objectives:** To assess patients' satisfaction with the primary health care services provided in 3rd tertiary allergy center and Salam primary health care center patients in Kirkuk, and to determine the relationship between patient satisfaction and their demographic data (age, gender, occupation).

**Methods:** A descriptive study was carried out at the 3rd tertiary allergy center and Salam primary health care center in Kirkuk city, Iraq. Data were collected through the use of the constructed questionnaire from 100 patients who visit those centers for routine care, through the period of the 1st December 2020 to 31st January 2021. Data analyzed through (Measures of central tendency and inferential statistics) by Statistical Package for the Social Sciences (V.22). Chi-square test for independence of two variables has been utilized to test the hypothesis, through the use of cross-classification table to examine the nature of the relationship between the variables. Tables show the method in which two variables are either related or unrelated. The null hypothesis ($H^0$) is a test of whether two categorical variables are independent, and that there is no relationship between two cross-tabulated variables. The alternative hypotheses ($H^1$) tests propose that the two categorical variables are related to each other. Data were grouped in terms of age of patients, gender and occupation.

## Findings

Overall, out of 100 clients responded to our questionnaire (Table 1.), age showed neutral score (Mean = 2.93, SD= 1.47).and the age group (21 -30 years) were the highest represented (n=27; 27%). Mean of score was used for reference about the client satisfaction, if the mean of score up to more than 3 that mean positive satisfaction with the available services otherwise it means dissatisfaction.

**Table 1: Demographic characteristics of the sample.**

| Items related to patients | | F | % |
|---|---|---|---|
| Age | less than 20 | 18 | 18.0 |
| | 21 -30 years | 27 | 27.0 |
| | 31 -40 years | 23 | 23.0 |
| | 41 -50 years | 15 | 15.0 |
| | 51 -60 years | 10 | 10.0 |
| | 61 years or more | 7 | 7.0 |
| | Total | 100 | 100 |
| Mean = 2.93 Std. Deviation = 1.47 | | | |
| Gender | male | 48 | 48.0 |
| | female | 52 | 52.0 |
| | Total | 100 | 100 |
| Mean = 1.52 Std. Deviation = ..5 | | | |
| Occupation | self-employer | 15 | 15.0 |
| | governmental employer | 28 | 28.0 |
| | Retired | 6 | 6.0 |
| | Housewife | 32 | 32.0 |
| | Jobless | 2 | 2.0 |
| | Student | 17 | 17.0 |
| | Total | 100 | 100 |
| Mean = 3.29 Std. Deviation = 1.65 | | | |

Continuity of Table 1

| **Items related to patients** | | **F** | **%** |
|---|---|---|---|
| Attended centers | Third tertiary allergy center | 67 | 67.0 |
| | Salam primary health care center | 33 | 33.0 |
| | Total | 100 | 100 |
| Mean = 1.33 Std. Deviation = .4 | | | |
| Marital status | single | 26 | 26.0 |
| | married | 72 | 72.0 |
| | divorced | 1 | 1.0 |
| | widowed | 1 | 1.0 |
| | Total | 100 | 100 |
| Mean = 1.77 Std. Deviation = .5 | | | |
| Referral types | referral | 51 | 51.0 |
| | self- referral | 49 | 49.0 |
| | Total | 100 | 100 |
| Mean = 1.49 Std. Deviation = .5 | | | |
| Patient diagnosis | Hypertension | 9 | 9.0 |
| | Diabetes mellitus | 6 | 6.0 |
| | cardiac diseases | 5 | 5.0 |
| | GIT diseases | 8 | 8.0 |
| | respiratory diseases | 32 | 32.0 |
| | others | 40 | 40.0 |
| | Total | 100 | 100 |
| Mean = 4.68 Std. Deviation = 1.6 | | | |
| Residency | Urban | 88 | 88.0 |
| | rural area | 12 | 12.0 |
| | Total | 100 | 100 |
| Mean = 1.12 Std. Deviation = .3 | | | |

**Table 2: Distributions of frequencies related patients' satisfactions.**

| No | Items | YES | | NO | |
|---|---|---|---|---|---|
| | | F | % | f | % |
| 1- | Patient satisfaction with nursing care services. | 95 | 95.0 | 5 | 5.0 |
| 2- | Patient satisfaction with medical services. | 94 | 94.0 | 6 | 6.0 |
| 3- | Patient satisfaction with the availability of medical services. | 89 | 89.0 | 11 | 11.0 |
| 4- | Patient satisfaction with the availability of prescribed medication | 78 | 78.0 | 22 | 22.0 |
| 5- | Patient satisfaction with the quality of prescribed medication | 79 | 79.0 | 21 | 21.0 |
| 6- | Patient satisfaction with no cost for prescribed medication | 95 | 95.0 | 5 | 5.0 |
| 7- | Patient satisfaction with the cooperation of pharmacist. | 95 | 95.0 | 5 | 5.0 |
| 8- | Patient satisfaction with the availability of laboratory services. | 81 | 81.0 | 19 | 19.0 |
| 9- | Patient satisfaction with the level of laboratory staff competence. | 86 | 86.0 | 14 | 14.0 |
| 10- | Patient satisfaction with the laboratory staff cooperation about diagnosis. | 76 | 76.0 | 24 | 24.0 |

**Chi-square analysis:** Chi-square test for independence of two variables was applied (**Tables 3,4, and 5**), and the probaility of high level of satisfaction were reported as there were a significat relationship (P <0.005) in items related to (nursing care services, availability of medical services, quality of prescribed medication, cooperation of pharmacist, availability of laboratory services and. laboratory staff cooperation about diagnosis than other items.Also, there were a statistical significant difference (P <0.005) between respondents age more than other demographic characteristics.

**Table 3: Group differences between patients age and his/her satisfactions.**

| | Items | $x^2$ | df | P-value | Significancy |
|---|---|---|---|---|---|
| 1 | Patient satisfaction with nursing care services. | 23.977[a] | 5 | .000 | Sig. >.05. |
| 2 | Patient satisfaction with medical services. | 8.392[a] | 5 | .136 | Not sig. |
| 3 | Patient satisfaction with the availability of medical services. | 19.296[a] | 5 | .002 | Sig. >.05. |
| 4 | Patient satisfaction with the availability of prescribed medication | 9.995[a] | 5 | .075 | Not sig. |
| 5 | Patient satisfaction with the quality of prescribed medication | 14.024[a] | 5 | .015 | Sig. >.05. |
| 6 | Patient satisfaction with no cost for prescribed medication | 7.653[a] | 5 | .176 | Not sig. |
| 7 | Patient satisfaction with the cooperation of pharmacist. | 23.977[a] | 5 | .000 | Sig. >.05. |
| 8 | Patient satisfaction with the availability of laboratory services. | 7.542[a] | 5 | .183 | Not sig. |
| 9 | Patient satisfaction with the level of laboratory staff competence. | 2.674[a] | 5 | .750 | Not sig. |
| 10 | Patient satisfaction with the laboratory staff cooperation about diagnosis. | 15.427[a] | 5 | .009 | Sig. >.05. |

**Table 4: Group differences between patients' gender and his/her satisfactions.**

| | Items | $x^2$ | df | P-value | Significancy |
|---|---|---|---|---|---|
| 1 | Patient satisfaction with nursing care services. | 1.653a | 1 | .199 | Not sig. |
| 2 | Patient satisfaction with medical services. | .550a | 1 | .458 | Not sig. |
| 3 | Patient satisfaction with the availability of medical services. | 2.127a | 1 | .145 | Not sig. |
| 4 | Patient satisfaction with the availability of prescribed medication | .073a | 1 | .787 | Not sig. |
| 5 | Patient satisfaction with the quality of prescribed medication | 1.045a | 1 | .307 | Not sig. |
| 6 | Patient satisfaction with no cost for prescribed medication | .304a | 1 | .582 | Not sig. |
| 7 | Patient satisfaction with the cooperation of pharmacist. | .304a | 1 | .582 | Not sig. |
| 8 | Patient satisfaction with the availability of laboratory services. | .202a | 1 | .653 | Not sig. |
| 9 | Patient satisfaction with the level of laboratory staff competence. | .173a | 1 | .678 | Not sig. |
| 10 | Patient satisfaction with the laboratory staff cooperation about diagnosis. | 1.395a | 1 | .238 | Not sig. |

**Table 5: Group differences between patients' occupation and his/her satisfactions.**

| | Items | $x^2$ | df | P-value | Significancy |
|---|---|---|---|---|---|
| 1 | Patient satisfaction with nursing care services. | 8.514a | 5 | .130 | Not sig. |
| 2 | Patient satisfaction with medical services. | 10.060a | 5 | .074 | Not sig. |
| 3 | Patient satisfaction with the availability of medical services. | 6.220a | 5 | .285 | Not sig. |
| 4 | Patient satisfaction with the availability of prescribed medication | 4.514a | 5 | .478 | Not sig. |
| 5 | Patient satisfaction with the quality of prescribed medication | 13.126a | 5 | .022 | Not sig. |
| 6 | Patient satisfaction with no cost for prescribed medication | 4.135a | 5 | .530 | Not sig. |
| 7 | Patient satisfaction with the cooperation of pharmacist. | 8.514a | 5 | .130 | Not sig. |
| 8 | Patient satisfaction with the availability of laboratory services. | 11.120a | 5 | .049 | Sig. >.05. |
| 9 | Patient satisfaction with the level of laboratory staff competence. | 13.319a | 5 | .021 | Sig. >.05. |
| 10 | Patient satisfaction with the laboratory staff cooperation about diagnosis. | 3.833a | 5 | .574 | Not sig. |

## Discussion

Quality measurement is a key issue in healthcare systems for assessing health system performance and desired outcomes for patients [(11)]. Assessment of patient satisfaction is vital, because patient satisfaction reflects the gap between the expected and received health service, being the result of health care and an indicator of treatment use and commitment to the care and support provided [(12)]. Patient satisfaction is useful for providing quality indicators in healthcare services. It helps determine the quality of health care delivery and the extent to which patients respond to it. Its higher levels are indicative of higher levels of patient empowerment, commitment and compliance, all of which can lead to desired health outcomes [(13)].

Job satisfaction tends to be the satisfaction with the job or its outcomes, and this is based on its characteristics. The finding of this study revealed a high frequency level of satisfaction in most of questionnaire items. The focus on value has become an objective for healthcare rather than volume when paying for care costs, and new care delivery models aim to harmonize it and improve its quality [(14)]. Patient experience in assessing the quality of healthcare received can be used to improve healthcare quality for those most in need [(15)]. E-health applications are designed to optimize the utilization of healthcare services and to actively participate in patients' recovery, thus reducing health care expenditures and improving their quality [(16)].Continuity of care (COC) is a fundamental and widespread principle of primary care and has been associated with patient satisfaction and use of health care [(17)].

A statistical significant difference (P <0.005) were founded between respondents age more than other demographic characteristics. Espically in relation to nursing care services, availability of

medical services, quality of prescribed medication, cooperation of pharmacist. Health literacy, self-efficacy and patient satisfaction are among the factors involved in healthcare. And that the relationship between these factors and their combined effect on self-rated patient health has been studied over decades in chronic disease groups [18]. Regional variation in patient satisfaction with health care systems and its impact on the quality of services provided is important to improving the quality and development of a health care system, as it focuses on patients by improving their response in particular to the cultural aspects of the health requirements of the community [19].

## Conclusion

The study highlighted the sources of patient satisfaction related to primary health care services. The medical and nursing staff have a crucial role in providing health services, so their job satisfaction can improve the quality of care for patients, and reduce health care costs. Additionally, these results can provide priorities for developing healthcare services that can improve patient satisfaction. The utilization of telemedicine services, patient-centered medical visits and eHealth applications can be adopted to increase patient satisfaction with primary health care services. The use of remote patient monitoring programs are also effective ways to treat the signs and symptoms of COVID-19 at home and protect health workers.

**Conflict of Interest:** Nil

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**Ethical Clearance:** Ethical approval and permission to access the samples was obtained from Kirkuk Health Directorate in Iraq. (see appendix)

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# Strengthening Human Resources as Lecturers Through the E-Learning Learning Method

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## Abstract

**Introduction**: The concept of e-learning is an alternative for students who are unable to attend face-to-face lectures. The use of e-learning as an alternative learning is increasing in line with technological developments. **Aim**: This study applies e-learning learning methods to strengthen educational human resources as lecturers using Computer-based Multimedia Communication (CMC). **Method:** This research is a pre-experimental study. The research was conducted in STIKes Yarsi Mataram, Indonesia in 2020. The measuring instrument used a questionnaire. The study population was all students, with a total sample of 81 students. The independent variables include a method of learning. The dependent variable in this study was knowledge. The analysis used was univariate and bivariate using paired t-test. **Result and Discussion**: frequency of e-learning based on CMC in the experimental group, namely 63% in the evaluation and 80% in the implementation process. While in the control group using conventional methods, 50% of the intervention and 23% of the implementation. **Conclusion**: Improving student learning outcomes in the community nursing process by using the CMC learning method is more effective than using conventional methods.

***Keywords:*** *E-Learning, Method, Learning, Human Resources, Lecturer*

## Introduction

Along with the development of technology, the need for a concept and mechanism for teaching and learning based on information technology is very necessary. One of these concepts is e-learning. The concept of e-learning has an influence on the change from conventional education into digital form. The concept of e-learning has been widely accepted by the community, especially in educational institutions. E-learning is an alternative for students who are unable to attend face-to-face lectures.[1]

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The use of technology for learning activities in educational institutions in Indonesia is getting better, supported by regulations regarding the implementation of distance education. This study uses e-learning as alternative learning in educational institutions. This learning method will certainly create human resources as skilled and competent lecturers in teaching.[2]

The application of e-learning for online learning at this time is very easy by utilizing various electronic media such as smartphones. personal computers and so on. The application of technology in learning is suspected to improve learning outcomes. Information and communication technology-based learning will run effectively if the teacher's role in learning is as a learning facilitator or making it easier for students to learn, not just as an information provider. The learning process by

utilizing information and communication technology is a guide from the teacher to facilitate effective learner learning.

Effective learning can be said to be learning that utilizes information and communication technology optimally in the learning process as a tool. One of the uses of information and communication technology in learning is to use e-learning, especially with the CMC approach which makes it easier for students and lecturers to be freer to communicate and discuss related subjects.

The purpose of this study was to compare the effectiveness of giving lectures with e-learning methods and conventional methods.

**Method**

This research is pre-experiment. The research was conducted in STIKes Yarsi Mataram in 2020. The measuring instrument used a questionnaire. The study population was all students, with a total sample of 81 people. The independent variables include a method of learning. The dependent variable in this study was knowledge. The analysis used was univariate and bivariate using paired t-test.

The stages carried out in e-learning research include planning, implementation, and evaluation. At the planning stage, it is expected to be able to analyze learning programs, make semester or annual programs, prepare lesson plans, and make learning assessment plans. At the implementation stage, it is carried out on aspects of approaches, strategies, techniques, and learning procedures. The type of e-learning that will be developed by the researcher is Computer-based Multimedia Communication (CMC). The developed e-learning focuses on community nursing courses.

## Result and Discussion

**Table 1 Initial Evaluation of the Community Nursing Process**

| Community Nursing Process | Control Group (n=40) | | Experiment Group (n=36) | |
|---|---|---|---|---|
| | Right Answer | % | Right Answer | % |
| Assesment | 15 | 37 | 13 | 36 |
| Diagnose | 13 | 32 | 17 | 47 |
| Intervention | 16 | 40 | 19 | 52 |
| Implementation | 20 | 50 | 24 | 66 |
| Evaluation | 17 | 42 | 16 | 44 |

Most of the respondents did not understand the community nursing process for both the control group and the experimental group before the learning method was carried out. In the experimental group, the components of assessment, diagnosis, and evaluation, less than 50% answered the questions correctly. Meanwhile, in the intervention and evaluation components, more than 50% answered correctly. For the control group, almost every component, less than 50% answered correctly.

**Table 2 Final Evaluation of the Community Nursing Process**

| Community Nursing Process | Control Group (n=40) | | Experiment Group (n=36) | |
|---|---|---|---|---|
| | Right Answer | % | Right Answer | % |
| Assesment | 21 | 52 | 28 | 77 |
| Diagnose | 18 | 45 | 25 | 69 |
| Intervention | 20 | 50 | 24 | 66 |
| Implementation | 23 | 57 | 29 | 80 |
| Evaluation | 19 | 47 | 23 | 63 |

Most of the respondents already understood the community nursing process for both the control group and the experimental group after the learning method was carried out. In the experimental group, the components of assessment, diagnosis, intervention, implementation, and evaluation, more than 50% answered the questions correctly. For the control group, only in the assessment and implementation component, more than 50% answered correctly.

**Table 3 Comparison of Evaluation Results of Community Nursing Process (Control Group)**

| Community Nursing Process | Control Group (n=36) Pre | | Control Group (n=36) Post | |
|---|---|---|---|---|
| | Right Answer | % | Right Answer | % |
| Assesment | 15 | 37 | 21 | 52 |
| Diagnose | 13 | 32 | 18 | 45 |
| Intervention | 16 | 40 | 20 | 50 |
| Implementation | 20 | 50 | 23 | 57 |
| Evaluation | 17 | 42 | 19 | 47 |

The results showed that there was an increase in the proportion who answered correctly before and after using conventional learning methods.

**Table 4 Comparison of Evaluation Results of Community Nursing Process (Experiment Group)**

| Community Nursing Process | Experiment Group (n=36) Pre | | Experiment Group (n=36) Post | |
|---|---|---|---|---|
| | Right Answer | % | Right Answer | % |
| Assesment | 13 | 36 | 28 | 77 |
| Diagnose | 17 | 47 | 25 | 69 |
| Intervention | 19 | 52 | 24 | 66 |
| Implementation | 24 | 66 | 29 | 80 |
| Evaluation | 16 | 44 | 23 | 63 |

The results showed that there was an increase in the proportion who answered correctly before and after the e-learning learning method was carried out

In the assessment component, the control group who answered correctly increased by 15%, while the experimental group who answered correctly increased by 41%. In the diagnosis component, the control group who answered correctly increased 13%, while the group who answered correctly increased by 22%. In the intervention component, the control group who answered correctly increased by 10%, while the experimental group who answered correctly increased by 14%. In the implementation component, the control group who answered correctly increased 7%, while the experimental group who answered correctly increased by 14%. In the evaluation component, the control group who answered correctly increased by 5%, while the experimental group who answered correctly increased by 19%. So it can be concluded that the increase in those who answered correctly in the experimental group who received the e-learning method was more significant than the control group who received the conventional learning method.

Student learning outcomes obtained through the pre-test and post-test showed that the difference between the pre-test and post-test of the two groups was significantly different. This is shown from the evaluation results obtained that learning outcomes using the CMC-based e-learning approach have better results when compared to conventional methods. This means that improving student learning outcomes in the community nursing process using the CMC learning method is better than using conventional methods. This means that student learning outcomes in the community nursing process using the CMC learning method are better than those using conventional methods such as the lecture method in a class. The difference in learning outcomes between the control class and the experimental class occurred because the experimental class used CMC learning and the control class used conventional methods. The advantages of using the CMC learning method are: (1) independent learning, (2) high interactivity, (3) increasing memory levels, and (4) reducing costs. Activities in both classes during the learning process include 3 types of activities, namely preliminary activities, core activities, and closing activities.[3]

The implementation of this research was carried out for 3 meetings. Learning in the experimental class uses the CMC learning method, in the initial preliminary activity, the lecturer in charge of the course greets and gives apperception, at the first meeting students are given an initial test to determine students' initial abilities, at the next meeting to determine students' initial abilities, it is done by giving initial questions about the material the community nursing process lesson, then the researcher through the subject lecturer conveys the plan of activities to be carried out in the learning experimental class with the CMC learning method and the researcher communicates the indicators of learning outcomes to be achieved so that students know the competencies to be achieved after receiving the lesson. Before the learning begins, the lecturer in charge of the course motivates to arouse student interest.[4]

The activity after the introduction is the core activity. This activity begins with the researcher through the lecturer in charge of the course explaining CMC. Then the lecturer in charge of the course began to step on the learning ministry, namely about the maintenance or service of manual transmission and components. To check students' understanding, the lecturer asks questions related to the material being studied and allows students to ask questions.[5]

The last activity is the closing activity in the form of concluding the community nursing process material that has been studied with the guidance of the course lecturer. In this activity, the lecturer explains the important parts to unify the students' frame of mind, so that students can correctly conclude the material that has been given. At the third meeting of the closing activity, students were given a final test to determine the improvement in student learning outcomes that had been carried out. The test is in the form of a theory test and the ability to carry out the community nursing process including assessment,

diagnosis, preparation of interventions, implementation, and evaluation. The theory test is carried out in the classroom. Each student faces a sheet of questions and must answer theoretical questions in e-learning.[6]

While the implementation of the practical test was held in the community who had health problems, each student was tested one by one to carry out all the community nursing care processes from assessment to evaluation. In this test, students are also required to be able to answer the questions asked by the examiner and must be able to practice it. The control class does not use the Browser Based Training method but uses a conventional method, namely learning is carried out as usual by lecturers teaching every day.[7]

The preliminary activities in the control class are the same as the preliminary activities in the experimental class. The next activity is the core activity where the lecturer in charge of the course explains the subject matter and students listen and take notes on the explanation from the lecturer in charge of the course. Because only listening to students learning becomes less fun. A conducive learning climate is a backbone and driving factor that can provide the greatest attraction for the learning process, otherwise a less pleasant learning climate will cause boredom and boredom.[8]

To check the students' understanding, the lecturer in charge of the course asks questions related to the material being studied and provides opportunities for students to ask questions. Lecturers who support courses answer student questions clearly without guiding students to find their answers so that students feel that the correct answer only comes from the lecturer in charge of the course. This is because the lecturer in charge of the course conveys all the information intended for the achievement of the material being taught without involving students to play an active role in learning so that the experience gained by students is only through listening to students only as passive recipients without contributing ideas in the learning process. Learning should involve students as much as possible so that they can explore to form competencies by exploring various potentials and scientific truths. The last activity is the closing activity in the form concluding the material that has been studied with the guidance of the course lecturer. In this activity, the course lecturer explains the important parts to unify the students' thinking framework, so that students can correctly conclude the material that has been given.[9]

At the third meeting of the closing activity, students were given a final test to determine the improvement in student learning outcomes that had been carried out. The tests are in the form of theory tests and practical tests. In the implementation of the control group theory test, students were given the same questions as the experimental group theory test, but the questions had been recorded. The implementation of practice tests in the control group is the same as the implementation of practical tests in the experimental group.[10]

Increasing student learning outcomes by using e-learning learning media in the community nursing process, especially with the CMC approach, is important to study or try to apply in other courses because e-learning is a form of learning model that is facilitated and supported by the use of information technology and information technology. communication. E-learning has the following characteristics: 1) has content that is relevant to the learning objectives; 2) using instructional methods, for example presenting examples and exercises to improve learning; 3) using media elements such as words and pictures to deliver learning materials; 4) allows direct learning centered on the teacher or designed for independent learning; 5) build understanding and skills related to learning objectives either individually or improve group learning performance.[5]

The application of e-learning for online learning at this time is very easy by utilizing various electronic media such as smartphones. personal computers and so on. The application of technology in learning is suspected to improve learning outcomes. Information and communication technology-based learning will run effectively if the teacher's role in learning is as a learning facilitator or making it easier for students to learn, not just as an information provider. The learning process by

utilizing information and communication technology is a guide from the teacher to facilitate effective learner learning.[11]

Effective learning can be said to be learning that utilizes information and communication technology optimally in the learning process as a tool. One of the uses of information and communication technology in learning is to use e-learning, especially with the CMC approach which makes it easier for students and lecturers to be freer to communicate and discuss related subjects.

## Conclusion

Based on the discussion above, it can be concluded that the E-Learning learning method based on Computer-based Multimedia Communication (CMC) is more effective on the learning achievement of undergraduate Nursing students, especially in the community nursing process course.

**Conflict of Interest:** None

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**Ethical Clearance:** This research has received ethical approval from STIKes Yarsi Mataram, Indonesia with Number: 5/KEP/STIKES/Y.III/II/2020

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# Study of Lipid Profile, Lipid Peroxidation and DNA Damage in Patients with Coronary Artery Disease

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## Abstract

**Background and Objective:** This study was conducted to investigate some clinical aspects of coronary artery patients, as it included some tests for lipid oxidation and DNA damage and the extent of their association with the risk of coronary artery disease in the Iraqi population. **Results**: The lipid showed significant differences between the preferred messages in measuring lipids, TG (P = 0.005), high-density lipoprotein P = 0.018 (HDL) and low-density lipoprotein (vLDL) P = 0.004. It was named in lipid profile, and it was named In seizure patients (nonSTEMI). The glutathione analysis showed a significant decrease in patients compared to healthy subjects, and the unstable, non-Stemi and Stemi-series results were significant, and the significant differences were at (P = 0.00036). As for the research results, the significant differences were when they were high (P = 0.00072). The results of the comet assay showed differences between the four groups, for the group of patients with unstable angina and who suffer from a heart attack (Non-Stemi), the results showed close to average levels in the genetic material (P = 0.00014). The highest damage in the genetic material of patients with coronary artery disease was in patients suffering from a heart attack (Stemi) where the level of significant damage was very large compared to the rest of the groups (P = 0.00025). The results obtained on the biochemical levels of GHs, VLDL, HDL, TG and MDA showed significant differences between the healthy and the groups of patients with coronary artery disease below the probability level (P> 0.05). Also, the incidence of DNA damage in patients with coronary artery disease is much greater than healthy patients, and this indicates a significant effect of DNA damage on blood vessels, and on the other hand, STEMI patients are the most vulnerable to infection.

***Key words****:Coronary artery disease, DNA damage, Antioxidant, Lipid profile.*

## Inroduction

Coronary heart disease (coronary heart disease) is the leading cause of death in developed countries,with its introduction as stable angina, myocardial infarction and sudden coronary death [1,2]. Coronary artery disease usually appears as a heart attack at first, and becomes more complicated with heart failure or an irregular heartbeat. The primary mechanism is coronary arteriosclerosis. Risk factors for coronary artery disease include high blood pressure, obesity, diabetes, increased blood cholesterol levels, smoking, lack of exercise, poor diet, excessive alcohol consumption, and depression [3]. Thus, atherosclerosis can be assessed by testing the levels of cholesterol, triglycerides and lipoproteins in the blood [4,5]. Acute coronary syndrome is a term used to describe a group of symptoms that lead to acute myocardial ischemia. ACS is produced in a myocardial

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injury called a myocardial infarction (MI). The ACS includes unstable angina, non-elevated myocardial infarction (NSTEMI), and elevation-induced myocardial infarction (STEMI) [6]. Patients with STEMI have an elevation in the ST segment seen on the ECG machine. Usually the symptoms are similar in nature to angina pectoris, such as chest pain, but it is more severe and prolonged and is not relieved by nitroglycerin treatment under the tongue[7].

## Material and Methods

This study is a case-control study of 80 samples divided into four groups: 20 healthy controls, 20stable angina patients, 20 STEMI patients and 20 Non-STEMI patients. The sample of this study was collected in The Ibn Al-Bitar center for cardiac surgery in Baghdad/Iraq. Between December 2018 and July 2020. The following detailed information was obtained: age, gender, date of onset of disease. Blood samples were taken from venous about 7ml for different analysis. After centrifuging, serums were separated and stored at -80 C until measuring the concentration lipid profile,glutathione peroxidase (GPX1) and Malondialdehyde (MDA). All biochemical tests were done by Kenza 240 TX (Biolabo, France) and biolabo kits.DNA damage was measured by Comet Assay Kit (Trevigen, USA) andSYBR Green Dye (Sigma, Canda) . We used the serum for all samples and whole blood EDTA to DNA extraction.Friedewald formula was applied for LDL cholesterol measurement.

## Staistical Analalysis

Statistical calculations were made using the Statistical Package for the Social Sciences (SPSS) (version 20.0) program (IBM SPSS Statistics, SPSS Inc., Chicago, Illinois, USA). The Anderson-Darling test was performed to test the adherence of continuous, parametric,variables to the normal distribution. Normally distributed continuous parametric variables, with no significant outlier, presented using their mean and standard deviation (mean±SD) and parametric tests were used; independent t-test was used to analyze the differences between the mean of two groups, while one-way ANOVA was used to analyze the differences between the mean of more than two groups. The statistical tests were approved by assuming a null hypothesis of no difference between the mean of variables, a P-value ≤ 0.05 was considered statistically significant.

## Result and Discussion

The obtained result in table (1) showed that the levels of total cholesterols in the four groups are not significantly changed atP> 0.05 and the groups recorded normal values of TC. The levels of triglycerides between the groups of patients and the healthy control group showed significant differences P = 0.005. As for the values of TG in Non-STEMI patients, which were very high compared to the other groups (250.10 + 134.89). This is indicated by a study conducted on patients with coronary artery disease, as (90.47%) of men with artery disease Coronary syndrome and (72.97%) of women with coronary artery disease had a cholesterol level less than 200 mg / dl [8]. Another study indicated that the results of lipids showed an increase in the levels of TC and LDL and a decrease in TG, and there was no change in the level of HDL in STEMI patients than non-STEMI patients[9].

**Table 1: Comparison of lipid profile between CAD and healthy control**

| Parameters / Groups | TC. (mean+SD) | TG (mean+SD) | HDL (mean+SD) | LDL (mean+SD) | vLDL (mean+SD) |
|---|---|---|---|---|---|
| Control | A 154.20+22.13 | A 92.80+26.25 | A 45.930+6.697 | A 89.70+23.88 | A 18.30+5.31 |
| Unstable | A 141.90+33.43 | A 150.60+121.30 | B 32.370+9.496 | A 78.00+22.22 | A 31.60+23.21 |
| Non STEMI | A 167.60+37.61 | B 250.10+134.89 | A 41.210+9.251 | A 83.90+31.09 | B 49.70+26.98 |
| STEMI | A 152.50+56.76 | A 138.40+34.43 | B 35.500+12.583 | A 93.50+46.01 | A 26.90+6.10 |
| LSD | Non Sign. | 70.225 | 7.328 | Non Sign. | 13.742 |
| P-value | 0.551 | 0.005 | 0.018 | 0.723 | 0.004 |

The concentrations HDL was significantly related to the disease, as the concentration of HDL decreased significantly in patients with unstable angina (32,370 + 9,496) and in the STEMI patients (35,500 + 12.583), while healthy controls was (45.930+6.697) ₹which indicated significant differences between the groups, P = 0.018. No significant increase in mean LDL values was observed in the patient groups when compared with control (P = 0.723). The average level of VLDL in CAD patients with Non-STEMI (49.70 + 26.98) which significantly increased over the rest of the other groups (P = 0.004). The results of previous studies proved that cases of hyperlipidemia in relation to the increase of all lipids except for HDL, which is low. Patients with comorbidities also had fatty features that deviated from the normal range.[10]Table (2) shows that the average values for the level of GPx decreased significantly in patients (P = 0.00036), as the average values in the healthy control were (112.65 + 5.67). Which means that the level of glutathione peroxidase decreases in patients with unstable myocardial infarction (88.42 + 5.60), but it decreases significantly in Non- STEMIpatients (74.00 + 4.74) and in STEMI patients (68.62 + 3.38). The average concentration of MDA in the blood serum of all patients increased with a significant difference (P=0.00072) compared to the healthy (6.045 + 0.803). Furthermore, the results showed an increase in the concentration of MDA in patients with Unstable angina (11.170 + 1.347) and a greater concentration in Non-STEMI patients elevation (13.435 + 0.979) and STEMI patients (13.435+0.979).

The results of the current study showed decrease in the efficacy of glutathione peroxide in all patients with

coronary artery disease, which in turn was attributed to the deficiency of glutathione and increased reactive oxidative stress (ROS). It also showed a significant increase in the concentration of melondialdehyde (MDA) in patients more than healthy controls, as the current results of this study are consistent with those of [11,12]. In a comparative study by [13] between 50 patients and 50 healthy subjects, which indicated that coronary artery disease is associated with oxidative stress, lipid peroxidation, inflammation, and elevated liver enzyme activity. Coronary artery disease is a fatal disease that requires proper medical care. Antioxidant treatment may prevent disease progression. Consistent with the results of the current study, the results of the study by [14]indicated that the concentration of melondialdehyde (MDA) was significantly increased in patients with cardiac arrest more than in healthy subjects, and it increased to a greater degree in STEMI patients more than Patients with myocardial infarction and non-STEMI patients. As for glutathione, the level of glutathione in patients with cardiac arrest is much lower than that of glutathione in healthy subjects, and it decreases to a greater degree in STEMI patients. In a study conducted by [15] on heart patients, which recorded a clear increase in MDA and a decrease in the effectiveness of Gpx in patients, and it indicated the effectiveness of antioxidants in reducing MDA and this would reduce the risk of infection. Cardiovascular disease.

Table (3) shows some criteria for measuring the damage caused by the DNA in the white blood cells of patients with coronary artery disease and comparing them with the apparently healthy ones, as they were divided into several groups according to the amount of damage. The current results indicated that there were significant differences between the group in which no harm occurred (P = 0.00016), so the percentage was the largest in healthy people, and the percentage of non-damage in the genetic material in STEMI patients was much less than the rest of the patients. .The incidence of little damage in the genetic material of healthy people was greater than that of patients, as the results showed that there were significant differences between the groups of patients and healthy subjects (P = 0.00033).[16] in a comparative study of coronary patients indicated that DNA damage in the leukocytes of coronary patients had higher values of percentage of tail DNA (2.1x), And Tail Mean Moment Migrations (3 x) and Tail Length (3 x), indicating high DNA damage in peripheral leukocytes of CAD patients in compared with normal controls. In addition, there was an increase in the irreversible and reversible genetic damage to the white blood cells / lymphocytes in the peripheral blood of patients with coronary artery disease. Since oxidative stress may be involved as a major contributor to the development of atherosclerosis, it may also be a causative factor in DNA damage in coronary artery disease where DNA damage occurs frequently in cells exposed to reactive oxygen species (ROS). Furthermore, the average damage in the genetic material, the results indicated that there was a very large significant decrease in healthy subjects compared to patient groups (P=0.00014). The results also indicate a significant increase in the damage of genetic material in patients with unstable myocardial infarction, with no significant differences between non-STEMI patients and STEMI patients that recorded the highest average damage rate at ($P \leq 0.05$).

As shown in Table (3), where the percentage of high damage in the genetic material of healthy people decreased compared to groups of coronary artery patients. STEMI and non-STEMI patients did not show significant differences between them, but they were greater than high damage in the genetic material for healthy subjects and all groups of patients and healthy subjects showed statistically significant significant differences (P = 0.00025). Some studies give evidence for the concept that patients with coronary artery disease show changes in DNA repair and DDR gene expression, and some are related to the functional form of the disease itself, as the results prove that the DNA damage occurring in the blood cells of patients is STEMI. It was much greater than the damage done in the rest of the patients with coronary artery and that the percentage of DNA damage in healthy people decreased significantly. In addition to that patients with coronary artery showed

changes in the process of repairing the damaged DNA and geneexpression such changes may be due to the amount of progression of the atherosclerotic process in patients [17]. The results of the current study are consistent with the results of many studies conducted to find out the amount of damage to the DNA of white blood cells for coronary artery patients in different geographic regions and many population groups, as these studies recorded a significant increase in the percentage of damage to the DNA of patients much greater. Of the healthy subjects that reported a small percentage of the damage may be due to the natural repair process that occurs to DNA[18, 19,20].

**Table 2: Comparison of glutathione peroxidase (GPX1) and Malondialdehyde (MDA) between CAD and healthy control .**

| Parameters / Groups | GPx pg/ml (mean+SD) | MDA nmol/ml (mean+SD) |
|---|---|---|
| Control | A 112.65+5.67 | A 6.045+0.803 |
| Unstable | B 88.42+5.60 | B 11.170+1.347 |
| Non STEMI | C 74.00+4.74 | C 13.435+0.979 |
| STEMI | C 68.62+3.38 | D 15.625+1.135 |
| LSD | 8.441 | 1.276 |
| P-value | 0.00036 | 0.00072 |

**Table (3): The levels of damage in the genetic material (DNA damage) in healthy people and groups of patients with coronary artery disease.**

| Parameters / Groups | No Damage % (mean+SD) | Low Damage % (mean+SD) | Medium Damage % (mean+SD) | High Damage % (mean+SD) |
|---|---|---|---|---|
| Control | A 47.837+1.623 | A 39.092+1.239 | A 6.522+0.370 | A 6.550+0.689 |
| Unstable | B 40.638+2.183 | B 32.407+1.223 | B 12.820+1.931 | B 14.140+1.319 |
| Non STEMI | B 40.870+0.937 | BC 30.115+2.095 | B 13.775+0.615 | B 15.237+0.967 |
| STEMI | C 33.467+1.581 | C 28.522+2.192 | C 19.340+1.199 | C 18.670+2.436 |
| LSD | 2.269 | 2.517 | 3.153 | 2.411 |
| P-value | 0.00016 | 0.00033 | 0.00014 | 0.00025 |

## Conclusion

High levels of lipid profile and MDA asscociate with the increase damge in DNA of CAD. Furthermore, the significant decrase in the activity of GPx enzyme in patients with CAD. The incidence of DNA damage in patients with coronary artery disease is much greater than that of healthy people, and this indicates a significant effect of DNA damage on blood vessels. On the other hand, STEMI patients are the most vulnerable to injury.

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# The Effect of Aquatic Exercises on Special Endurance (Strength and Speed) and Achievement Run (800m) for Youth

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## Abstract

The research aims to use Aquatic exercises to develop special endurance and run (800m) for youth to identify the level of (speed endurance and force endurance) of the participants of the sample members of the experimental group. The researchers used the experimental method for its suitability to the nature of the study, and the sample was from Wasit Governorate clubs racers. The participants were (12) racers. We were divided into two control and experimental groups. The duration of the experiment was (8) weeks from (28/10/2020 to 10/3/2021). The place of the experiment is in the Al-Gharraf River (a branch of the Tigris River) and the Kut Olympic Stadium. The researchers used tests to speed endurance of a distance (600m), a test to endurance the strength of running by jumping for one minute (Bousing), and an achievement test (800m). The researchers concluded that training in the aquatic medium exercises affected the development of special endurance (speed endurance and force endurance) and it had a positive effect in completing the 800m race for youth.

***Keywords:*** *Aquatic, Medium Exercises, Special Endurance, Achievement, Run (800m).*

## Introduction

The 800m race is one of the track and field athletics in which performance is affected by the development of special endurance, which constitutes the most important capabilities for this race. Therefore, researchers and those interested in the field of training have tended to invent advanced training methods in order to achieve positive effects in the development of sports achievements by emphasizing special endurance.Therefore, the two researchers decided to use a new training method to develop the endurance of using a new environmental that constitutes resistance against internal strength. Increasing the water resistance raises and improves the level of the racer from the technical side and the physical side, as it shows its real importance in improving the imbalance between the different muscle groups, as well as improving the mechanics of movement for the joint of the body [(1)].

As it is known that the resistance shown by the water medium is one of the methods that can cause the development of muscular strength through the use of exercises that are directly related to the stages of performance of the activities depending on the speed of movement of the body when using various strength exercises with body weight, the (800m) race is one of the medium distance activities that need to speed endurance and force endurance.

Therefore, the importance of the research comes through the uses of the Aquatic, which provides a better opportunity, in the opinion of the researchers, when applying different exercises to develop these qualities.

The researchers noted that most of the training units given to the athletes of Wasit Governorate clubs

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in particular and Iraq clubs, in general, did not shed light on the use of the aquatic during training despite the importance of this type of training, which leads to a great development for athletes at the level of special endurance, so it was used The researchers exercise the resistance of the aquatic with bodyweight to be an aid method in developing special endurance and running (800m).

The research objective to prepare the aquatic exercises to develop special endurance (speed endurance and force endurance) and to identify the level of special endurance for the (800m) youth athletes (experimental group).

## Methods

### Participants

12 of the young running players and the sample was divided into two groups in a random way by drawing by numbers (double and odd) an experimental group (6) and a control group (6) athletes. The experimental group used exercises in the water medium that were prepared and organized by the researchers, while the control group in its training, depends on the application of the training curriculum by the trainer.

**Table 1. The homogeneity of research sample (age, weight, length and training age )**

| Basic variables | Statistical significance | | | |
|---|---|---|---|---|
| | Arithmetic mean | Median | Standard deviation | Skewness |
| Age (year) | 17.22 | 18 | 1.231 | 0.732 |
| Length (cm) | 174 | 172 | 4.123 | 0.723 |
| Weight (kg) | 60.122 | 58 | 2.321 | 0.921 |
| Training age (month) | 29.5 | 28 | 3.232 | 0.632 |

The above table shows that there are not statistically significant differences between the research sample individuals in some variables (age, height, weight, and training age). the skewness is ±1 which indicated the Homogeneity of the research sample.

**The procedure of measurement:** the following devices and tools were used in this study:Electronic scale for measuring weight and height PESAPERSONE, type of German origin, Metric Tape Length (50) Count (1), shot tool (1), Plastic cones (20), An athletics stadium with international legal dimensions, Electronic stopwatches of Chinese origin (12).

**Main experiment: consisted of the following tests:**

**Pretests:** The tribal tests for the research sample were conducted at exactly three o'clock in the afternoon for two days, starting from Saturday (31/10/2020) to Monday (01/11/2020)at the Kut Olympic Stadium.

On the first day: an achievement test (800m).On the second day: a running speed endurance test (600m) and a jumping power endurance test (Bousing) for one minute.

**Aquatic exercises:**

The training protocol contained (24) training units, it was applied to the research sample for a period of (8)

weeks, with three training units per week (Saturday-Tuesday-Thursday),the duration of one training unit is (40-50) minutes. The training protocol included exercises within the water to train the special physical abilities of the race (800 m), knowing that the heights of the water for speed-endurance exercises not more than 10 cm below the knee, and for strength-endurance exercises, the water height is not more than 10 cm above the knee. Arms and trunk Rise of the water at chest level.The researchers used the high intensity and low intensity interval training units.Was applied exercise in rural water in the pre-competition preparation period, because in this period must be given where the exercises are similar in terms of Kinetic performanceof power and speed as well as the direction of muscular work in with those movements that lead in the competition to develop the physical capabilities of active sports [(2)].

**Post-Test:** The researchers conducted post-tests for the special physical abilities of the research sample at exactly four o'clock in the Kut Olympic Stadium, for two days on (5/1/2021) on Tuesday and (6/1/2021) on Wednesday, under the same conditions as the pretests.

**Statistical Analysis :**the researchers used the static bag SPSS.

## Results

### View and Discuss Results

**Table2:** It shows the results of the arithmetic means, standard deviations, the calculated t-value, the level of significance, and the type of significance of the physical variables of the pre and posttests of the experimental group.

| significant | Significant level | t-test | posttest | | pretest | | Units | Tests |
|---|---|---|---|---|---|---|---|---|
| | | | Standard deviation | Arithmetic mean | Standard deviation | Arithmetic mean | | |
| Sig. | 0.021 | 4.391 | 1.21 | 86.66 | 2.07 | 89.50 | s | Speed-endurance 600m |
| Sig. | 0.003 | 2.821 | 9.93 | 221.67 | 9.50 | 212.67 | m | Strong-endurance |
| Sig. | 0.021 | 17.639 | 1.38 | 119.50 | 2.45 | 122.00 | s | achievement |

**Table3: It shows the results of the arithmetic means, standard deviations, the calculated t-value, the level of significance, and the type of significance of the physical variables of the pre and posttests of the control group.**

| significant | Significant level | t-test | posttest | | pretest | | Units | Tests |
|---|---|---|---|---|---|---|---|---|
| | | | Standard deviation | Arithmetic mean | Standard deviation | Arithmetic mean | | |
| Sig. | 0.001 | 4.391 | 1.72 | 88.83 | 1.83 | 90.83 | s | Speed-endurance 600m |
| Sig. | 0.018 | 2.821 | 4.89 | 218.50 | 8.61 | 212.83 | m | Strong-endurance |
| Sig. | 0.00 | 17.639 | 2.00 | 121.00 | 1.60 | 122.17 | s | achievement |

**Table 4: It shows the results of the arithmetic means, standard deviations, the calculated t-value, the level of significance, and the type of significance of the physical variables of the posttests of theexperimental&control group.**

| Significant | Significant level | t-test | Control group | | Experimental group | | Units | Tests |
|---|---|---|---|---|---|---|---|---|
| | | | Standard deviation | Arithmetic mean | Standard deviation | Arithmetic mean | | |
| Sig. | 0.02 | 4.42 | 1.72 | 88.83 | 1.21 | 86.66 | s | Speed-endurance 600m |
| Sig. | 0.42 | 3.99 | 4.89 | 218.50 | 9.93 | 221.67 | m | Strong-endurance |
| Sig. | 0.33 | 3.81 | 2.00 | 121.00 | 1.38 | 119.50 | s | achievement |

## Discussions

Tables (2-3-4) show that the results of the tests for the variable of endurance of speed, endurance of strength and achievement. We note that there is a remarkable development for the members of the research sample for the two groups between the results of the pre and post tests and for all the variables under study, and this seems clear through the significance of the statistical results and this is due to theenforcement of The training protocol developed by the trainers and their following appropriate training methods and means.

We also note that the experimental group superiority the control group in all the studied variables for the post-tests, and the researchers attribute this to the use of the aquatic exercises, as the aquatic exercises had a wonderful effect and the training load was appropriate and organized in a way that suits the development of endurance of speed and endurance of force.

The water medium is one of the external resistances imposed on the runner, and this increases the intensity of training on it. The intensity that was used within the exercises ranged between (55 - 90%) and was sufficient for the development to occur. The athlete reaches a state of fatigue, otherwise the adaptation process will not occur, and this was confirmed by (Holman) "that the use of the appropriate training load leads to the process of fatigue that is also commensurate with the level of that load." (3)

The exercises used depended on the number of repetitions and speed of movement, as well as the use of various exercises such as strength with body weight, jumping from one leg to another and alternating back and front stretch, which gave a positive in strengthening the muscles of the legs, thigh and leg, and through the process of effective exchange between contraction and muscle relaxation within The water medium during the exercise, this is confirmed by (Mohammed Hassan Allawi and Abu Al-Ela Abdel-Fattah) that "the ability to stretch in the muscles contributes to increasing the endurance of the speed of the motor performance of the exercises used" () and the researchers believe that the exercises that led to the development of speed endurance is a natural result of giving incomplete rest between repetitions for the purpose of Adaptation of organs and muscles to perform efficiently under conditions of muscle fatigue and this is what is characterized by endurance of speed, which was confirmed by (Hamdi) if training aims to develop and improve speed endurance, incomplete rest should be used.

As for the force endurance variable, we note that the statistical results indicate that there are significant differences between the experimental group and the control group in favor of the experimental group that used special exercises in the aquatic.The researchers also see that the ratios that were given within the exercises in the water medium were taking into account the principles of the training volume, and this is what Karim Aziz and Nass indicated that the studied and consistent training in terms of the components of the training load and identical with the possibility of the research sample in terms of application duration and formation of intensity, size and comfort will lead To develop the sample positively and that the low-intensity interval training leads to the development of strength endurance in addition to other physical abilities, which was applied in the aquatic [4], The researchers believe that developing a variable endurance force for the working muscles of the body and assisting in performance is one of the basic pillars to achieve the effectiveness of running (800 m). Similar to competition exercises, and this was confirmed by (Mohamed Abdel Hassan) that strength endurance exercises are primarily done through competition exercises or special exercises where the conditions of external forces are difficult by linking them to competition exercises such as running in a difficult field such as water medium, sand or clay ground[5].

As for the achievement test for the effectiveness of running (800m), the results indicated that there are significant differences between the experimental and control groups in favor of the experimental, and the researchers attribute this to the development of speed endurance and strength endurance for the players and thus led to the development of achievement as it appeared in the dimensional results of the tests as there is a high correlation between The evolution of endurance speed and endurance force and the achievement of running (800m), as this is one of the most important abilities that he focuses on in training (800m), which means maintaining speed with a high intensity and sufficiently for the length of the period of performance of physical effort by resisting fatigue, and this is what was focused on in the exercises, as its development had an impact on improving achievement and this is what (David) confirmed that these variables are among the important pillars of running (800m) if they are used regularly and the intensity is high and the rest is short and increases when approaching the stage of competitions, (Shaker Al-Sheikhly) proved that there is a very high correlation between the development of speed endurance, endurance of force and the achievement of running (800m)[6].

## Conclusions

1-Exercising in the aquatic had a significant impact on the development of special physical abilities through the results obtained.

2- The training in the aquatic had an effective effect on the development of special endurance (endurance of strength and endurance of speed) and had a positive effect on the achievement of the (800m) race for youth.

## Recommendations

1- Encouraging the trainers to use the water medium as a training method that was not used in the (800m) game because of its effective role in the development of special endurance.

2- Conducting similar studies using the water medium as an unused training method in the field of middle-distance running training to develop physical abilities and special endurance (strength endurance and speed endurance).

**Financial Disclosure**: There is no financial disclosure.

**Conflict of Interest**: None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Faculty of Physical Education and Sports Sciences and all experiments were carried out following approved guidelines.

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# Association of Adipoq +45 T>G Gene Polymorphism with Insulin Resistance and Icam-1 Value in Obese Adolescents

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## Abstract

**Background:** Obesity is defined as the accumulation of excess body fat tissue which harms health, such as cardiovascular disease, metabolic syndrome, diabetes mellitus type 2 and dyslipidemia. Lower adiponectin levels are strongly associated with overweight, obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular risk factors in adulthood. ADIPOQ +45 T>G gene polymorphism is one of a genetic variations that affect plasma adiponectin levels.

**Methods:** A cross-sectional study of 180 obese adolescents aged 13-18 years in Surabaya and Sidoarjo. Obesity is defined as body mass index (BMI)>p95 on the BMI for age curve according to Centers for Disease Control and Prevention (CDC) 2000. Blood samples were taken for ICAM-1 examination using the ELISA method, HOMA-IR with fasting insulin and glucose calculations, and ADIPOQ + 5 T>G gene polymorphisms by PCR-RFLP. Data were analyzed using the Chi-square, Mann-Whitney, and T-test using SPSS version 21, p-value<0.05 was considered significant.

**Results:** There was a significant difference in the mean BMI by age group (p <0.001). There was no statistically significant difference between the median BMI in the three groups (wild, mutant homozygote, and mutant heterozygote) of genotype distributions. ADIPOQ +45 T>G gene polymorphisms occur with the same frequency in both males and females. There was no association between the genotype distribution of the ADIPOQ +45 T>G gene polymorphism with insulin resistance and ICAM-1 value, but the ICAM-1 value was found to be higher in the ≥ 15 years age group. There was no association between insulin resistance and ICAM-1 values.

**Conclusion:** There was no association between ADIPOQ + 45 T>G gene polymorphisms with insulin resistance and ICAM-1 values in obese adolescents. There was no association between insulin resistance and ICAM-1 values.

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***Keywords:*** *obese adolescents, ADIPOQ +45 T>G gene polymorphism, HOMA-IR, insulin resistance, ICAM-1*

## Introduction

Obesity is defined as the accumulation of excess body fat tissue which harms health. This condition is more experienced by adolescents and adults. The lifestyle of today's teenagers often skip breakfast and

prefer to consume fast food. and tends to a sedentary lifestyle, making adolescents at risk for obesity.[1] Being overweight and obese are risk factors for several life-long complications, such as cardiovascular disease, metabolic syndrome, type 2 diabetes mellitus, and dyslipidemia.[2] Obese adolescents tend to be obese adults, the condition of obesity will have an impact on metabolism.[3]

Several studies explaining the role of genetics in the pathogenesis of obesity have been conducted. Lower adiponectin levels are strongly associated with overweight, obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular risk factors in adulthood.[4] In children and adolescents, hypoadiponectinemia has been shown to predict obesity, metabolic syndrome, hypertension, insulin resistance, and visceral fat accumulation.[5] Obese adolescents will experience insulin resistance. Research on obese adolescents aged 13-18 years found 78% of children with insulin resistance.[6] Four polymorphisms in ADIPOQ have been studied, two located in the promoter region of the gene (−11391 G>A and −11377 C>G), one in exon 2 (+45 T> G), and one in intron 2 (+276 G>T).[7] In humans, hypoadiponectinemia is associated with lower vasodilator response in diabetic patients, and adiponectin administration increases nitric oxide (NO) production in aortic endothelial cells.[8] Initial stimulation of inflammation, such as a diet high in saturated fatty acids, hypercholesterolemia, obesity, insulin resistance, hypertension, and smoking stimulates adhesion molecules such as P-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular cell adhesion molecule 1 (ICAM- 1) so that monocytes and lymphocytes that are in the bloodstream can stick to the endothelial surface which is an early sign of endothelial dysfunction that can cause cardiovascular abnormalities.[9]

In Indonesia, gene research still has several challenges. The ADIPOQ gene polymorphism in obese adolescents has never been studied. The finding of the relationship between gene polymorphisms and insulin resistance and tilapia ICAM-1 can be used as prevention and management in obese adolescents. This prompted researchers to analyze the relationship between ADIPOQ +45 T>G gene polymorphisms with insulin resistance and ICAM-1 values in obese adolescents.

## Methods

### Data Collection

A cross-sectional study was conducted on 180 obese adolescents in junior and senior high schools in Surabaya and Sidoarjo, East Java, Indonesia in May-September 2020. Subjects were recruited with a total population sampling method that eligible for the inclusion and exclusion criteria. The inclusion criteria in this study were adolescents aged 13-18 years with obesity, as well as both parents and subjects who agreed to participate in this study. Adolescents with a history of corticosteroid consumption for more than two months up to a period of 6 months before the study took place or subjects who were in a sick condition, alcohol and smoking consumption, have diabetes mellitus were excluded. Measurement of body weight is performed with digital weight scale Seca, Germany with a precision of 0.1kg. Height measurement was performed using stadiometers Seca, Germany with an accuracy of 0.1cm. The subjects had been fasting for 12 hours before the blood samples were taken (plasma insulin and plasma glucose). ICAM-1 examination was carried out in the laboratory of the Institute Tropical Diseases (ITD) Airlangga University with the Human intercellular adhesion molecule 1 Elisa kit from the Bioassay Technology Laboratory. Analysis of ADIPOQ +45T>G is using polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method.

### Definitions

Obesity is defined as BMI>p95 on the BMI for age curve according to CDC 2000. Insulin resistance was calculated using the Homeostatic model assessment for insulin resistance (HOMA-IR) with the formula insulin (μU/mL) x glucose (mg/dL) /405) and defined if HOMA-IR ≥ 3.4.[10] The cut-off reference value used the median ICAM-1 in obese children (284.4 ng / ml) for male and female sex.[11]

## Statistical Analysis

Some data were reported as mean, standard deviation (SD), and median. The association of gene polymorphism, insulin resistance, and ICAM-1 value were calculated using IBM-SPSS statistics version 21.0.

## Result and Discussion

This study is an analytic observational study with a cross-sectional approach to obese adolescents in secondary schools in Surabaya and Sidoarjo. The total of subjects was 180 students.

**Table 1 Age and Sex Distribution**

| Base Characteristics | n | % |
|---|---|---|
| Age | | |
| 13-<15 | 77 | 42,80 |
| ≥15-18 | 103 | 57,20 |
| Sex on age 13-<15 | | |
| Male | 44 | 57,10 |
| Female | 33 | 42,90 |
| Total | 77 | |
| Sex on age ≥15-18 | | |
| Male | 61 | 59,20 |
| Female | 42 | 40,80 |
| Total | 103 | |

The sample of this study was more in adolescents aged ≥15-18 years than in the 13- <15 years age group. The largest sample was found in males in both age groups. These findings were similar to studies on the prevalence of overweight and obesity in Argentine adolescents with a mean age of 15 reported 10.9% and 2.2%, respectively, with a higher prevalence in males than females.[12]

The reason of male adolescents are more obese than female is that adolescent male consume more calories than the recommended calorie intake per day, consume more sugary drinks, and spend more time watching television than girls. Teenage girls tend to feel fat, so they make more weight loss efforts through diet patterns.[13]

**Table 2 Status Data of Weight, Height, IMT, Blood Sugar, Insulin, HOMA-IR, and ICAM-1 based on Ages**

| Variables | Age 13-<15 years | | | Age ≥15-18 years | | | p |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Median (min-max) | Mean | SD | Median (min-max) | |
| Weight (kg) | 78,11 | 12,37 | 77,90 | 89,67 | 13,26 | 88,60 | *<0,05 |
| Height(cm) | 160,95 | 161,50 | 161,50 | 160,93 | 10,35 | 161,00 | *0,98 |
| BMI | 30,17 | 3,58 | 29,80 | 34,48 | 4,45 | 33,56 | **<0,05 |
| Blood sugar | 87,74 | 7,30 | 85 | 85,77 | 6,04 | 85 | **0,09 |
| Insulin | 25,53 | 17,93 | 20,33 | 21,82 | 9,54 | 19,45 | **0,71 |
| HOMA-IR | 5,52 | 3,88 | 4,32 | 5,52 | 3,88 | 4,32 | **0,55 |
| ICAM-1 | 600,75 | 474,47 | 568 | 662,53 | 459,05 | 663 | **0,04 |

*T-*test* , **Mann-Whitney *test*

Table 2 shows a significant difference between body weight and BMI with the sample age group. The age group ≥15-18 years is greater than 13- <15 years. There was a significant result of ICAM-1 score in the age group. The ICAM-1 score in the ≥15-18 years group was greater. There was insignificant result between blood sugar, HOMA-IR, and ICAM-1 insulin values with age groups.

**Table 3 Status Data of Weight, Height, IMT, Blood Sugar, Insulin, HOMA-IR, and ICAM-1 based on sex**

| Variables | Male | | | Female | | | p |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Median | Mean | SD | Median | |
| Weight (kg) | 87,25 | 14,91 | 86,80 | 81,19 | 12,03 | 80,50 | *<0,05 |
| Height (cm) | 163,98 | 7,90 | 163,50 | 156,68 | 9,18 | 157 | *<0,05 |
| BMI | 32,43 | 4,39 | 31,40 | 32,92 | 4,93 | 31,60 | **0,62 |
| Blood sugar | 87,03 | 6,36 | 85 | 86,02 | 7,07 | 84 | **0,15 |
| Insulin | 23,18 | 14,02 | 19,57 | 23,72 | 13,68 | 19,66 | **0,71 |
| HOMA-IR | 4,99 | 3,11 | 4,19 | 5,04 | 2,89 | 4,32 | **0,76 |
| ICAM-1 | 592,81 | 458,50 | 560 | 696,72 | 471,29 | 656 | **0,06 |

*T-*test* , **Mann-Whitney *test*

In table 3 there is a significant difference between body weight and height for gender. The weight and height of the male sex are greater than female in obese adolescents. BMI, blood sugar, insulin, HOMA-IR, and ICAM-1 were insignificant.

The difference in the mean BMI between the age groups in this study was influenced by sexual maturity which generally differed in each group, besides that it could be due to changes in dietary patterns with age. In children and adolescents who are still developing, BMI is interpreted differently from adults. BMI changes with age and sex, as body weight and height increase. Besides, other factors such as ethnicity, social, and culture also affect obesity.

**Table 4. Gene ADIPOQ +45T>G Polymorphism Genotype Distribution Analysis Based on Sex**

| Genotype Distribution | n(%) | Sex | | |
|---|---|---|---|---|
| | | M | F | |
| *Wild type* (TT) | 111(61,70) | 65 | 46 | *p=1,00 |
| Mutant *heterozygote* (TG) | 62 (34,4) | 36 | 26 | |
| Mutant *homozygote* (GG) | 7 (3,9) | 4 | 3 | |

* Chi-square

The relationship of ADIPOQ + 45T> G gene polymorphisms and sex is shown in table 4. The results of the analysis had no relationship between genotype distribution and sex. Analysis of correlation data between alleles and sex is shown in table 5. There was no relationship between allele distribution and sex.

In this study, heterozygote and homozygote mutants were found in some obese children. There was no significant difference between sex, body mass index, and age group on the genotype distribution of ADIPOQ +45T>G gene polymorphisms. This is also similar to the research of Kasap et al. there was no significant difference in each adiponectin genotype on obesity. There was also no relationship between the polymorphism of the +45 T>G SNP gene from the adiponectin gene and obesity in children and its complications.[14] In this study, we did not group ethnically that may made bias in the result.

**Table 5. Correlation Analysis of Polymorphism ADIPOQ +45T>G and HOMA-IR**

| Genotype Distribution | HOMA-IR | | p |
|---|---|---|---|
| | Insulin Resistance | Non-resistance insulin | |
| Wild | 77 | 34 | *0,74 |
| Mutant | 46 | 23 | |

*Chi-square

Table 5 shows no correlation between the distribution of ADIPOQ +45 T>G gene polymorphisms and the HOMA-IR value in obese adolescents. In this study, there was no significant difference between the HOMA-IR values based on gender, age group, and the distribution of the genotype of the ADIPOQ + 45T> G gene polymorphism. Similar to this study, several previous studies did not find a relationship between ADIPOQ + 45T> G gene polymorphisms and HOMA-IR.[15–17]

Some studies conclude that the G allele in + 45T> G SNP of the adiponectin gene is associated with decreased fasting insulin levels and lower HOMA-IR scores.[7,18] In another study, adiponectin concentrations were positively correlated with insulin sensitivity and significantly decreased with worsening glucose tolerance in Pima Indians and Caucasians.[19]

**Table 6. Correlation Analysis of Gene Polymorphism ADIPOQ+45T>G and ICAM-1**

| Genotype Distribution | ICAM-1 | | p |
|---|---|---|---|
| | Normal | High | |
| Wild | 30 | 81 | *0,60 |
| Mutant | 16 | 53 | |

*Chi-square

Table 6 shows that there is no correlation between the genotype distribution of the ADIPOQ + 45T> G gene polymorphism and the ICAM-1 value in obese adolescents. Intercellular adhesion molecule-1 / ICAM-1 is a member of the immunoglobulin superfamily and is expressed on a wide variety of cells under inflammatory conditions. One of the functions of ICAM-1 is the leukocyte adhesion receptor in response to inflammatory stimuli, usually expressed on the surface of endothelial cells as a mediator for transferring leukocytes to tissues.[20]

In previous study of sICAM-1 (Serum ICAM-1) levels were analyzed in the serum of obese mice due to a long-term (6 months) high-fat diet. The mean level of sICAM-1 was higher in male rats than in female rats ($p < 0.05$). When associated with body weight, sICAM-1 levels increased by an average of 10 mg/ml for every 10g of body weight gain, with a correlation of $r = 0.50$ ($P < 0.001$). This relationship did not differ statistically between men and women.[21] It is consistent with human studies, that elevated levels of sICAM-1 have been associated with several pathological conditions, including obesity and its complications.

Several human studies have also shown that sICAM-1 levels are also increased in obesity, and are positively correlated with central obesity[22] and insulin resistance.[23,24] The difference in the results of this study was due to the endothelial inflammatory process in adolescents which did not occur at the time of examination but could arise over time. Also, adolescents are still classified as active in physical activity which is a factor in increasing insulin sensitivity to suppress the inflammatory process in the endothelium, but in this study, physical activity factors were not recorded and analyzed.

**Table 7. Correlation between HOMA-IR and ICAM-1 groups**

| HOMA_IR | ICAM-1 | | p |
|---|---|---|---|
| | normal | high | |
| Non-insulin resistance | 17 | 40 | *0,46 |
| Insulin Resistance | 29 | 94 | |

*Chi-square

In Table 7, there is no correlation between the HOMA-IR and ICAM-1 groups in obese adolescents. Insulin resistance has been recognized as an independent risk factor for cardiovascular disease by causing dyslipidemia.[25] Besides, insulin resistance is often associated with metabolic syndrome, whose components are central obesity, high blood pressure, high triglycerides, low HDL, and impaired fasting plasma glucose.[26]

Obese children who are resistant to insulin have a significantly greater risk of cardiovascular disease. Insulin resistance in childhood predicts cardiovascular risk.[27] Insulin resistance in early vascular smooth muscle abnormalities can be seen with markers of endothelial dysfunction (ICAM and E-selectin).[28]

There was an association between ICAM-1 and insulin resistance found ($p < 0.0001$) in other studies that were consistent with clinical evidence related to insulin resistance and inflammation.[23] No studies have directly measured in vivo insulin sensitivity and its association with atherosclerotic abnormalities in children. Very limited observation shows an association between HOMA-IR, arterial stiffness, and fasting insulin levels

in children.[29]

The difference in this study could be due to differences in the classification of insulin resistance using the HOMA-IR value with different cut-off values, while other studies have used FSIVGTT so that it affects the results obtained. Also, the physical activity factor of the sample was not analyzed in this study so that it could be the one that affects the results on insulin and ICAM-1 values.

## Conclusion

There is no association between ADIPOQ +45T>G gene polymorphisms to insulin resistance and ICAM-1 value. No association of insulin resistance and ICAM-1 values. If further research is carried out, it is preferable to add non-obese subjects to the study sample for control. Physical activity and race of each research subject are recorded and analyzed on the research results.

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# Hemorrhagic Varicella in a 41-Year-Old Woman with Evans Syndrome : Case Report

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## Abstract

**Background:** Varicella is an infection caused by the *varicella-zoster virus* (VZV) with symptoms of an exanthematous vesicular rash and systemic symptoms. Hemorrhagic varicella commonly seen in immunocompromised patients. Evans syndrome (ES) is an autoimmune with two or more cytopenias,including autoimmune hemolytic anemia (AIHA) and immune thrombocytopenia (ITP).

**Case:** 41-year-old woman complains of swelling filled with reddish fluid almost all over her body,sometimes painful and accompanied by fever. Patient also experienced vaginal bleeding resulting in anemia (Hb 8.8 g/DL) and thrombocytopenia (platelets 34,000/uL). Dermatological status of the generalized,multiple hemorrhagic vesicles with an erythematous base,partially ruptured. Tzank test revealed multinucleated giant cells. Patient suffered AIHA and received therapy with methylprednisolone 4 mg/day and mycophenolic acid 2x500 mg/day.

**Conclusion:** We report a case of hemorrhagic varicella in a 41-year-old woman with Evan's syndrome with concurrent features of AIHA and ITP. Dermatological status of the generalized, multiple hemorrhagic vesicles with an erythematous base,some of ruptured with erosions. Tzank test revealed multinucleated giant cells. Patient was treated with acyclovir 5x800 mg for 7 days,2% salicylic acid and 0.5% menthol applied every 12 hours and mupirocin 2% ointment applied twice a day on the erosion area and clinical improvement was found after 17 days of therapy.

***Key words:*** *Acyclovir, Evans Syndrome, Tzank test, Haemorrhagic Varicella*

## Introduction

Varicella is an infection caused by *Varicella-zoster virus* (VZV) with symptoms of an exanthematous vesicular rash and highly contagious. Varicella is more common in children, but can also occur in adults or in certain conditions like immunocompromised.[1] Immunocompromised patients may develop severe complications such as hemorrhagic varicella, encephalitis, pneumonitis and thrombocytopenia.[2] Evans syndrome is an autoimmune condition that presents with AIHA and/or ITP with or without immune neutropenia. Evans syndrome was first reported in 1951 and often accompanies varicella.[3]

Sharma et al. reported the incidence of hemorrhagic varicella worldwide was estimated at 3.3% of the total varicella cases with an increase in mortality of 7-10%.[4]

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Wu et al. reported that there are 2,794 varicella cases in the period 2008 -2017.[5] Sondakh et al. reported that there were 77 varicella cases occurred in 2010-2015 at Cipto Mangunkusumo Hospital Jakarta.[6] Incidence of Evans Syndrome is quite rare found. Mannering et al. reported that the incidence of Evans Syndrome in the world ranged from 0.5-1.2/1,000,000 people per year.[7] Tanaka et al. reported the occurrence of 8 cases of Evans Syndrome associated with varicella infection.[8]

Clinical manifestations of hemorrhagic varicella can appear all over the body. The clinical manifestations of Evans syndrome are associated with anemia and thrombocytopenia which include pallor, weakness, fatigue, jaundice, petechiae, ecchymosis, bleeding gums, epistaxis and other bleeding.[9] The diagnosis of VZV is made based on the clinical picture, Tzanck test, polymerase chain reaction (PCR), or enzyme-linked immunosorbent assay (ELISA).[9] The diagnosis of Evans syndrome can be established by a Coomb's test which is positive for hemolytic anemia.[9] Differential diagnoses of varicella include herpes zoster and disseminated herpes zoster.[10]

Hemorrhagic varicella therapy is acyclovir 5x800 mg/24 hours which is useful for reducing the rash, constitutional symptoms and inhibiting viral replication.[11] There are no specific guidelines for the management of Evans Syndrome, it's just symptomatic therapy with corticosteroids and intravenous immunoglobulin in severe cases.[9]

The purpose of this case report is to know that varicella can show atypical clinical manifestations in the form of hemorrhagic varicella, association with AIHA and Evans syndrome.

Case

Patient Mrs. H, 41 years old, came with swelling filled with reddish fluid in several parts of the body. Complaints appeared since 4 days ago in the form of blisters around the groin and itching, which then spread to body and face. She also felt weak one day later accompanied by fever and difficulty sleeping. Patient has a history of AIHA since 4 years ago.

The patient then went to a general practitioner and was advised to do laboratory tests. The results showed a hemoglobin value of 4.6 g/dl (14.0-17.5 g/dl), then the doctor recommended blood transfusion, but after blood transfusion the hemoglobin level became lower, 2.9 g/dl. The patient was consulted to an internal medicine specialist and diagnosed with AIHA, received oral methylprednisolone therapy 4 mg/day and cellcept® (mycophenolate mofetil) 500 mg orally twice daily routinely. The patient was declared cured in April 2018 and oral medication was discontinued, but in December 2018 there was vaginal bleeding of approximately 500 ml. Patient experienced a clinical deterioration, so she was hospitalized again at the PKU Muhammadiyah Surakarta Hospital for five days and from the laboratory result, it was found that platelets were 30,000 gr/dl (150,000 - 450,000 gr/dl). Patient underwent a Coomb's test and the results were positive, then she was routinely monitored and received Cellcept® (mycophenolate mofetil) 500 mg orally 2 times a day. Patient had no history of allergies, atopy, hypertension or diabetes mellitus.




**Figure 1. A-J. The generalized region showed multiple polymorphic hemorrhagic vesicles with an erythematous base and varied in size with a diameter of 0.2-0.5 cm which were partially eroded and covered with blackish crusts.**

Physical examination showed that the patient was seriously ill with normal vital signs and a pain scale of 8. Dermatological status in the generalized region showed multiple polymorphic hemorrhagic vesicles with erythematous base with varying diameter of 0.2-0.5 cm and some had erosions covered with black crusts (Figure1). Patient was diagnosed with hemorrhagic varicella and differentially diagnosed with disseminated

herpes zoster based on the history and physical examination.

The Tzanck test showed multinucleated giant cells (Figure 2). Laboratory result showed anemia with a hemoglobin level of 8.8g/dL (14.0-17.5g/dL), leukocytosis with a result of 15,800/µL (4,500-14,500/µL) and thrombocytopenia with a result of 34,000/µL (150,000-450,000/µL), while other examination results were within normal limits. Based on the results of blood chemistry examination, there was an increase in SGOT and SGPT values, the SGOT value reached 361u/l (<35u/L), while the SGPT value reached 804u/l (<45u/L). The urinalysis showed macroscopic examination results pH 5.0 (5.0-7.5), leukocytes 500/uL (negative), protein +2 (negative), urobilinogen +2 (normal), bilirubin +1 (negative) and erythrocytes +2 (negative). , while microscopic examination showed leukocytes 63.6/lpb (0-12/lpb), crystals 5.2/uL (0.0/uL), yeast like cells 226.6/uL (0.0/uL) and erythrocytes 40-42/lpb (0- 8.7/lpb). There was an increase in LDH levels of 2243 u/l (210-425 u/l), reticulocyte levels of 2.06% (0.5-1.5%), total bilirubin levels of 1.41 mg/dl (0.2-1.2 mg/dl) with a direct bilirubin value of 0.77 mg/dl. dl (0-0.4 mg/dl) and indirect bilirubin 0.64 mg/dl (0.3-1.1 mg/dl) and PT and APTT were within normal limits.

The patient was hospitalized and treated together with internal medicine and anesthesia for the management of Evans Syndrome and pain complaints. Based on the history, physical examination and supporting examination, our patient was diagnosed with hemorrhagic varicella. The patient was treated with oral acyclovir 800 mg every 4 hours for 7 days, 2% salicylic acid cream + 0.5% menthol topical every 12 hours applied to the lesion of the vesicle, mupirocin ointment applied to the area of erosion every 12 hours and paracetamol 500 mg given if the patient had a fever. The patient was managed by the internal medicine department with injection of methylprednisolone 250 mg 24 hours, levofloxacin injection 750 mg 24 hours, cetirizine oral tablet 10 mg every 24 hours, folic acid tablet 1 mg oral 24 hours and calvit D (Ca hydrogen phosphate 500 mg, cholecalciferol 133 IU) tablet orall y every 12 hours. Anesthesia department provided additional therapy in the form of injection of morphine 680 mcg per 24 hours for pain relief.

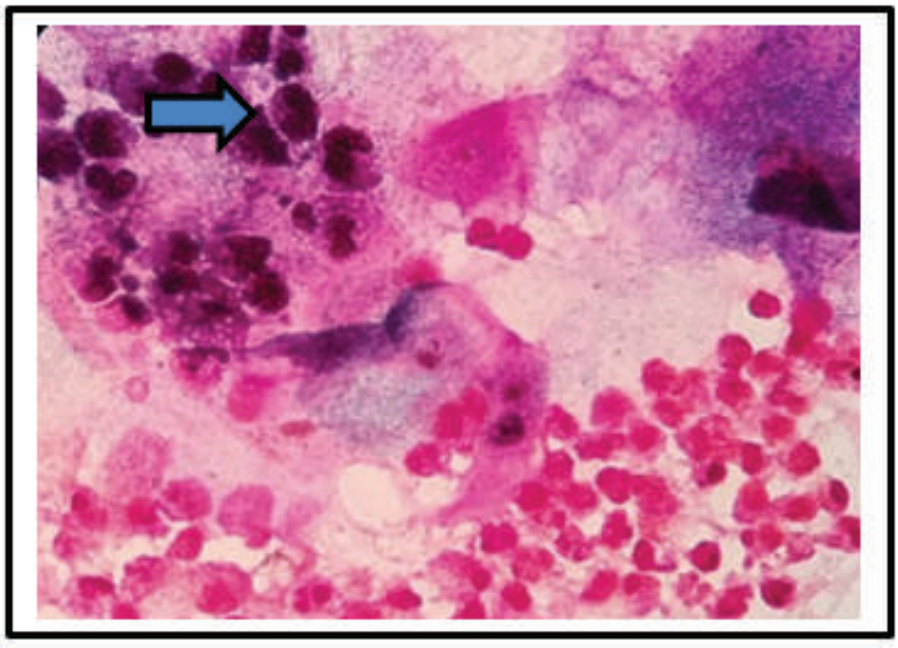

**Figure 2. In Tzanck's smear, multinucleated giant cells were found (blue arrow).**

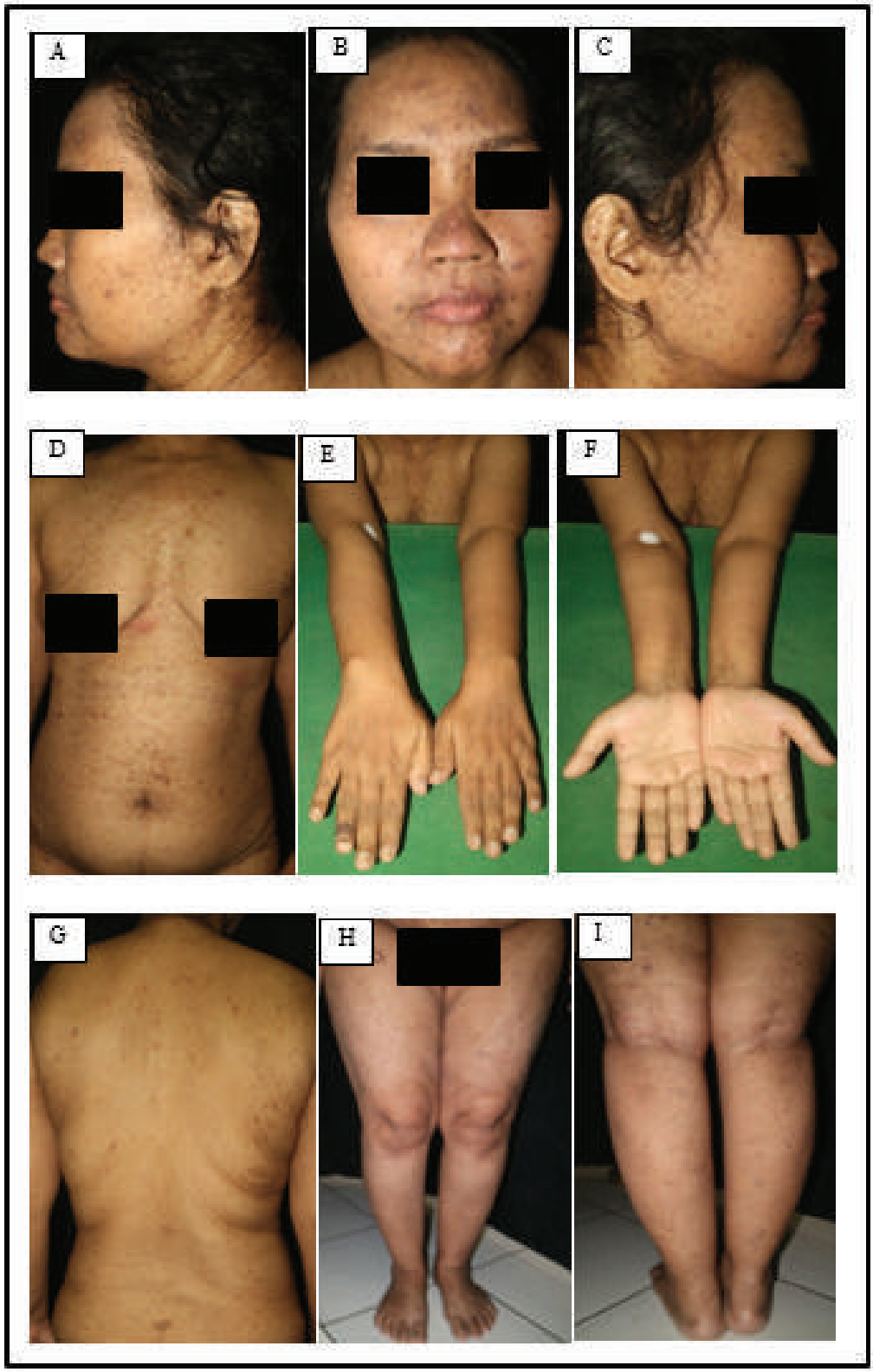


**Figure 3. A-I. Day 17 after therapy. The generalized region shows macules and multiple discrete hyperpigmented patches.**

## Discussion

Varicella is caused by primary infection of VZV. Varicella is a self-limiting disease, but it can be life-threatening. Varicella is a highly contagious disease. Varicella spreads through respiratory tract and direct contact with lesions. The incubation period for varicella ranges from 10-21 days with an average incubation period of 14 days.[4,9] Evans syndrome is a rare condition that is a continuation of ITP and AIHA with the result positive Coomb's test of unknown etiology. Evans syndrome mostly occurs at a young age, more often in women than men, age range of 23-50 years.[9]

Varicella in immunocompromised patients may have atypical manifestations in the form of hemorrhagic varicella.[4] Patients with Evans syndrome have main symptom, namely thrombocytopenia. The pathological process of thrombocytopenia is the formation of autoantibodies that react with platelet surface antigens. If these autoantibodies bind to platelets, they will cause platelets to exit the circulation through phagocytosis by the reticuloendothelial system. This results in shortened platelet lifespan resulting in thrombocytopenia.[9] Hemorrhagic varicella is associated with thrombocytopenia where vesicular eruptions in varicella turn into hemorrhagic vesicles if the patient suffers from thrombocytopenia.[4]

Clinical manifestations of varicella begin with prodromal symptoms followed by the appearance of skin lesions. Varicella skin lesions begin with the appearance of macules that develop into papules, vesicles, pustules, and crusts.[12,14] Vesicles in varicella are round-shaped oval, filled with clear fluid, and overlying erythematous skin. The vesicle fluid can turn into pustules, then rupture and dry into crusts. The skin rash is dominant on the trunk and head region which then spreads to the extremities centrifugally. At first, blisters appeared around the groin that felt itchy, then appeared vesicles in the groin area, body and face accompanied by fever. Dermatological status of patient in the generalized region showed multiple polymorphic hemorrhagic vesicles with an erythematous base and varying diameter of 0.2-0.5 cm, some were eroded and covered with black crusts. This clinical picture is consistent with that described in the literature.

The diagnosis of varicella is made based on the clinical manifestation which is reinforced by a history of contact with varicella sufferers within a period of 2-3 weeks.[9] Tzanck test that shows multinucleated giant cells will confirm the diagnosis.[12] In this case, the Tzanck test results from a new vesicle lesion on the patient's right arm revealed multinucleated giant cells. The Tzank test of the patient is consistent with the literature.

The diagnosis of Evans Syndrome is made based on clinical manifestations and anamnesis which include a history of disease progression, family history of autoimmune disorders and exclude risk factors that may predispose infection, malignancy, autoimmune disease, vaccination history and drug use. The physical examination focused on signs of anemia or thrombocytopenia. Supportive laboratory examinations such as complete blood count, peripheral blood test will show anemia, thrombocytopenia, reticulocytosis, poikilocytosis, especially due to the presence of spherocytes. Elevated indirect bilirubin and lactate dehydrogenase may also be found. Positive results from the Coombs test indicate ongoing immune hemolysis.[3,9] In this case, the clinical picture of vaginal bleeding found indicated the presence of thrombocytopenia which was in line with the laboratory results obtained, namely a platelet count of 34,000/µL (150,000-450,000). /L). The clinical manifestation of pallor and weakness which is a symptom of anemia is also found and hemoglobin level was 8.8g/dL. This patient exhibited concurrent clinical features of AIHA and ITP suggestive of mixed-type Evans syndrome. The lab result showed an increase in LDH, namely 2243u/l (210-425u/l), Reticulocyte levels 2.06% (0.5-1.5%), PT and APTT were within normal limits. The definitive diagnosis of Evans Syndrome is related to a positive Coomb's test for hemolytic anemia.[13] In this case, a positive Coomb's test result, physical examination, and laboratory finding was used as the basis for diagnosing mixed type Evans Syndrome.

The differential diagnosis in this case was disseminated herpes zoster (HZD). The clinical picture of HZD is the initial lesion in the form of herpes zoster localized in one or several dermatomes in 90% of cases which then in 1-12 days will spread generally. This is the main difference between disseminated herpes zoster and varicella that occurs in adulthood. In this case, the initial lesion of herpes zoster was not localized in dermatome, although the Tzanck test revealed multinucleated giant cells, so the diagnosis of disseminated herpes zoster could be ruled out.[1,10]

The first-line therapy for VZV is acyclovir. The dose for adult patients is 20 mg/kg with a maximum dose of 800 mg 5 times a day. Antiviral aims to stop the formation of new lesions and shorten the duration of rash, fever and constitutional symptoms. Immunocompromised patients are recommended to be given acyclovir injection therapy at a dose of 10-15 mg/kg given every 8 hours intravenously for 7 days.[12] In some cases that do not respond well to acyclovir, other therapeutic options can be given, namely brivudine, foscarnet, or vidarabin.[15] Topical therapy is symptomatic therapy and can be given depending on the stage of the disease. Powdering can be applied to vesicle lesions and to erosion, topical antibiotics can be given to prevent secondary infection. Antipyretics are sometimes needed if there are complaints of fever.[12]

An exact algorithm for Evans Syndrome treatment has not been postulated to date. Some literature mentions that the first-line therapy is corticosteroids. In acute life-threatening conditions, packed red cell (PRC) transfusions can be given. Intravenous immunoglobulin (IVIG) may be given in cases with thrombocytopenia as the main laboratory finding in these cases. We treated this patient with an internist and anesthesiologist. Our management is oral acyclovir 800 mg every 4 hours for 14 days and administration of 2% salicylic acid cream + 0.5% menthol every 12 hours for vesicular lesions and mupirocin ointment for erosion areas every 12 hours and additional 500 mg paracetamol is given if needed. Management given by internal medicine colleagues is injection of methylprednisolone 250 mg per 24 hours, injection of levofloxacin 750 mg per 24 hours, cetirizine oral tablet 10 mg per 24 hours, folic acid tablet oral 1 mg per 24 hours, calvit D (ca hydrogen phosphate) 500 mg, cholecalciferol 133 IU) tablet orally every 12 hours, while an anesthesiologist friend gave morphine injection therapy 680 mcg per 24 hours to treat pain in patients.

The patient was allowed to go home by the internal medicine department after the tenth day of treatment in an improved condition. The patient was re-controlled to the skin clinic after one week after hospitalization with the condition of the lesions having improved with the remaining lesions in the form of hyperpigmented macules and plaques and erosion covered by blackish crusts in some areas. (Figure 3)

## Conclusion

In this paper, we report a case of hemorrhagic varicella in a 41-year-old woman with Evans syndrome. The diagnosis is based on history, physical examination and investigations. Autoanamnesis, there were complaints of watery splinting which began with the appearance of abrasions in the groin area since 4 days before admission to the hospital. Physical examination revealed that the dermatological status in the generalized region showed multiple polymorphic efflorescence of hemorrhagic vesicles with an erythematous base and varied sizes (0.2-0.5 cm in diameter), some of which had erosions covered with black crusts. The results of the Tzanck test revealed multinucleated giant cells. The patient was treated with oral acyclovir 800 mg every 4 hours, 2% salicylic acid + menthol 0.5% topical every 12 hours for vesicular lesions, mupirocin ointment for eroded areas every 12 hours and additional 500 mg paracetamol was given if the patient developed a fever. The prognosis for this patient is dubia. The control patient one week later with the condition of the lesion has improved.

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# Estimation of Interleukin-10 and Interleukin-22 Levels in the Advances of Breast Cancer

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## Abstract

**Background:** Breast cancer is the secant-kind of carcinoma in women with higher incidence in Iraq. There are many prognostic and predicitive factors used for management of the breast cancer. Serum levels of the cytokines may be utilized as a marker of immunity status and prognosis in CA breast.

**Aims of the Study:** Measuring the IL-10 and IL-22 in breast cancer patients and association IL-10 and IL-22 with stage and grade for breast cancer. Materials and Methods: The case-control study was conducted on 60 women with CA. Breast and 60 controls group, patients with CA. Breast were referred to Middle Euphrates cancer center in Najaf during the period November 2019-October 2020. Measurement of Interleukin-10 and Interleukin-22 by using ELISA. Results: The showed a levels of IL-10 and IL-22 in breast cancer group higher than levels of IL-10 and IL-22 in control, and showed a high levels of IL-22 in advances stage and grade III. The showed non-significant in IL-10 levels between four stages and three grade. Conclusions: A significant elevation levels of IL-10 and IL-22 in breast cancer groups comparison with control, elevation significant IL-22 levels in stages and grades of breast cancer.

***Keywords:*** *IL-10, IL-22, Breast cancer, ELISA.*

## Introduction

Breast cancer is a form of malignancy caused by abnormal growth and unregulated division of cells within the terminal and lobular units of breast that can infiltrate and kill the surrounding normal tissue. as well as spread across the body by blood or lymph fluid to new locations[1,2]. It is the most-frequent malignant disease and the leading cause of death from cancer among women worldwide[3]. Breast cancer is the most-common cancer for women around the world, accounting for 25% of all cases[4]. In 2018 it resulted in two million new cases and 627,000 deaths[5]. Breast cancer division splits breast cancer into divisions based on a variety of factors that has a specific reason. The histopathological type, tumour grade, tumour stage, and protein and gene expression are the most important categories[6]. As the cells lose the characteristics found in typical breast cells, pathologists identify them as well differentiated(low-grade), moderately differentiated(intermediate-grade), and poorly differentiated(high-grade).Cancers that are poorly differentiated have a poor prognosis[7,8]. The current breast cancer staging schemes are dependent on the clinical size and degree of invasion of the primary tumour(T), the clinical absence or presence of palpable axillary lymph nodes and signs of local invasion(N), as well as clinical and imaging proof of distant metastases(M)[9].

IL-10 is produced by TH0,TH2, cytotoxic T cells, Treg, γδ-T cells, NK cells, NK T cells, B cells, dendritic cells,mast cells, and activated monocytes. It was originally known as the cytokine synthesis inhibitory factor because of its capacity to inhibit the production

of certain cytokines. IL-10 is the most studied and well-known anti-inflammatory cytokine[10]. Interleukin-10, that plays important coordinated role in breast cancer[11], which regulates immune response[12] and inhibits proinflammatory roles of antigen-presenting cells by expressing antagonising costimulatory molecules. Its low expression is linked with poor survival outcome[13].

Interleukin-22 is a type of cytokine that has a-helical structure. IL-22 binds to a cell surface receptor that is composed of two subunits:IL-10R2 and IL-22R1[14]. Elevated expression of IL-22 has been observed in many human tumours, including breast[15]. However, anticancer effects of IL-22 have been reported in cancer, where it slows cancer cell growth by arresting the G2/M cell cycle, resulting in reduced cell proliferation and tumour weight[16]. There was also a good positive association with IL-22, linked to a high grade. That IL-22 was upregulated in serum and tissues of BC patients and that this was linked to clinical stages[17].

## Materials and Methods

The case-control study was conducted on 60 women with CA. Breast and 60 women of controls group. Patients with CA. Breast were referred to Middle Euphrates cancer center in Najaf during the period November-2019 to October-2020..Some information were gathered from each woman such as grade and stage. The distribution of patients according to these criteria was shown in(Table1). Three ml of serum samples were taken from patients. All sera were stored at -20ºC for future immunological analysis. Measurement of cytokines(Interleukin-10 and Interleukin-22) by using ELISA

**Table(1): Breast cancer patients and criteria used in this study**

| Criteria | No. CA. Breast patients(60) |
|---|---|
| Tumor stage | |
| I | 12 |
| II | 19 |
| III | 18 |
| IV | 11 |
| **Histological grade** | |
| G1 | 17 |
| G2 | 25 |
| G3 | 18 |

## Statistical-Analysis

The statistical significance was done by using SPSS version 17.The ANOVA test was used to determine the statistical significance of the difference in mean between more two groups. Z-test was also used to differences between two groups. P-value less than 0.05 level of significance was considered statistically significant[18].

## Results

### Mensuration Mean of IL-10 and IL-22 in Groups

The present results revealed high significant differences(P<0.001) in mean of IL-10 in patients of breast cancer compared with control group. In addition to that showed high significant differences(P<0.001) in mean of IL-22 in CA Breast patients compared with control group as shown Table(2).

**Table(2): Comparison the mean of IL-10 and IL-22 between groups**

| Interleukins | Breast Cancer Group | Healthy Control Group | P-value |
|---|---|---|---|
| | | | |
| IL-10 pg/L M±SD | *186.3±39.5 | 91.8±30.6 | 0.0001 |
| IL-22 ng/L M±SD | *187.1±54.7 | 82.6±28.7 | 0.0001 |

Evaluation the Mean of IL-10 and IL-22 based on Cancer Stages

In regard to the tumor stages, there no significant differences in mean of IL-10 between in stages patient of CA. Breast group. The highest significant differences(P<0.01) in mean of IL-22 patients of breast cancer compared between stages in group, the highest IL-22 levels in stage II, III and IV than in stage I, the mean of IL-22 levels was higher in advance stages(stage IV and III) of breast cancer as shown Table(3).

**Table(3):The IL-10 and IL-22 mean in stages of CA. Breast patients**

| Stages | IL-10 pg/L M±SD | IL-22 ng/L M±SD |
|---|---|---|
| Stage I | 180.3±34 | 137.6±49.1 |
| Stage II | 176.5±42 | *181.1±48.1 |
| Stage III | 193.3±28.5 | **195.3±44.9 |
| Stage IV | 198.3±54.1 | ***239±41 |
| P-value | 0.396NS(no-significant) | 0.00012 |

*(P<0.05),**(P<0.01),***(P<0.001)

Evaluation the Mean of IL-10 and IL-22 based on Cancer Grades

According to the grade status, in Table(4) showed that there no significant differences in mean of IL-10 was shown in patients of breast cancer group compared among three grades. Furthermore, the mean of IL-22 in breast cancer in grades(G1,G2 and G3)with statistically significant differences(P<0.01) when compared among grades.

**Table(4):The IL-10 and IL-22 mean in grades of CA. Breast patients**

| Grades | IL-10 pg/L M±SD | IL-22 ng/L M±SD |
|---|---|---|
| Grade I | 174.5±40.1 | 151.4±57.4 |
| Grade II | 189±32.8 | *196.2±45 |
| Grade III | 193.6±46.9 | **208.2±50.5 |
| P-value | 0.332NS | 0.0005 |

*(P<0.01),**(P<0.001)

## Discussion

Cancer is a major cause of death in economically developed countries and in developing countries, which is the second-major cause of death[19]. Cancer-related deaths are projected to increase worldwide, with 11 million deaths expected by 2030[20]. Despite the fact that this malignant has a good prognosis, it was the most frequent cause of cancer-related death[21].

Interleukin-10 is a pleiotropic anti-inflammatory cytokine that causes immunosuppression and assists tumour immune-surveillance escape.IL-10 has a dual proliferative and inhibitory effect on breast tumour cells, indicating a complex role for IL-10 in the initiation and progression of breast cancer[22]. Anti-inflammatory cytokines play an important role in tumour development; for example, IL-10, a potential anti-inflammatory cytokine, stimulates the forming of a microenvironment, that suppresses anti-tumor immune responses and promotes cancer cell growth[22].The result of study demonstrated a highly significant(P<0.01) increase IL-10 level in breast cancer women in comparison with control group. The result of study agreed with study by Abeer, who found increase level of IL-10 in breast cancer women in comparison with control group[23],this is agrees with Kozlowski et al. that found a strong relationship between concentration of IL-10 and breast cancer, where interleukin-10 levels in serum of women with breast cancer were statistically higher than in control[24].

Interleukin-22 is upregulated in a variety of human cancers, and several studies have shown that IL-22 plays a tumor-supporting role in the growth of these cancers[25]. IL-22 has been shown to stimulate epithelial cell proliferation, transformation, and migration in breast cancer[17,26]. This study showed high significant differences in mean level of IL-22 between breast cancer patients group and control group. The results of study agreed with results in Tunisia, the found high IL-22 level in breast cancer patients[27]. However, the few studies which reported tumour suppressive effects

of IL-22 were generally carried out over a period of time in a non-physiological environment with exogenous IL-22 injections, and cannot necessarily reflect the natural role of the endogenous host IL-22 in tumorigenesis modulation[28].Given that commensal microbial elements have recently been associated with inflammation mediated-tumor development[29], it can affect the understanding of whether IL-22 plays a role directly or indirectly in tumour promotion, especially at tissue sites where epithelial-microbiotic interactions are intense[10]. IL-22 also has key a role on cancers that arise from non-mucosal sites such as the breast or prostate, as well as its effects on metastases[11].

Cancer is typically diagnosed at a late stage, where the prognosis is low and the efficacy of therapy is limited. Furthermore, there are problems with distinguishing four stages, including the TNM classification[9]. Thus, there is a huge opportunity to improve cancer patients' outcomes by enhancing diagnosis and care approaches, as well as ongoing research and assessment of biomarkers in relation to therapeutic efficacy and overall survival[12]. The results in the study that demonstrated the no significantly difference($P>0.05$) IL-10 levels with tumor stages in breast cancer patients. The results agreed with study in Baghdad-Iraq, who found no correlation of IL-10 level with stages of breast cancer[23], other the study in China, found no significant differences in IL-10 levels between I, II and III stages of breast cancer[23]. These results were consistent with those reported that IL-10 levels in gastric cancer patients were not correlated to tumour stage[24].Those results were incompatible with those reported in China, reported a significantly increase in serum IL-10 levels in patients with TNM stage II and III ductal carcinoma than in stage I ductal cancer($P<0.001$) in malignant breast[15]. In certain epithelial cancers, such as breast and lung cancer, the role of IL-22 in cancer progression has been recognized. When immune cells release IL-22, it can promote tumour growth, aggressiveness, and treatment resistance by acting on cancer cells[25]. The results in the study that demonstrated the significantly difference($P<0.01$) IL-22 levels with tumor stages in breast cancer patients. Other study showed IL-22 absenteeism in TME during initiation and hyperplasia stages of breast cancer. It was expressed in the early stages of carcinoma and increased significantly as the tumour advanced to the malignant stage[27]. Another research observed that IL-22 levels in stage III-IV patients were significantly higher than in stage I-II in RCC[18]. The current understanding of IL-22 function is dependent on advanced-stage cancer cell line models in which IL-22 has been shown to promote cell proliferation, transformation, and migration in human cancer cell lines[17]. IL-22 and HOXB-AS5 were shown to upregulated in the serum and tissues of BC patients and were linked with clinical stages of cancer[19].

Breast tumours are classified into grade-I(well-differentiated), grade-II(moderately-differentiated) and grade-III(poorly-differentiated)[8].

IL-10 is essential for the suppression of pro-tumor inflammation mediators[20]; however, IL-10 may have a potential role in tumour angiogenesis regulation[21]. The results in the study that demonstrated the no-significantly difference($P>0.05$)IL-10 levels with tumor grade in breast cancer patients. The results agreed with a study by Abeer, found no association of IL-10 level with grades of breast cancer[23]. The complex role of IL-10 in determining the immune response seems to be influenced by the tissue microenvironment and the expression of IL-10 receptors on different immune cells[22]. The results in the study that demonstrated the highest significantly difference($P<0.01$) IL-22 levels with grades in breast cancer patients. The results agreed with study in Tunisia, found the high IL-22 levels was significantly associated with a high histopathological grade III[22]. Some studies have indicated elevated IL-22 level was correlated with breast cancer progression[26]. In tumor tissue, IL-1 and IL-23 increased production of IL-22. Thus,IL-1 and IL-23 promoting breast cancer progression via IL-22 be one possible mechanism[12].

## Conclusions

A significant elevation IL-10 and IL-22 levels in breast cancer women comparison with control group, that showed the importance of these cytokines to promote

or suppress immunity toward breast cancer . Elevation significant of immunological IL-22 in breast cancer women were also shown in patients with an advanced CA stage and grade III, which may be considered as a non-invasive primitive marker for earlier prediction of breast cancer staging and grading.

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**Ethical Clearance**: Not required

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# Occupational Health Hazards among Workers at Government Garment Factory in-Al Najaf City

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## Abstract

Descriptive design cross-sectional study was conducted in assess the occupational health hazards among workers in Al-Najaf in garment factory and its association with socio-demographic characteristics of workers in the province of Najaf during period from December 23th 2020 to February 9 the 2021. 180 male and female workers were selected through non-probability sampling (purposive sampling) and the data collected using the questionnaire that was designed earlier from three main parts, first part included socio-demographic data characteristic and the second part was included health care facilities that include health services to workers and the three part (health hazards) and that consist of (physical hazards, chemical hazards, biological hazards, psychological hazards The study found that the overall risk score was moderate, the higher risk was for psychological risks, and the lowest risk was for safety measures and protective equipment, The overall hazard score was found to have a statistically significant relationship with each job title and work location (P 0.05). Further research, as well as an education program and regulations for the use of safety measures, were strongly recommended.

***Keywords-*** *Occupational health, Hazard, Workers, Garment Factory*

## Introduction

According to the studies of the World Health Organization, which stipulates and is concerned with the safety of workers and avoiding them from health risks in the field of occupational health and safety to reduce the work risks for workers they are exposed to while working in the garment factory and apply the field of an integrated health system in building (good health - in terms of mental health - physical health - And mental health - as well as improving the economic situation in terms of worker safety and avoiding all risks to which they are exposed in the work environment) (1) .

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Occupational health is a very important science aims to protect all aspects of workers health in workplace from hazards that may cause injuries to the workers and threaten their lives (2) .

Occupational health was developed from mono-disciplinary to multi-disciplinary and comprehensive approach that deals with person's Physical, mental and social heath .the work environment may impose positive or negative influences on person's health and the productivity is influenced by physical and psychological of workers well-being(3) .

One of the main reasons for increased occupational hazards level environment is double the costs of care and the lack of commitment by the private sectors in addressing environmental policy and therefore workers exposed to occupational hazards and failure to provide for that slide care (private industry) and the failure to

provide health insurance for the class labor by the government and with the piece increases injury rate among

Workers of this side and the other hand must coordinate with the industrial government to work to reduce the risks and increase the proportion of occupational safety and health [4] .

The well-being of more workers because it represents important segment on the level of industry and social he is human part of life must provide psychosocial support through the equivalent or through training courses on how hazard assessment and increased health awareness to prevent injuries and to improve worker health and increasing trends, skills and knowledge of those risks and thus serve the interests productivity and health, a goal of global safety for the workers organization [5] .

## Materials and Methods

Samples were collected for workers from the garment factory in Najaf due to the health risks present in it from all sections of the factory, where the average life expectancy of workers was (41-50) years and their rate was 78.1%, where about 180 samples were collected out of 350 workers from different sections of The laboratory and the sample was collected in the interview method and the sample was not probable (purposive sample)

## Results

In general, the results or overall showed that the workers in terms of physical hazards, chemical hazards and biological hazards, as they had no such risks, had little to do with the workers, or as for the psychological risks, they were high among the workers, as shown in the table.

**Table (1) Overall Assessment and mean of scores of occupational health hazards domains**

| Items | MS | Assessment |
|---|---|---|
| Physical Hazards | 1.63 | Moderate |
| Chemical Hazards | 1.61 | Moderate |
| Biological Hazards | 1.65 | Moderate |
| Psychological Hazards | 1.33 | High |
| Overall Occupational Hazards | 1.55 | Moderate |

**Table (2) Relationship between physical health hazards and demographic data**

| Items | Chi Square | P value | Sig. |
|---|---|---|---|
| Age / Years | 17.91 | 0.0001 | HS |
| Gender | 0.64 | 0.42 | NS |
| Residence | 1.67 | 0.19 | NS |
| Levels of Education | 3.94 | 0.68 | NS |
| Marital Status | 2.4 | 0.49 | NS |
| Economic Status | 0.44 | 0.8 | NS |
| Job Title | 7.0 | 0.42 | NS |
| Duration in Service/ Years | 2.3 | 0.33 | NS |

NS: Non-significant; HS: High Significant

**Table (3) Relationship between chemical health hazards and demographic data**

| Items | Chi Square | P value | Sig. |
|---|---|---|---|
| Age / Years | 12.62 | 0.001 | HS |
| Gender | 0.95 | 0.32 | NS |
| Residence | 0.09 | 0.75 | NS |
| Levels of Education | 3.94 | 0.68 | NS |
| Marital Status | 2.53 | 0.47 | NS |
| Economic Status | 3.57 | 0.16 | NS |
| Job Title | 6.51 | 0.48 | NS |
| Duration in Service/ Years | 3.22 | 0.22 | NS |

**NS: Non-significant; HS: High Significant**

**Table (4) Relationship between biological health hazards and demographic data**

| Items | Chi Square | P value | Sig. |
|---|---|---|---|
| Age / Years | 7.38 | 0.02 | S |
| Gender | 4.95 | 0.12 | NS |
| Residence | 0.44 | 0.45 | NS |
| Levels of Education | 2.84 | 0.58 | NS |
| Marital Status | 4.22 | 0.37 | NS |
| Economic Status | 1.57 | 0.26 | NS |
| Job Title | 4.51 | 0.38 | NS |
| Duration in Service/ Years | 1.22 | 0.28 | NS |

**NS: Non-significant; S: Significant**

**Table (5) Relationship between psychological health hazards and demographic data**

| Items | Chi Square | P value | Sig. |
|---|---|---|---|
| Age / Years | 1.13 | 0.56 | NS |
| Gender | 6.61 | 0.01 | HS |
| Residence | 2.09 | 0.14 | NS |
| Levels of Education | 14.63 | 0.02 | S |
| Marital Status | 0.74 | 0.86 | NS |
| Economic Status | 1.77 | 0.41 | NS |
| Job Title | 7.67 | 0.36 | NS |
| Duration in Service/ Years | 2.33 | 0.25 | NS |

**NS: Non-significant; HS: High Significant; S: Significant**

## Discussion

The current study found a moderate level of the physical, chemical hazards, biological hazards among workers while there was high psychological hazards (table) . Biological hazards include infections caused by bacteria and molds , while psychological hazards include stress, anxiety, depression, and work burden . These results are consistent with the study or research that was conducted in Nigeria [6] .

According to the table (2) about relationship between physical hazards and demographic data. It shows that there is no significant relationship (P > 0.05) between physical hazards and demographic data except for age which was high significant relationship (P<0.01). And according to the table (3) about relationship between chemical hazards and demographic data. It shows that there is no significant relationship (P > 0.05) between chemical hazards and demographic data except for age which was high significant relationship (P<0.01). This is due to the fact that workers have not a few years of service in the factory this result is represented by the age of the workers who are more experienced than other workers. Therefore, workers represent a high percentage of all samples this research contradicts my current study, which states that the age relationship is in relation between chemical hazards and demographic information, as there is no significant relationship the age relationship related to physical risks and demographic information also indicated that there is no statistically significant relationship [7] .

According to the table (4) about relationship between Biological hazards and demographic data. It shows that there is no significant relationship (P > 0.05) between biological hazards and demographic data except for age which was significant relationship (P<0.05) this is due to the fact that the workers have not a few years of service in the factory. As this result is considered and it represents the majority for the age characteristic as they are more experienced than other workers, the current study is consistent with the research conducted in Dhaka [8] .

According to the table (5) about relationship between psychological hazards and demographic data. It shows that there is no significant relationship (P > 0.05) between psychological hazards and demographic data except for gender which was high significant relationship (P<0.01); This is due to the fact that women love the profession of sewing and are creative in the art of sewing, and because the risks in this profession are much less than the risks, for example in the field of health Or was the reason for women joining this profession because of the poor social and economic conditions within the family due to the lack of family income He current study agrees with the research conducted in the city of Savar in Bangladesh, which found that the percentage of women is the most statistically speaking in this field.

In addition the Levels of Education also was significant (P<0.05). The reason for this is because the profession does not depend on the obtaining an academic certificate, but rather on the acquisition of experience and skill from others and the art of creativity in this profession. His current study agrees with the research conducted in the city of Saver in Bangladesh, Which states that the educational level of the workers was an acceptable percentage [9] .

## Conclusion

Form a study group Overall, the threat level was moderate, and the health condition was chronic in the workplace for the study population, with a rise in morbidity, In terms of the physical, chemical, and social factors, the results were moderate, while the psychological factors were high**,** There is a great need for occupational safety and health and training programs for employers and workers in order to improve the level of perception about safety measures.

**Ethical Clearance**: Taken from University of Kufa ethical committee

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# Relationship between Parenting Stress and Risk of Attention Deficit Hyperactivity Disorder (ADHD) in Elementary School Children

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## Abstract

**Background**: Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder during child development with three symptoms which are comprised of inattention, hyperactivity, and impulsiveness that may persist into adulthood. This condition can be a stressor for parents in performing parenting and may lead into a state of parenting stress. **Objective:** To analyze the relationship between parenting stress and the risk of developing Attention Deficit Hyperactivity Disorder (ADHD) in elementary school children. **Methods:** This research was conducted in Surabaya from November 2020 to January 2021 with respondents consisting of parents of elementary school children who have ADHD risk and agreed to participate in this study with a total of 55 samples selected using a purposive sampling technique. The research design was cross-sectional using demographic questionnaire, Abbreviated Conners Rating Scale (ACRS), and Parenting Stress Index-Short Form (PSI-SF) which were filled out by respondents through an online form. The data were processed and analyzed using One-Sample Chi-Square analysis test program with a p-value <0.05, considered statistically significant. **Results:** The majority of parents experienced moderate parenting stress levels (58.2%). There was a significant relationship between each level of parenting stress and the risk of ADHD in children ($p < 0.001$). **Conclusions:** There is a significant relationship between parenting stress and the risk of ADHD in children.

***Keywords:*** *Attention Deficit Hyperactivity Disorder, Parenting Stress, Children, Elementary School*

## Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder in which a developmental disorder occurs causing emotional and behavioral disorders. The three main symptoms of ADHD which are inattention, hyperactivity, and impulsiveness, typically appear in children before the age of 12 years old and can persist until the child reaches adulthood. The symptoms exhibited in ADHD can result in performance problems in social, educational, or work aspects. The prevalence of ADHD is estimated to range from 3 to 7% in the US with manifestations of ADHD symptoms at least occurring in two different situations, such as at school and home[1].

Parents who have children with ADHD tend to feel anxious, fearful, and more irritable, so they are at greater risk of falling into parenting stress[2,3]. Parenting stress conditions that arise will result in an inadequate skill of parenting to a child with ADHD. Parents will tend to use negative parenting approach, more control towards

child behaviour, and give a lot of punishment due to inappropriate behavior from their children. If this is not resolved, it can result in the worsening of child condition and deterioration of relationship between parent and child[2,4]. Such situations are further dampened with the current conditions, which is the Corona Virus Disease 2019 (COVID 19) pandemic, that requires children to study at home remotely through online learning. This condition will make children feel bored and also affect stressors which will cause parenting stress on parents because they have to oversee learning activities as well as double the role of being a teacher for their children while at home. Parents who have to do work from home (WFH) will be burdened by bigger thoughts and energies which will lead to the risk of anxiety and depression, that subsequently will have an impact on parenting stress[5,6].

## Method

This research is an observational analytic study with a cross-sectional design. The population of students aged 9 to 12 years old came from five elementary schools in Surabaya, namely Semolowaru I/261, Semolowaru IV/614, Keputih 245, Kertajaya, and Baratajaya. The sample of this study consisted of 55 students from the five schools at the 4th, 5th, and 6th grades with a purposive sampling method that was determined by the inclusion and exclusion criteria of the researcher. All required data was taken through a questionnaire which was filled in by the parents of students through an online platform from November 2020 to January 2021. Ethical clearance is obtained from the Faculty of Medicine, Universitas Airlangga (Ref: 284/EC/KEPK/FKUA/2020).

The demographic questionnaire contains data of students, which are age and gender, as well as data of parents, which are age, gender, education, occupation, and income. The Abbreviated Conners Rating Scale (ACRS) questionnaire for early detection of ADHD risk in children listed in the Stimulation, Detection, and Early Intervention of Child Growth and Development (SDIDTK) book consists of 10 questions. A score that is more than equal to 13 means that the child has a high risk of ADHD[7]. The Parenting Stress Index-Short Form (PSI-SF) questionnaire measuring the level of parenting stress in parents consists of 36 questions that have been translated into Indonesian and have been tested for validity and reliability[8,9]. The results of the total scores obtained will be categorized into low, moderate, and high levels. The data were processed and analyzed with the aid of an analysis test program with univariate analysis techniques and bivariate analysis techniques with the One-Sample Chi-Square test which was considered significant if the p-value was <0.05.

## Results

### Characteristics of Children and Parents

The characteristics of the child and the parents are presented in table 1 below. The composition of sex in children is dominated by the male which is 56.4% and age is dominated by the age of 10 which is 32.7%. Most parents are between 31 until 40 years old (60%) with the most recent education in senior high school (70.9%). The occupation is dominated by housewives (47.3%) and the majority income is between 0 to 1.500.000 Rupiah (50.9%).

**Table 1. Characteristics Socio-Demographic of Children and Parents**

| Children | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Gender | Boys | 31 | 56.4 |
| | Girls | 24 | 43.6 |
| Age | 9 Years Old | 4 | 7.3 |
| | 10 Years Old | 18 | 32.7 |
| | 11 Years Old | 17 | 30.9 |
| | 12 Years Old | 16 | 29.1 |

***Cont...*** **Table 1. Characteristics Socio-Demographic of Children and Parents**

| Parents | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Education | Junior High School | 7 | 12.7 |
| | Senior High School | 39 | 70.9 |
| | Associate Degree | 1 | 1.8 |
| | Bachelor Degree | 8 | 14.5 |
| Age | 21-30 Years Old | 3 | 5.5 |
| | 31-40 Years Old | 33 | 60 |
| | 41-50 Years Old | 17 | 30.9 |
| | 51-60 Years Old | 2 | 3.6 |
| Occupation | Housewife | 26 | 47.3 |
| | Entrepreneur | 10 | 18.2 |
| | Private | 7 | 12.7 |
| | Laborer | 4 | 7.2 |
| | Government Employees | 3 | 5.5 |
| | Others | 5 | 9 |
| Income | 0 – 1.500.000 Rupiah | 28 | 50.9 |
| | 1.500.001 – 2.500.000 Rupiah | 12 | 21.8 |
| | 2.500.001 – 3.500.000 Rupiah | 8 | 14.5 |
| | More than 3.500.000 Rupiah | 7 | 12.7 |

**Prevalence The Risk of ADHD in Children**

Early detection of ADHD using the ACRS questionnaire was performed with a cut-off score of 13. There are 55 children (12.2%) who score more than or equal to 13 and can be classified as at high risk of experiencing ADHD, from the total sample in this study which is 449 children. The data are presented in table 2 below.

**Tabel 2. Prevalence The Risk of ADHD in Children**

| | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Risk of ADHD | Yes | 55 | 12.2 |
| | No | 394 | 87.8 |
| Total Sample | | 449 | 100 |

**Parenting Stress and Risk of ADHD in Children**

Measuring the level of parenting stress using the PSI-SF questionnaire was divided into three categories which are low, moderate, and high. Stress levels of parents with children at risk for ADHD are dominated at moderate levels (58.2%) and only 5.5% of parents experience low levels of parenting stress. The data are presented in table 3 below.

**Table 3. Overview of Parenting Stress and Risk of ADHD in Children**

| | Category | Frequency (n) | Percentage (%) |
|---|---|---|---|
| Level of Parenting Stress | Low | 3 | 5.5 |
| | Moderate | 32 | 58.2 |
| | High | 20 | 36.4 |
| Total Sample | | 55 | 100 |

In this study, the relationship between each category was tested on parenting stress on the risk of ADHD in children. It was found that there was a significant relationship between each level of parenting stress and the risk of ADHD in children ($p < 0.001$). The data are presented in table 4 below.

**Table 4. Relationship Between Parenting Stress and Risk of ADHD in Children**

| | | Level of Parenting Stress | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | Low | P | Moderate | P | High | P | |
| Risk of ADHD | Yes | 3 | 0.000* | 32 | 0.000* | 20 | 0.000* | 55 |
| | | 5.5 % | | 58.2 % | | 36.4 % | | 100% |
| | No | 113 | | 259 | | 22 | | 394 |
| | | 28.7 % | | 65.7 % | | 5.6 % | | 100 % |

*significant level $p<0.05$

## Discussion

The sample at high risk for ADHD had more male sex frequencies than female (Table 1). Previous research in Bali also showed the same result, where the frequency of male sex with ADHD was more than in girls with a ratio of 2:1[10,11]. This is consistent with the evidence that boys have ADHD more often because they show more challenging and aggressive traits than girls[11]. The age range in this study was between 9 and 12 years according to the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM) - 5 which provides explaination that some of the symptoms of ADHD in these individuals appear before turning 12 years[1]. The results obtained were that the highest ages were 10 years and followed by 11 years, 12 years, and 9 years (Table 1). Another study by Novriana (2013), also obtained results that were not much different, in which more children with ADHD were found in the 11-13 years age range[12]. ADHD can persist into adulthood in several situations and this can have serious consequences, such as failure to complete school, depression, problems with social relationships, substance abuse, violation, accidental injury, and failure

to do a job. All of that can happen if ADHD conditions are not detected as early as possible and are not treated properly[1,13]. The higher the age of the child, the greater the risk of ADHD, coupled with the consistently high prevalence of ADHD, which shows the importance of early detection in children so that this ADHD condition does not get worse and can be treated early.

The parenting stress description in this study shows that most of the parents are included in the moderate level of stress, followed by high and low categories (Table 3). This is consistent with the previous meta-analysis conducted by Theule (2013) which elucidates that parents with ADHD children will experience high levels of parenting stress[14]. This occurs because parents have inadequate knowledge of their child's condition so that in carrying out parenting there will be a feeling of discomfort and anxiety if the results obtained are not in line with the wishes of the parents as caregivers[14]. The condition of the COVID-19 pandemic that is currently occurring when this research was conducted, where the school learning process and a lot of work done online at home can add to the burden on the mind and affect stressors in parents[6]. So that this condition also accelerates the parenting stress condition where there is a relatively high level of parenting stress.

The results of the bivariate analysis test to determine the relationship between variables tested in this study using the One-Sample Chi-Square test technique obtained p-value = 0.000 ($p < 0.001$). So, it can be concluded that there is a significant relationship between parenting stress and ADHD in children (Table 4). These results are consistent with previous research conducted by Perez *et al*. (2018) in which the results showed association between parenting stress in parents and the condition of children with ADHD[15]. Another study, Setiawati *et al*. (2018), which discusses the level of parental anxiety about children with ADHD also showed a significant relationship[3].

The existence of a relationship at low parenting stress levels can be seen from the data showing that only a few parents of children with ADHD risk have low parenting stress levels and the rest tend to be at moderate and high levels. This is in line with the theory that the symptoms of children with ADHD will affect the psychopathological conditions of the parents as caregivers, where there are higher levels of stress[2,16]. At high parenting stress levels, there was also a significant relationship and strengthened with the percentage at high parenting stress levels found in parents with children at risk for ADHD than parents who did not have children at risk for ADHD. This condition will make parents have difficulty in teaching and children will receive less attention, so that cognitive stimulation in children will be reduced and this makes the risk of ADHD even higher[11,17].

The three symptoms of ADHD consist of inattention, impulsivity, and hyperactivity. Inattention is a symptom of decreased attention to something characterized by the child being unable to hold their attention both while learning and playing for a long time. Impulsiveness is a symptom of ADHD shown by a child who often imposes their will or likes to interrupt other people's conversations. Hyperactivity itself can be shown by the behavior of children who are unable to stay still in certain situations for a long time and are easily influenced by environmental conditions. The three symptoms are shown in children with ADHD if allowed to persist into adulthood and of course, will be detrimental to both themselves and the environment around them[1]. These three symptoms can arise or get worse due to many influencing factors like genetic and environmental factors. Environmental factors can come from exposure to heavy metals, chemicals, and psychosocial conditions[18]. The discussion in this study is to focus on the psychosocial conditions around the child that one of which is due to inappropriate environmental conditions such as parenting stress on parents while caring for children.

The mechanism of parenting stress with the risk of ADHD in children can be explained because stress conditions in parents will affect the neurotransmitters dopamine and norepinephrine in children, where there will be a decrease in dopamine and norepinephrine

which cause symptoms of decreased attention or inattention and impulsivity[19]. If the stress condition of the parents is not resolved immediately, then the children will indirectly be affected by stress from their parents when doing inappropriate parenting. This stress condition can inhibit action potential inhibition on pre-synaptic neurons so that the production of dopamine and norepinephrine will be produced in large quantities and cause symptoms of hyperactivity in these children[19]. Parents who have children with ADHD experience confusion and misunderstanding about the conditions that occur in their child, resulting in anxiety which can be a stressor while doing parenting[3]. This will indirectly affect the way of caring for children, where parents who ignore their child's condition will make the child grow more impulsive and act according to their wishes without thinking about the impact that arises afterward[20].

Parents who do not have children at risk for ADHD show a tendency towards low to moderate parenting stress levels with the predominance remaining at moderate parenting stress levels. This is of interest because it should be expected that parents without children who are at risk for ADHD tend to have low levels of parenting stress. It should be noted that the urgency at this time is the influence of the COVID-19 pandemic condition, wherein previous research by Hiraoka (2020), it was found that there was a significant relationship between parenting stress and the school learning process during COVID 19. This happens because parents as caregivers need adjustments both in their daily work and in the school learning system for children where parents will be more involved in accompanying children and this condition can add stressors to parents while caring for their children[6,21,22].

## Conclusion

There is a significant relationship between parenting stress and the risk of ADHD in children ($p < 0.001$). The majority of parents with children at high risk for ADHD experience moderate to high levels of parenting stress. Parenting stress levels are more likely to be experienced by parents who have children at high risk of ADHD than parents who do not have children at high risk of ADHD. More research is needed about the condition of ADHD in children so that the level of parenting stress on parents can be minimized.

Future research is expected to be focussed on to other risks which are thought to also affect the risk of ADHD in children so that these conditions could be identified and managed early.

**Conflict of Interest:** There is no conflict of interest.

**Source of Founding:** The study is self-funded.

**Ethical Clearance:** This study had been approved by Ethical Commission of Health Research Faculty of Medicine Universitas Airlangga (Ref: 284/EC/KEPK/FKUA/2020).

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# Quantitative Risk Assesment of Benzene Exposure in Printing Industry X Surabaya City

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## Abstract

Benzene is a compound chemically volatile and lasts a long time in the air and quickly inhaled into the human body. The use of benzene is widely used in various industries, one of which is the printing industry. Benzene produced right in the printing industry are ink, cleaning fluid, and when the machine in operation. Workers exposed to benzene can risk cancer and non-cancer health effects that are harmful to the human body. This study aimed to assess cancer and non-cancer risk of workers in the printing industry X Surabaya City. This research is observational, cross-sectional with the same sample with a total population of 11 workers in the printing industry X Surabaya City. The risk assessment method uses four stages: hazard identification, exposure analysis, dose and response analysis, and risk characterization. This research shows that the average concentration of benzene is 1.0729 mg/m$^3$ or 0.34 ppm. RQ value > 1 both real-time and lifetime. As many as 63.6% of workers have real-time ECR >10 $^{-4,}$ and 81.8% of workers have ECR lifetime >10$^{-4}$. This study concludes that workers are in an unsafe condition and have non-carcinogenic and carcinogenic health risks.

***Keywords:*** *benzene, printing, assessor a risk, Risk Quotient, Excess Cancer Risk*

## Introduction

Benzene is a compound that has the chemical formula $C_6H_6$. Benzene including organic compounds, volatile or *Volatile Organic Compounds* (VOC). Due to the volatile nature of benzene and lasts a long time in the air, it is quickly inhaled and enters the human body. About 50% of benzene in the air is quickly absorbed through the lungs[4]. Benzene as a vapor can last for hours or even days, depending on the environment, climate, and other pollutants[20].

Benzene is used in a variety of industries, including petroleum, chemical production, and manufacturing. In some countries, it still occurs in shoemaking, painting, printing, and rubber manufacturing[14]. In Indonesia, benzene is still used in the petroleum industry, gas stations (*SPBU*), printing, shoes factory, workshops[17,23,25,27,29]. The use of benzene in printing is proven in[16]research that benzene is released during printing machine operation, depending on the printing speed and temperature

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equipment. Benzene is damaging indoor air quality and adversely affects health workers[16]. Several studies also show that there is a concentration of benzene in the printing industry[3,10,17,22].

Each chemical has its safe value or Threshold Value (NAV). For example, benzene has a NAV of 0.05 ppm, according to Manpower Regulation Number 5 of 2018. Meanwhile, according to NIOSH, benzene has a NAV of 0.1 ppm[4]. Based on the research of[17], shows that there is a concentration of benzene in the X printing press in Semarang City with an average of 0.1322 ppm, which has a NAV above 0.1 ppm according to NIOSH[17]. Based on the research of[3] showed that the benzene concentration in the Printing X Surabaya had a NAV that exceeded 0.5 ppm, and the highest benzene concentration was 15.6418 ppm[3].

Exposure to benzene which humans inhale then distributed throughout the body through the blood. Once distributed, benzene is metabolized to be excreted or will produce harmful substances to the body and cause health problems.

Health disorders caused by benzene could be cancerous or non-cancerous. Based on research by[11] conducted in a newly renovated building in Beijing showed that inhalation exposure to formaldehyde and benzene could result in both cancer and non-cancer risks[11]. The risk of cancer caused by benzene that often occurs is Acute Myeloid Leukemia (AML). Based on the research of[28] showed that exposure to benzene, diesel fuel, metals, insecticides, fertilizers, glues and adhesives, paints and other coatings, as well as inks and pigments, is associated with an increased risk of *Acute Myeloid Leukemia* (AML) (all subtypes combined) and subtypes individuals[28]. For non-cancer health risks in the form of headaches, *fatigue,* and other non-cancer health problems. Based on the research[10] shows that workers in the X printing office in Semarang City experience several health complaints, namely headaches and fatigue[10].

Based on the explanation above, the purpose of this study is to assess the level of cancer and non-cancer risk of workers in the printing industry X Surabaya City.

## Method and Material

This research is an observational study. Only observations were made without intervention, and according to time, this study used a cross-sectional research design. This research was conducted in the printing industry X Surabaya City in 2019. The sample in this study is the same as the total population of 11 workers.

Data collection for benzene concentration was using the 1501-2003 gas chromatography method and the High Volume Dust Sampler (HVDS). At the same time, the data collection characteristics of respondents using questionnaires and interviews. Data analysis using risk assessment. There are four stages in the risk assessment, namely hazard identification, exposure analysis, dose and response analysis, and risk characterization.

Intake calculation who obtained from the following formula:

$$I = \frac{C \times R \times tE \times fE \times DtC \times R \times tE \times fE \times Dt}{W_b \times t_{avg} \qquad W_b \times t_{avg}}$$

Where:

C = Concentration (mg/m$^3$)

R = Respiratory Rate (m$^3$/hour)

tE= Daily Exposure Time (hour/day)

fE=Annual Exposure Frequency (days/year)

Dt=Exposure Duration (real-time or lifetime)

$W_b$ = Body Weight (kg)

$t_{avg}$= Average Time Period, 30 years x 365 days/ year (non-carcinogenic) or 70 years x 365 days/year (carcinogenic)

The difference between real-time and lifetime is the duration of exposure (Dt). For real-time is the actual time achieved during work, while lifetime is an estimated 30 years.

There are two results from risk characterization, namely, non-carcinogenic risk (RQ) and carcinogenic

risk (ECR).

To determine the RQ (Risk Quotient) the formula is:

$$RQ = \frac{I_{nc}}{RfD \text{ atau } RfC}$$

Where:

$I_{nc}$ = Intake Non-Carsinogenic (mg/kg/day)

RfD or RfC = Reference Dose (mg/kg/day)

After obtaining the RQ value, assumed that the RQ value 1 indicates a safe condition that allows no risk of non-carcinogenic health effects. While RQ > 1 indicates an unsafe condition that enables non-carcinogenic health effects, risk control or management is needed.

Meanwhile, to determine the carcinogenic risk, the ECR (Excess Cancer Risk) calculation is carried out with the formula:

$$ECR = I_c \times CSFI_c \times CSF$$

Where:

$I_c$ = Carcinogenic Intake (mg/kg/day)

CSF = Reference Dose (mg/kg-day)

If the ECR $10^{-4}$, then the exposure concentration is safe, and there is no risk of carcinogenic health effects. If the ECR>$10^{-4}$, then the exposure concentration is not safe for the body and can cause carcinogenic health effects.

## Finding

This study resulted in the concentration of benzene at each measurement point in table 1

**Table 1. Distribution of Benzene Concentration in Printing Industry X Surabaya City 2019**

| Measuring Point | Benzene Concentration (mg/m3) | Total | |
|---|---|---|---|
| | | f | % |
| Point 1 | 0,5056 | 5 | 45,4 |
| Point 2 | 0,1320 | 3 | 27,3 |
| Point 3 | 2,9593 | 3 | 27,3 |
| Total | | 11 | 100 |
| Mean | 1,0729 | | |

In table 1, know that the highest concentration of benzene in the production section was 2.9593 mg/$m^3$ or 0.93 ppm, and as many as three workers (27.3%) of benzene were exposed. On the other hand, the lowest concentration was 0.1320 mg/$m^3$ or 0.04 ppm, and as many as three workers (27.3%) of benzene were exposed. The workers who were most benzene exposed were at point 1 as many as five people (45.5%) with a concentration of 0.5056 mg/$m^3$ or 0.16 ppm. In comparison, the average concentration of benzene in the printing industry X Surabaya City is 1.0729 mg/$m^3$ or 0.34 ppm.

**Table 2. Distribution of Workers Weight in Surabaya City Printing Industry X 2019**

| Body Weight (kg) | Frequency (person) | Percentage (%) |
|---|---|---|
| ≤ 54 | 2 | 18,2 |
| 55-64 | 3 | 27,3 |
| 65-74 | 4 | 36,4 |
| >74 | 2 | 18,2 |
| Total | 11 | 100 |

The distribution of body weight showed that the highest percentage was in the 65-74 kg group, which was 36.4%. While the lowest rate in the two weight groups, 54 and > 74, is 18.2%. The average body weight of workers in the printing industry X Surabaya City is 64.2 kg, which means that the worker's weight is less than the US-EPA standard, 70 kg for adults.

**Table 3. Distribution of Activity Pattern in Printing Industry X Surabaya City Workers 2019**

| Activity Pattern | N | Min. | Max. | Mean | Std Deviation |
|---|---|---|---|---|---|
| Daily Exposure Time (hour/day) | 11 | 8 | 9 | 8,09 | 0,302 |
| Annual Exposure Frequency (days/year) | 11 | 260 | 260 | 260,00 | 0,000 |
| Exposure Duration (year) | 11 | 2 | 20 | 10,09 | 5,522 |

Based on table 3, know that the average exposure time is 8.09 hours/day, with the longest time being 9 hours/day. Meanwhile, the average duration of exposure is 10.09 days/year, with the most prolonged period being 20 days/year. Therefore, for the intermediate frequency of exposure, which is the same as 260 days/year, there is no variation.

**Table 4. RQ (Risk Quotient) Real-time and Lifetime Surabaya City Printing Industry X 2019**

| Respondent | RQRealtime | RQ Lifetime |
|---|---|---|
| 1 | 237,356 | 474,7114 |
| 2 | 63,1498 | 94,7246 |
| 3 | 195,308 | 585,9252 |
| 4 | 13,3743 | 26,7486 |
| 5 | 37,8899 | 94,7246 |
| 6 | 85,1794 | 511,0765 |
| 7 | 11,6436 | 87,3268 |
| 8 | 6,9054 | 23,0180 |
| 9 | 5,54687 | 27,7343 |

**Table 4. RQ (Risk Quotient) Real-time and Lifetime Surabaya City Printing Industry X 2019**

| Respondent | RQRealtime | RQ Lifetime |
|---|---|---|
| 10 | 58,5976 | 878,9633 |
| 11 | 29,9133 | 69,0306 |

Table 4 shows that all 11 workers have an RQ value > one both real-time and lifetime, which indicates their condition is not safe and has a risk of non-carcinogenic health effects.

**Table 5. ECR (Excess Cancer Risk) Real-time and Lifetimein Printing Industry X Surabaya City Workers 2019**

| Respondent | ECR*Realtime* | ECR *Lifetime* |
|---|---|---|
| 1 | 0,00092 | 0,00184 |
| 2 | 0,00025 | 0,00037 |
| 3 | 0,00076 | 0,00228 |
| 4 | 0,00005 | 0,00010 |
| 5 | 0,00015 | 0,00037 |
| 6 | 0,00033 | 0,00199 |
| 7 | 0,00005 | 0,00034 |
| 8 | 0,00003 | 0,00009 |
| 9 | 0,00002 | 0,00011 |
| 10 | 0,00023 | 0,00342 |
| 11 | 0,00012 | 0,00027 |

**Table 6. Distribution of Excess Cancer Risk (ECR) Printing Industry X Surabaya City Workers 2019**

| No. | ECR | f | % |
|---|---|---|---|
| Real-time | | | |
| 1 | Safe ($\leq 10^{-4}$) | 4 | 36,4 |
| 2 | Unsafe ($>10^{-4}$) | 7 | 63,6 |
| Total | | 11 | 100 |
| Life-time | | | |
| 1 | Safe ($\leq 10^{-4}$) | 2 | 18,2 |
| 2 | Unsafe ($>10^{-4}$) | 9 | 81,8 |
| Total | | 11 | 100 |

Based on table 6, from the ECR real-time calculation of benzene exposure, seven workers (63.6%) were in an unsafe condition and at risk of having carcinogenic health effects. While the results of the calculation of the ECR lifetime of benzene exposure were more extraordinary, as many as nine workers (81.8%) in unsafe conditions and at risk of having carcinogenic health effects

## Discussion

Benzene in the printing industry X Surabaya arises from chemicals containing benzene in the production process. Based on the research of[26], benzene in the printing industry is produced by solvent cleaners[26]. Chemicals are containing benzene, one of which is printing equipment, in addition to solvents, thinners, cleaners, inks, and anti-rust oils[7].

The concentration of benzene in the printing industry X Surabaya City is divided into an average value of 1.0729 mg/m$^3$ or 0.34 ppm. The average value of benzene concentration in the printing industry X Surabaya City, by Ministry of Manpower 5 of 2018, shows that it is still below the Threshold Value (NAV) for benzene chemicals, which is 0.5 ppm. However, one point with a concentration exceeding the Threshold Limit Value (TLV) of 0.5 ppm equals 2.9593 mg / m $^3$ or 0.93 ppm. The results are in line with research by[10] that the average concentration of benzene in the printing industry X Semarang was under NAB is equal to 0.4226 mg / m $^3$ or 0.1323 ppm. There is one sample that exceeded NAB, i.e., 0.553 ppm. In addition, the research by[22] showed that 64% of workers in the printing industry in Medan who were exposed to benzene concentrations were below the NAV and 36% above the NAV.

The daily exposure time shows the number of hours worked in a day that the worker has. The highest working hours of workers in the printing industry X Surabaya City is 9 hours/day. While the duration of exposure shows how long workers are exposed to benzene in years. The workers in the printing industry X Surabaya longest worked for 20 years and most recently worked for two years. The duration of exposure affects the effect of benzene on the body, which can interfere with workers' health this is in line with research by[2], who showed that long-term or long-term exposure to benzene could cause dermatological effects that can increase skin diseases, besides that blood disorders and liver disorders are also caused by long-term benzene exposure[2].

The RfD or RfC value or reference dose represents the daily exposure level in the human population, which is unlikely to have an adverse effect. RfD and RfC are that the RfD is for chronic exposure by mouth, while RfC is for chronic exposure through inhalation. Each chemical has a different RfD and RfC. The RfC of benzene in this study was 0.00031 mg/kg/day.

To get to know the value of RQ or non-carcinogenic risk characteristics, it is necessary to value *Intake* Non-carcinogenic ($I_{nc}$) and the value of the RfC, then dividing the value $I_{nc}$ RfC. From the calculation results in table 4. Real-time and lifetime Risk Quotient (RQ), which has a value of more than one (RQ> 1) which means unsafe conditions that allow the risk of non-carcinogenic health effects for workers in the printing industry X Surabaya City. This is in line with research by[21] conducted at the printing and photocopying center of the city of Ardabil, Iran, that the *hazard quotient* (HQ) of benzene in indoor air with *an inkjet printer* is > 1, which indicates that the risk of non-hazardous The carcinogenicity associated with benzene exposure is enormous. Non-carcinogenic risks are health disorders that attack the human body but do not cause cancer. Such as cardiovascular risk, headaches, fatigue, and others. The results of research conducted by[1] showed that there was a relationship between benzene exposure and cardiovascular risk[1]. In addition, the study by[6] showed that benzene exposure was associated with fatigue at work[6].

CSF (*Carcinogenic Slope Factor*) is the risk value obtained by the average lifetime amount of one mg/kg/day of chemical carcinogens and specific contaminants. The CSF benzene value in this study was 0.029 mg/kg/day.

To get the value of ECR and determine the characteristics of the required value of carcinogenic risk *Intake* carcinogenic ($I_c$ ) and the value of CSF,

then dividing the value $I_c$ by the CSF. *Realtime* ECR of benzene exposure of 7 workers in unsafe conditions and at risk of carcinogenic health effects. At the same time, the results of the calculation of the ECR *lifetime of* benzene exposure are more significant, namely as many as nine workers in unsafe conditions and at risk of having carcinogenic health effects. Most of the workers in the printing industry X Surabaya City are at risk of having carcinogenic health effects. This is in line with research[10] which showed that the average ECR benzene *real-time* at 0.00028 to 0.0012 and *lifetime* of 0.001 to 0.0043, then the value of the ECR> 1 which means that workers in the industry X printing Semarang is at risk of having cancer health effects. Benzene is a chemical that has carcinogenic properties that can cause cancer. It is proven that benzene is included in the A-1 category, which is carcinogenic to humans[12]. Benzene causes Acute Myeloid Leukemia (AML) which is a malignant disease (cancer) that attacks the blood system and bone marrow[19]. This is evidenced in a study[15] which showed that the concentration of benzene in the ambient air of Tehran, Iran, can increase the risk of AML, especially in the southern and central areas of Tehran, Iran. Benzene exposure can increase the AML. There are men between the ages of 30 and 40. In addition to AML, benzene also can cause *non-Hodgkin lymphoma* (NHL), *Chronic Lymphocytic Leukemia* (CLL), and *Multiple Myeloma* (MM), which is also cancer. *Non-Hodgkin Lymphoma* develops in the lymphatic system (lymph), which is part of the human immune system[18]. According to[5] , there was an increased risk of *Non-Hodgkin Lymphoma* in Chinese women exposed to benzene in Shanghai[5]. While *Chronic Lymphocytic Leukemia* (CLL) is a type of cancer which is a small lymphocyte in the bone marrow and peripheral tissues that will overgrow with medium-sized (immature) tumor cells slowly but with accumulation[13]and *Multiple Myeloma* (MM) is cancer in which the cell immunoglobulin production pl asthma long-lived, and the end of the differentiation pathway for B cell neoplasm[9]. This is by research on[8] showed that exposure to benzene is associated with the risk of *Chronic Lymphocytic Leukemia* (CLL) and *Multiple Myeloma* (MM)[8]. In addition to attacking the blood system, benzene can also cause colon cancer, according to Talibov *et al.* (2018), which shows a relationship between benzene exposure at work and an increased risk of colon cancer. Exposure to benzene can cause cancer, especially in the proximal colon, but women can also be at risk of developing cancer rectal[24].

Smoking habits in workers can increase the risk of carcinogenic and non-carcinogenic health effects due to benzene exposure. The smoking habit of workers exacerbates the work environment that has been exposed to benzene. This is important to prevent to minimize the risk of health effects due to benzene on workers. In addition, the use of PPE (Personal Protective Equipment) that workers rarely use, such as gloves and masks, increases the risk of workers being exposed to carcinogenic and non-carcinogenic health effects. Therefore, it is essential to educate workers to maintain health, especially not smoking and using PPE and exercising every day, and consuming nutritious food.

## Conclusion

Benzene concentration in the printing industry X Surabaya City is still below the Threshold Limit Value (NAV) with an average concentration of benzene which is 1.0729 mg/m$^3$ or 0.34 ppm.Realtime and lifetime RQ values (in the next 30 years) exceed one, which means workers risk non-carcinogenic health effects.For real-time ECR calculation, 63.6% of workers have ECR > $10^{-4}$. Meanwhile, the calculation result of ECR lifetime is 81.8% ECR > $10^{-4}$. Both mean that workers are in unsafe conditions and are at risk of having carcinogenic health effects.

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**Ethical Clearence**: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University

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# Age Estimation with Cemental Incremental Lines in Normal And Periodontally Diseased Teeth Using Phase Contrast Microscope

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## Abstract

**Background:** Forensic age estimation defines an expertise in forensic medicine, which aims to define in the most accurate way to determine the unknown chronological age of the person involved in judicial or legal proceedings. Dental cementum is a vital tissue which demonstrates continuous apposition throughout the life of the tooth. Literature studies have revealed that tooth cemental annulations(TCA) would serve as a reliable tool in establishing the age of an individual. The use of specialized microscopic methods are been employed to enhance the assessment of the cemental annulations. The study aims at assessment and evaluation of cemental annulations in normal and periodontally diseased extracted teeth using phase contrast microscopic method. **Methodology**: A total of 60 teeth were included in the study and out of which thirty teeth were normal and 30 were periodontally involved teeth respectively. Longitudinal ground sections were prepared using micromotor and diamond disc. Sections were mounted and observed under phase contrast microscope. Measurements were made using an image analyzer software. The total width of cementum was divided by the distance between two incremental lines. The eruption age of the tooth was then added to this to obtain the chronologic age for each individual. The results of the study showed no significant deviation of estimated age from the actual age in both periodontally sound and periodontally diseased teeth.

**Conclusion**: The study concludes that the use of phase-contrast microscopy in conjunction with image enhancement procedures improves the accuracy of age estimation and may serve as a reliable aid in forensic identification.

***Key Words:*** *Age estimation, cementum, forensic odontology, microscope*

## Introduction

Human individuality is the trademark of civilization, and the identification of this individualism plays an important role in human identification. In this modern speedy epoch, several incidents are taking place, varying from natural calamities to unwanted disasters. Identification of the victims of such disaster is of paramount importance in forensic medicine.[1] Forensic odontology is considered as reliable and trustworthy for identification of the deceased as well as living individuals. In the oro-facial region tooth serves as the best source in forensic science. Teeth are one of the hard tissues which undergo least amount of degenerative changes when exposed to environmental factors.[2]

Age estimation plays a vital role in the recognition of an individual. Assessment of age is not only done in the deceased but it may be many a times necessary to determine the age of living persons who are either unwilling or unable to reveal their identity.[3] Teeth undergo structural changes throughout the life, these

changes form the core essence for age estimation. Cementum is a hard tissue of tooth root which is deposited regularly throughout the life of an individual, even when individual growth is completed.[4] This regular deposition of cementum occurs in a rhythmic pattern ensuing the appearance of dark and light bands. One pair of dark and light band represents one increment which is deposited annually.[5] The counting of cemental annulations i.e. the Tooth Cementum Annulation(TCA) method was applicable to historical skeletons and cremations to assess the age at death and has now been extended to forensic cases.

Counting cemental annulations require histological examination of thin tooth sections. Previous studies have demonstrated the use of various microscopic methods and reported that incremental lines are best viewed through phase contrast microscopy. Technical improvements and new technologies to help differentiate the lines have been proposed[6], but decreased accuracy of the technique in more advanced ages and the influence of periodontal diseases are still factors that require better understanding.

Few studies have applied TCA method on periodontally affected teeth and demonstrated varying results. Therefore, the purpose of this study was to evaluate the correlation between the number of incremental lines in cementum and age of the individual with the aid of phase contrast microscope; and also, to analyze the influence of periodontal health on the age estimates.

## Methodology

The present study was carried out in the Department of Oral Pathology and Microbiology. The study was conducted on extracted teeth from individuals of age ranging from 20 - 50 years. Extracted teeth were obtained from the Department of Oral and maxillofacial Surgery. The study samples comprised of a total 60 teeth, of which 30 were sound teeth and remaining 30 were periodontally diseased teeth, respectively. The chronological age of the patients from whom the teeth were extracted was recorded. The teeth selected for the study were fulfilling the following criteria:

**Inclusion criteria:**

Teeth extracted for the following reasons were included in the study: -

- Orthodontic treatment
- Prosthetic treatment
- Impactions
- Periodontal disease

**Exclusion criteria:**

Teeth extracted for the following reasons were excluded in the study: -

- Teeth with periapical pathologies
- Root canal treated teeth
- Teeth with history of trauma

**Preparation of ground sections:**

The extracted teeth were preserved in 10% buffered formalin. Formalin fixed teeth were rinsed in water for several hours. Teeth were then treated with sodium hypochlorite and hydrogen peroxide to remove the organic debris and stains. Longitudinal sections of teeth were prepared using low speed micromotor and diamond disc. The sections were then ground using arkansas stone. The sections were then mounted using DPX.

**Microscopic analysis:**

These sections were examined under phase contrast microscope at a magnification of 10X, using Olympus research microscope (BX53). On microscopic examination the cementum showed alternately arranged dark band and light band. One dark band along with a light band following it was considered as one annulation.

The middle portion of the root was selected for counting the annulations.[7] The apical part of cementum was excluded because of the increase in thickness and cellularity of cementum in this region.[8]

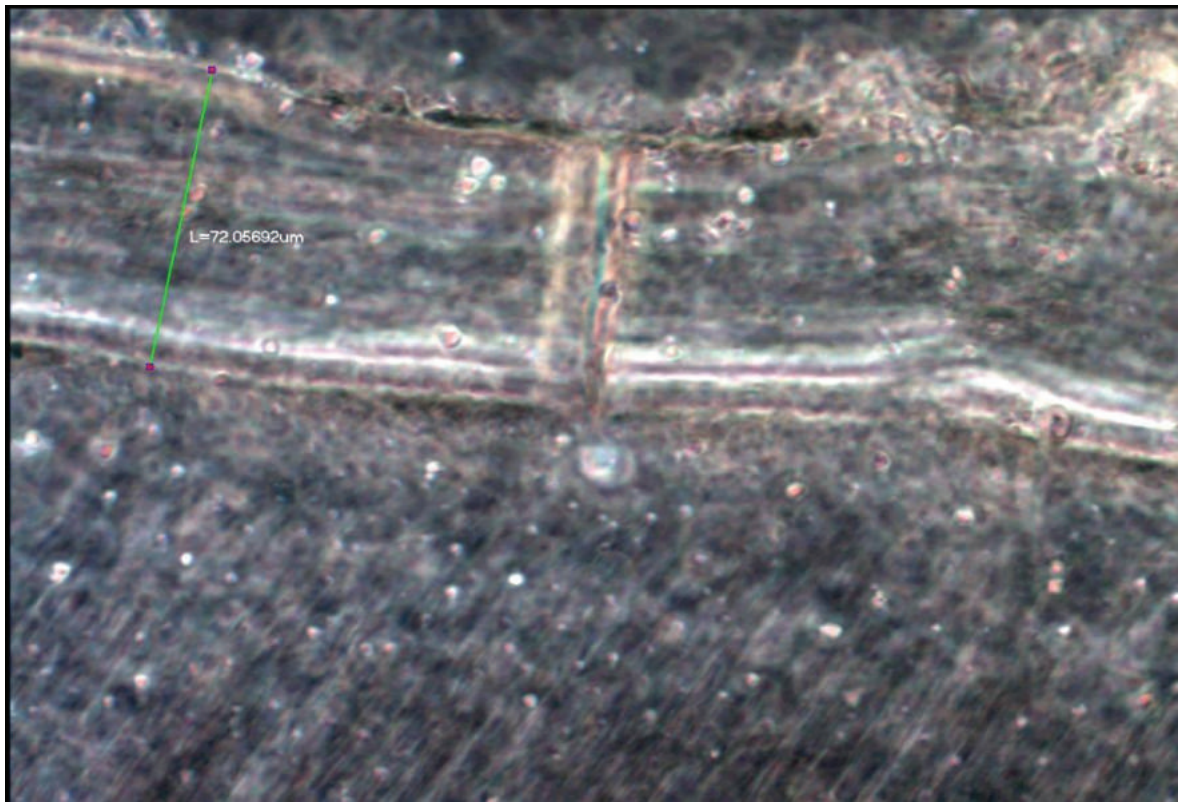



**Figure 1: Photomicrograph of normal tooth showing measurement of total width of cementum (ground section 10x)**

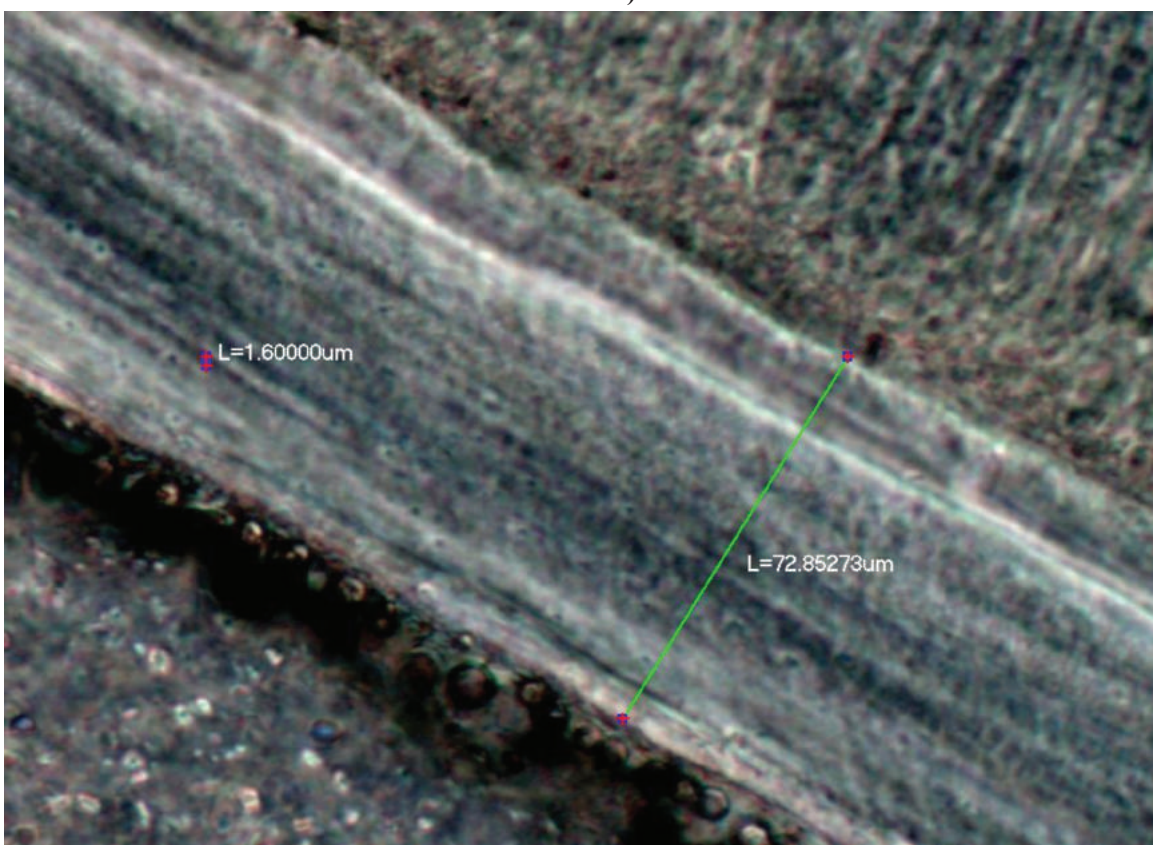



**Figure 2: Photomicrograph of periodontally diseased tooth showing measurement of total width of cementum (ground section 10x)**

The measurements were made for the total width of cementum and the width between two adjacent incremental lines. (Figure 1 and 2). The width of cementum was taken from the surface of cementum to the dentinocemental junction (DCJ) and the width between two incremental lines was taken between two adjacent lines which were easily recognizable and seemed to run approximately parallel to each other. (Figure 3 and 4).

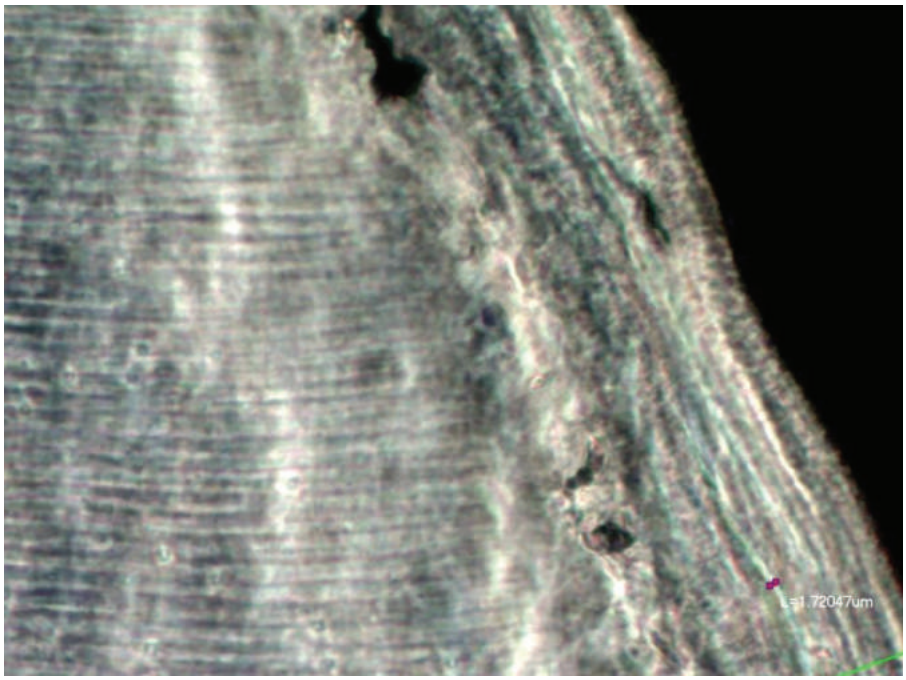



**Figure 3: Photomicrograph of normal tooth showing measurement of width between two incremental lines of cementum (ground section 10x)**

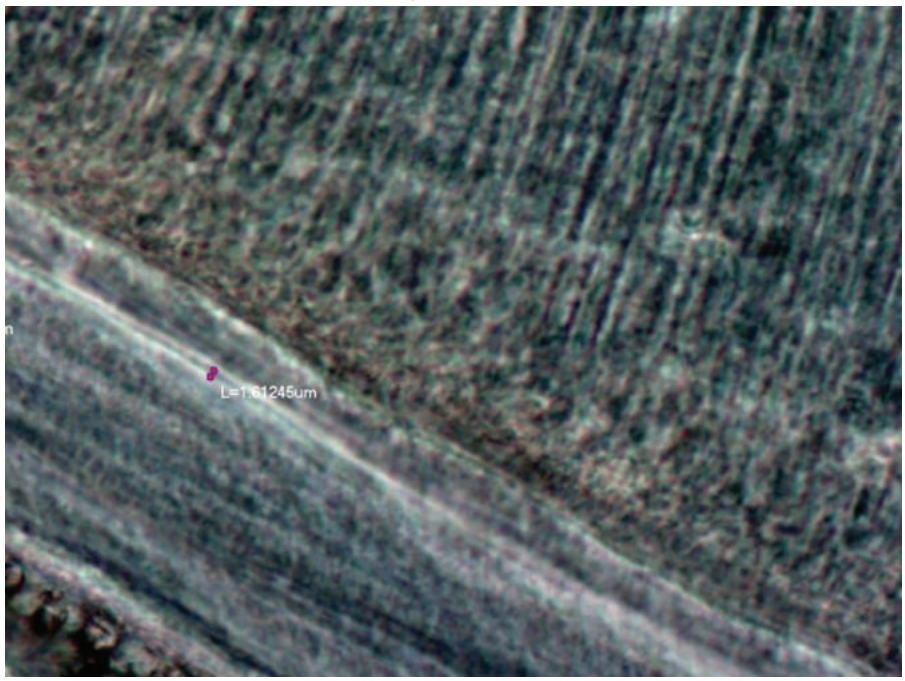



Figure 4: Photomicrograph of periodontally diseased tooth showing measurement of width between two incremental lines of cementum (ground section 10x)

The area selected for counting was photographed under 10X magnification, using a digital camera. The images were then transferred from the microscope to a computer, and counting was done with the help of image analyzer software (ScopeImage 9.0). To reduce interobserver variability, the counting of cemental lines was done by three observers. The mean value of the three observations was used for statistical evaluation.

Estimation of age:

The number of incremental lines in the total cementum width was calculated by the formula:

Number of incremental lines (n) = X/ Y

where, X is the total width of cementum (from DCJ to cementum surface) and Y is the width of cementum between the two incremental lines.

The chronological age of the individual was obtained, by adding average age of eruption in years for each tooth to the counted number of incremental lines.[9]

Estimated age (E) = number of incremental lines(n) + eruption age of tooth (t)

Statistical analysis:

The data obtained was subjected to statistical analysis. Data was analyzed using Statistical Package for the Social Sciences (SPSS) Ver 23.

1. Descriptive, student paired t test was done for intragroup comparison of actual age and estimated age.

2. Regression analysis was done for reliability of the procedure of age estimation in normal and periodontally diseased teeth.

## Results

Dark and light incremental bands of cementum were observed in all the longitudinally sectioned specimens. Cemental annulations in longitudinal sections of sixty teeth respectively were counted and analyzed for their correlation with actual age of the person using phase contrast microscope. The measurements of cemental annulations, calculated and the actual chronological age of individuals were tabulated for sections of normal teeth and sections of periodontally diseased teeth.

**Table 1: Comparison of mean values of actual age and predicted age in both groups**

| Group | N | Actual Age | | Estimated Age | | Mean Difference in Age | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | t value | p value |
| Normal Teeth | 30 | 27.833 | 5.9659 | 28.903 | 6.1250 | -1.07 | 1.7 | -3.442 | 0.002** |
| Periodontally compromised teeth | 30 | 39.367 | 8.4261 | 40.393 | 8.4818 | -1.02 | 1.67 | -3.354 | 0.002** |

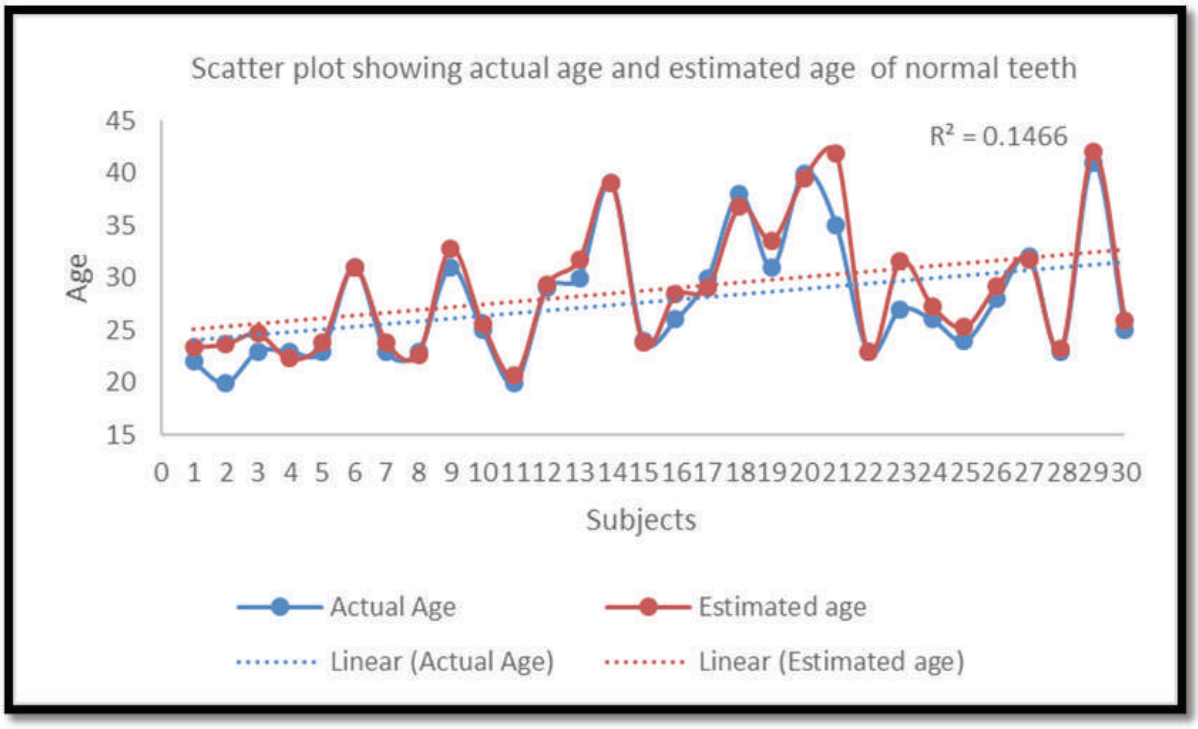
**-Statistically Highly significant ($p<0.01$)

The mean and SD of actual age and estimated age in both the groups are compiled in Table 1. There is statistically significant differences present in the mean values of actual age and estimated age in both the groups (p=0.002), (-) value indicates that actual age is less than the predicted age.

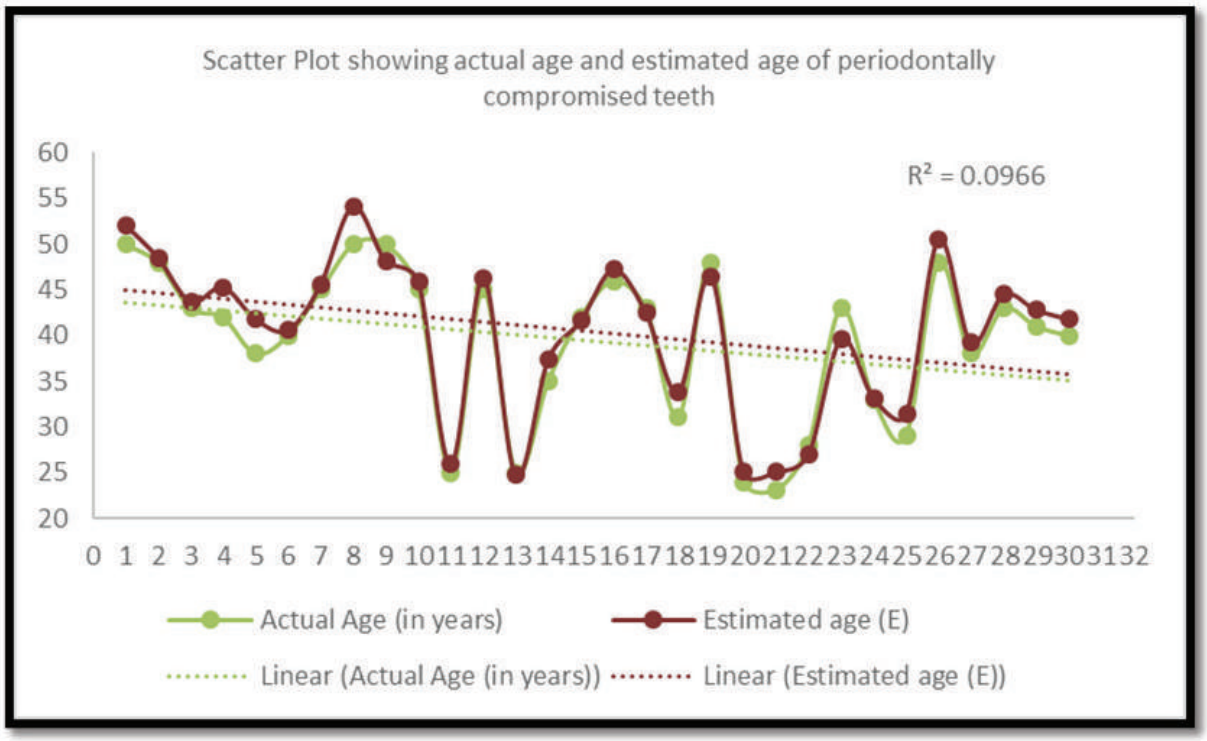
**Table 2:** *Regression analysis for validation of the estimated age*

| Group | Variables | (B) | Standard Error | t Stat | P-value | Lower 95% | Upper 95% | R square | Std Error | F value | Sig |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | Intercept | 0.79 | 1.51 | 0.52 | 0.61 | -2.30 | 3.88 | 0.922 | 1.685 | 335.12 | <0.001** |
| | Actual Age | 0.94 | 0.05 | 18.31 | 0.00 | 0.83 | 1.04 | | | | |
| Group 2 | Intercept | 1.54 | 1.51 | 1.02 | 0.31 | -1.55 | 4.64 | 0.961 | 1.702 | 691.91 | <0.001** |
| | Actual Age | 0.99 | 0.04 | 26.30 | 0.00 | 0.91 | 1.06 | | | | |

**-Statistically Highly significant (p<0.01)




**Graph 1: Scatter plot showing actual age and estimated age of normal teeth**




**Graph 2: Scatter plot showing actual age and estimated age of periodontally diseased teeth**

Table 2 is a consolidated Regression analysis test output. For normal teeth, the reliability value of 0.92 which indicates the equation for age estimation is 92% accurate and the standard error of the estimate is 1.68 which indicates that out of 100 entries there is a chance of error in 2 individuals. ($p<0.01$). For periodontally diseased teeth, the reliability value of 0.96 which indicates the equation for age estimation is 96% accurate and the standard error of the estimate is 1.70 which indicates that out of 100 entries there is a chance of error in 2 individuals ($p<0.01$). The scatter plot revealed only minor deviations from the normal distribution and allowed regression analysis.

Regression equations:

Group 1: Normal teeth

Estimated Age (Y) = 0.79 + 0.94 * Actual Age (X)

Group 2: Periodontally diseased teeth

Estimated Age (Y) = 1.54 + 0.99 * Actual Age (X)

## Discussion

Age estimation is a domain of the forensic sciences which forms an important part of every identification process, especially when information relating to the deceased is unavailable. Teeth are considered to be a source of abundant data in the process of somatic development and the vicinity in which they are formed. The periodic incremental features present in teeth provide information on the developmental rate and can be used to assess the developmental age of a tooth.[10]

Cementum is one such specialized calcified structure of teeth that covers the entire surface of root. Cementum is deposited in layers during and after eruption of the tooth. Continuous apposition of cementum leads to formation of two types of layers with different optical properties. Histologically, these layers are visible as alternate light and dark lines or bands.[11] The layered appearance is due to the structural differences in the mineral phase, an optical phenomenon that is possibly related to altered mineral crystal orientation and reflects a cyclic annual formation pattern.[12]

Each pair of these alternate bands accounts for one incremental deposit. The number of incremental deposits when added to the chronological year of eruption of the respective tooth gives the histologic age of the individual under study.[5] Cementum comprises of a biological evidence that can be used to estimate the age of the individual.[13] Many researchers have suggested the use of cementum of teeth for determination of human chronologic age.[8]

Cementum annulations have been studied in previous literature extensively in animals and humans.[14] Several authors have studied TCA for age estimation and reported varied findings ranging from failure in applying the technique to humans, to a significantly low margin of error in age estimations for this technique.[15] So, the present study was designed to evaluate the age using TCA method.

In the current study, a sample of sixty extracted teeth was included from patients with an age range between 20 and 50 years old since by the age of 20 years growth and dental development were completed as confirmed by Someda et al.[16]

The method of tooth sectioning is also an important consideration. Some authors prefer cross sections and others reported longitudinal sections to be appropriate, each section having some advantages and disadvantages over the other.[17] Longitudinal sections allow examining the whole root surface such as advocated by Klevezal and Kleinenberg[18]. Stott et al.,[14] and Avadhani et al.,[8] prefer cross sections as they allow a series of observations. Maat et al.,[19] recommended to cut the sections perpendicular to the exterior of root not perpendicular to the root axis. Mallar et al.,[17] compared longitudinal sections with cross sections for age estimation and found that longitudinal sections were better than cross sections for estimating age.[17] So, in the present study, longitudinal sections have been used to evaluate cemental annulations.

Sectioning of tooth can be done either manually by preparing ground sections on Arkansas stone or by using a hard tissue microtome. Hard tissue microtomes can be used to maintain uniform thickness of the sections. Structures which are visible in ground sections of about 100-µm thickness may disappear in thin sections. Use of hands in grinding was injurious to the fingers.[20] Although hand grinding is tedious and injurious method, some investigators prefer this and are of the opinion that it is not replaceable by any other method.[17] The present study used manual sectioning.

Various authors have compared the feasibility of counting cemental annulations using different microscopes. Pundir S et al., estimated age based on TCA method using bright field, polarizing and phase contrast microscopy and suggested that the incremental lines are best viewed through phase contrast microscopy.[21] Similar findings were suggested in other studies conducted by Bhondey A et al.,[22] Gowda CBK et al.,[3] and Kaur P et al.,[23] who observed that phase contrast microscopy was better than light microscopy for evaluating the cemental lines. The clarity of TCA by phase contrast microscope was explained by Sanderson who discussed how phase contrast microscope can properly distinguish between two types of layers with different optical properties. He found that phase contrast microscope depends on refraction of light by the specimen as it utilizes the difference between light rays propagating directly from the light source and light rays refracted by the specimen when light passes through it to add bright/dark contrast to images of transparent specimens and this done by the aid of a phase-contrast objective and a condenser fitted with phase contrast microscope for observations. He depicted that phase contrast microscope is suitable for viewing colorless and transparent specimens (as cementum).[20] So, the present work illustrated that the use of phase contrast microscopy served as a good observatory for assessing cementum annulations.

Manual counting of cemental lines is time consuming and is potentially subjective. The use of an image analyzer has got an added advantage of enhancing the view for measuring the cemental lines. In the present study, phase contrast microscopy in conjunction with image analyzer were used to enhance the cemental annulations, thereby reducing the margin of error of using simpler microscopic method.

In the present study, we used the middle third region of the root for counting cementum annulations similar to the studies conducted by Mallar et al.,[17] and Aggarwal et al.[9] In the mid-root region of a tooth, the cementum present is usually acellular and the annulations are evenly placed and less compressed than the cementum near the cementoenamel junction (CEJ).[11,24] Acellular cementum examination in the mid root section minimizes hindering factors such as cementocytes, has adequate thickness because of its slow and constant growth and is thus more useful in estimating age of death.[20]

The results of our study are in consensus with Charles et al.,[24] Condon et al.,[25] Maat et al.,[19] and Witwer Backofen et al.,[26] who reported a well-correlated connection between cementum layering and chronological age in their studies with a significant p value of $< 0.05$. However, our results contradicts the studies done by Lipsinic et al.,[26] Lucas and Loh and Miller et al., who did not reveal a relationship between chronological age and count of tooth cementum annulations in their studies.[23]

In the current research, we assessed the TCA method for age estimation in normal teeth and periodontally compromised teeth. Wittwer Backofen et al.,[26] used the computer software for counting the cemental annulations and found that the variation between the actual and estimated age was found to be in the range of 2-3 years. Aggarwal P et al.,[9] in their study found a mean error of 1-2 years. In our study, the variation between the actual and estimated age was found to be 1 year in both normal and periodontally compromised teeth. The calculated probability is 0.002 for both the groups which is statistically highly significant ($p<0.01$).

The variability in age of eruption of a certain tooth type, also the incremental line count will always lead to an age determination within a certain range, but in our study we considered the mean eruption age rather than the eruption age range. If the age range of eruption of tooth was considered it would correspond to the variation range in calculated age.

The statistics also revealed the effect of periodontal health on age estimates. The results in the present study showed that TCA is applicable to periodontally sound teeth as well as in periodontally diseased teeth. It is observed that normal teeth showed a reliability value of 92% and periodontally compromised teeth showed 96% respectively. Our study is found to be in accordance with Wittwer Backofen,[26] Aggarwal P[9] and Tyagi N[7] who concluded that the cemental annulation count did not vary in the presence of periodontal disease, but our findings contradicted the results of Dias PEM[6], who suggested that a correlation exists between incremental cemental lines and actual age, but however, this correlation decreased if individuals have periodontal problems. Kvaal and Solheim[28] found that teeth extracted because of periodontal disease showed a weaker correlation with age than did the sound teeth. Kagerer and Grupe[5] depicted that periodontally affected teeth with sufficient nutritional support of their roots showed minimal deviation of histological age from the known age.

In our study, we assessed a lower mean age (age range 20-50years), as literature recommended an age-limited applicability of the TCA method for age estimation.[56] According to studies conducted by **Kvaal** and **Aggarwal**, in lower age groups the correlation coefficients between chronological tooth age and incremental lines were stronger than in the higher age groups.[9,28] The accuracy of tooth cemental annulation method decreased with increasing age. Usually this was interpreted as a metabolic disorder of higher age with the influence of periodontal regression, dental caries, or other individual characters cumulating over age.[29] Previous studies indicated that predicted age counts for those over 55 years of age showed greater divergence from actual age.[26] Similar to the previous studies we have also found a stronger correlation in lower age group.

Our study sample included 3 samples of third molar teeth, 2 in normal teeth group and 1 in periodontally involved group. Deviation of calculated age from actual age was observed in these teeth. Third molars are teeth with highest variability concerning anatomy, agenesis

and age of eruption and therefore its significance as developmental marker has been questioned. Age estimation by means of third molars is limited due to its biological variance.[30]

In the current research we also observed that there was no substantial influence of periodontal health on the estimated age. This provides a strong support for application of TCA method in archeological skeletal samples in which most individuals suffered from extreme dental disease.

According to our study we found that tooth cementum annulation method is the most reliable method for age estimation when used with special microscopic methods such as phase contrast microscopy and digital image enhancement procedures.

## Conclusion

Teeth are particularly useful in age estimation as they display a number of observable age related variables and they tend to remain intact under circumstances which might alter or obliterate the rest of the skeleton. Countable cemental annulations are present in human teeth, and which when appreciated can be used for estimation of age of an individual with accuracy. The histological assessment of cemental annulations using phase contrast microscope and digital enhancement narrows down the error rate to just over one year making this method more reliable. Moreover, no significant influence on annual production of incremental lines was observed in the periodontally diseased teeth. This technique may be extremely valuable in forensic medicine, forensic dentistry, and anthropology. Further studies are needed to determine the availability of TCA for individuals older than 50 years. A much larger sample size would shed more light on evaluating the impact of periodontal disease on the estimated age.

**Ethical approval:**

Ethical approval was obtained from the Institutional Ethical Review Board. Reference number: IERB/2015-16/16.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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# Vascular Endothelial Growth Factor (VEGF) Expression on Placenta Accreta Spectrum (PAS) FIGO Grading

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## Abstract

This study aims to analyze the role of placenta VEGF level using immunocytochemical techniques and comparing the obtained results with PAS FIGO grading. This retrospective cross sectional study on total sampling of formalin fixed paraffin-embedded tissue from placenta sampling of elective surgery which were diagnosed as PAS. Sample were immunostained using VEGF and semi-quantitatively Immuno Reactive Score (IRS) measured based on modified Remmele scale index. The result obtained 32 samples that meet inclusion criteria which are found into grade I (n=5), grade II (n=11) and grade III (n=16). This study showed average VEGF at grade I (4.68±1.03), grade II (3.81±1.41), grade III (4.46±1.82) and no significant difference of VEGF expression between grade I, grade II and grade III (p = 0.264). Intraplacental VEGF do not describe the severity of PAS FIGO grading.

***Keywords:*** *Grading, placenta accreta, VEGF*

## Introduction

Major cause of maternal mortality in Indonesia are postpartum hemorrhage, hypertension during pregnancy and infection. One of the causes of heavy bleeding and difficult to control during surgery were placenta accreta spectrum (PAS). The incidence of PAS in Indonesia has risen from 2013 to 2016 with the highest rate being in Dr. Soetomo Surabaya, amount to 0% to 4% of total deliveries[1].

One of the placental angiogenic factors is Vascular Endothelial Growth Factor (VEGF). VEGF is secreted in the placenta, causing vasodilation, vasculogenesis and angiogenesis[2,3]. Marked increase in VEGF has been postulated as the cause of placental hypervascularity in PAS[4,5,6]. PAS grading describes the severity of villi invasion into the myometrium. FIGO (The International Federation of Gynecology and Obstetrics) in 2018 divides into grade 1-3C based on clinical and histological[7]. The molecular mechanism of this grading remains unknown. This study aims to analyze the role of placenta VEGF level using immunocytochemical techniques and comparing the obtained results with PAS FIGO grading.

## Method

Retrospective analytic study using total sampling of pregnant women with PAS who underwent elective surgery at Dr. Soetomo Hospital Surabaya in 2020. Subjects compiled using inclusion criteria of paraffin block derived from placenta accreta tissue of patients with third trimester gestational age with PAS which was performed elective surgery that had examined by transabdominal sonography prior to surgery with complete data. Meanwhile, the exclusion criteria were incomplete data using previous criteria. From 149 PAS subjects, only 32 subjects have met the inclusion criteria. The confirmation of PAS was

done during surgery performed by a Maternal-Fetal Medicine staff, Dr. Soetomo hospital. Tissue collected during surgery, namely the placenta samples were microscopically examined using VEGF immunohistochemical staining were obtain pathology anatomy Department, Dr. Soetomo Hospital. The paraffin blocks of samples were cut into 4 µm sections with Leica microtome into slide. The slides then were staining with monoclonal antibodies for VEGF (C-1–sc7269 dilution 1:200; Santa Cruz Biotechnology, Dallas, TX, USA). For the evaluation of VEGF expression, a modified semiquantitative IRS scale of Rammele was applied. The method takes into account both percentage of positive immunoreactive cells and intensity of reaction colour (Table 2)[8,9]. Data were analyzed using SPSS version 24. Kruskal Wallis test was used to measure the comparison between variables. A p-value of less than 0.05 was considered statistically significant.

## Results

### Subject Characteristics

In this study the mean age of the mother, gestational age at ultrasound, age at termination, BMI, parity, in grade I, grade II and grade III groups did not differ significantly or homogeneous. Subjects' characteristics of the study were shown in the table below:

**Table 1. PAS Subjects Characteristics.**

| Variables | PAS( Grades 1-3C) (n=32) | | | |
|---|---|---|---|---|
| | Grade I (n=5) | Grade II (n=11) | Grade III (n=16) | P-value |
| **Details:** | **(mean±SD)** | **(mean±SD)** | **(mean±SD)** | |
| Maternal age (years) | 34.80±3.76 | 32.27±6.38 | 33.80±3.36 | 0.544 A |
| Gestational age at US | 33.0±2.44 | 34.08±4.20 | 33.40±3.04 | 0.807 A |
| BMI (mean±SD) | 28.43±4.65 | 27.21±4.56 | 25.88±3.68 | 0.585 A |
| Normal | 1 (20%) | 3 (25%) | 6 (40%) | 0.388 C |
| Overweight | 2 (40%) | 6 (50%) | 8 (53.3%) | |
| Obesity I | 2 (40%) | 2 (16.7%) | 0 (0%) | |
| Obesity II | 0 (0%) | 1 (8.3%) | 1 (6.7%) | |
| **Parity** | | | | 0.059 C |
| GII | 1 (20%) | 4 (33.3%) | 2 (13.3%) | |
| GIII | 2 (40%) | 1 (8.3%) | 8 (53.3%) | |
| GIV | 0 (0%) | 5 (41.7%) | 3 (25%) | |
| GV | 0 (0%) | 2 (16.7%) | 0 (0%) | |
| GVI | 2 (40%) | 0 (0%) | 1 (6.7%) | |
| GVII | 0 (0%) | 0 (0%) | 1 (6.7%) | |
| Gestational age during termination | 33.60±2.07 | 34.58±3.91 | 34.67±2.05 | 0.768 A |

***Cont...*** **Table 1. PAS Subjects Characteristics.**

| | | | | |
|---|---|---|---|---|
| **Risk Factors** | | | | |
| **History of curettage** | | | | 0.704 C |
| No | 5 (100%) | 11 (91.7%) | 13 (86.7%) | |
| Yes | 0 (0%) | 1 (8.3%) | 2 (13.3%) | |
| **C-section number** | | | | |
| Prev CS 1x | 4 (80%) | 8 (72.7%) | 5 (31.3%) | 0.045 C |
| Prev CS 2x | 1 (20%) | 2 (18.2%) | 10 (62.5%) | 0.042 C |
| Prev CS 3x | 0 (0%) | 1 (9.1%) | 1 (6.3%) | 0.785 C |
| Placenta previa | 5 (100%) | 11 (100%) | 16 (100%) | - |

Result were analyzed by C : chi-squared test; A : one way-anova test

**Table 2. Semiquantitative IRS scale taking into account both percentage of positive cells (A) and intensity of the reaction colour (B), with the final score representing product of two variables (A×B).**

| A | B |
|---|---|
| 0 pts - no cells with positivie reaction | 0 pts no colour reaction |
| 1 pt - to 10% cells with positivereaction | 1 pt - low intensity of colour reaction |
| 2 pts - 11% - 50% cells with positve reaction | 2 pt - moderate intensity of colour reaction |
| 3 pts - 51% - 80% cells with positive reaction | 3 pts - intense colour reaction |
| 4 pts- > 80% cells with positive reaction | |

**Comparison Analysis of VEGF levels and PAS Grading**

**Table 3. Comparison Results of VEGF in Grade I, Grade II, dan Grade III Groups.**

| Variables | PAS Grading (n=32) | | | P values |
|---|---|---|---|---|
| | Grade I (n=5) | Grade II (n=11) | Grade III (n=16) | |
| VEGF | 4.68±1.03 | 3.81±1.41 | 4.46±1.82 | 0.264* |

Notes: * = Significant at alpha 0.05. Result were analyzed by Kruskal Wallis test.

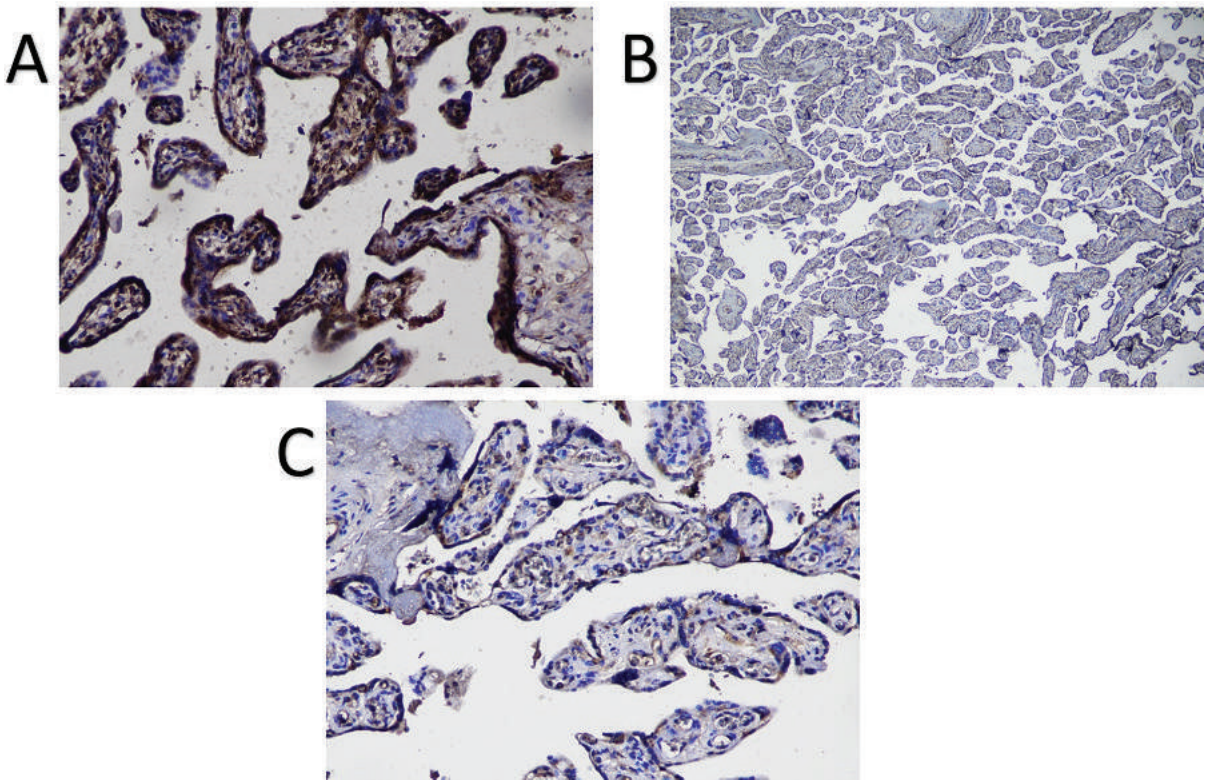


**Figure 1. Immunohistochemical expression of VEGF in various grade of PAS. A: 80% placenta cell were stained with strong intensity, 200× magnification; B: 51 % placenta cell were stained with moderate intensity, 40× magnification; C: 25% placenta cell were stained weak intensity, 200× magnification.**

## Discussion

In cases of PAS, trophoblast invasion occurs through the decidua basalis to the uterine myometrium, uterine serosa or even to the bladder serosa, extending to the parametrium and its surroundings. Based on the histopathological classification, it is divided into three, namely the placenta accreta when trophoblast cells attach to the surface of the myometrium and no decidual layer is found, placenta increta when trophoblast cells invade into the myometrium, and placenta percreta when trophoblast cells invade to the uterine serosa or to the bladder[10]. Meanwhile, the grading of the PAS from FIGO is clinical and histopathological[7]. In PAS, there is an imbalance in angiogenesis, namely an increase in angiogenic factors and a decrease in antiangiogenic factors[11,12,13]. One of the angiogenic factors was vascular endothelial growth factor (VEGF), which is produced by syncytiotrophoblast in greater numbers than in normal placentations, thereby increasing the degree of placental invasion of the uterine wall[14,15]. In our study, based on the results of the Kruskal Wallis test, it showed a significance value of 0.264 ($p > 0.05$), which means that there was no significant difference in VEGF between grade I, grade II, and grade III groups. It may be not only intraplacental VEGF that affect the increase in angiogenesis in PAS, but it is also affected by other factors, such as decreased expression of mRNA 34a, E-cadherin (E-CAD), epidermal growth factor EGF c-(erbB-2), transforming growth factor beta (TGF B), vascular endothelial growth factor receptors-2 (VEGFR-2), the endothelial cell receptor tyrosine kinase (RTK), Tie-2 or increased for the epidermal growth factor receptor (EGFR) and the TIMP-1 tissue inhibitor of matrix metalloproteinase[16].

## Conclusion

In summary, there is no difference in placental VEGF between grade I, grade II, and grade III, because Intraplacental VEGF do not describe the severity of PAS FIGO grading.

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

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**Ethical Approval:** This study was approved by the Health Research Ethic Commitee of Dr. Soetomo General Hospital, Surabaya, Indonesia (Approval number: 0274/LOE/301.4.2/I/2021).

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*Original Article*

# Sexual Dimorphism and Clinical Relevance of Thickness and Angulation of Lateral Lamella of Cribriform Plate-A Multidtector Computed Tomographic Study

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## Abstract

**Background:** The aim of this study was to establish the morphometry and sexual dimorphism of lateral lamella of cribriform plate in the North Karnataka region.

Method: Six hundred and forty four (644) Multidetector computed tomographic scans were collected from North Karnataka region and analyzed by using RadiAnt DICOM Viewer. The thickness and angulation of lateral lamella of cribriform plate of olfactory fossa were calculated on both the sides in both genders. Statistical analysis was done by using Student unpaired‘t’ test.

**Conclusion:** There was statistically significant difference in the angle of lateral lamella of cribriform plate when compared between right side (mean=106.25˚) and left side (mean=109.38˚) in males. There was no such difference among females. Statistically significant difference was observed in the angle of lateral lamella of cribriform plate in male patients (mean=106.25˚) when compared to female patients (mean=109.61˚). The thickness of lateral lamella of cribriform plate was not significant when compared between two sides in males and females. Hence, these variations in the morphometry of the lateral lamella of cribriform plate of ethmoid bone will be of importance for the surgeons in Functional endoscopic sinus surgeries and anterior skull base surgeries.

***Key-words:*** *Angle, Ethmoid bone, Lateral lamellae of cribriform plate, Sexual dimorphism, Thickness.*

## Introduction

The lateral lamella of the cribriform plate (LLCP) is a part of the ethmoid bone in the anterior skull base. The LLCP joins laterally with the orbital plate of the frontal bone which protects the olfactory fossa[1] LLCP forms the lateral boundary of the olfactory fossa. The lateral lamella of the cribriform plate is significantly shorter and less oblique in men than in women. The length of the lateral lamella was greater anteriorly than posteriorly in both sexes.[2] The two most common sites of skull-base injury associated with endoscopic sinus surgery are the lateral lamella of the cribriform plate and the posterior ethmoid roof near the anterior medial sphenoid wall.[3] The thin LLCP[4] offers less resistance to perforation which

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can lead to complications like direct penetration, trauma to dura matter, intracranial and intracerebral injuries during surgeries.[5] Hence, the morphometry of LLCP provides assessement during various surgical procedures like medial orbital wall decompression.[6]

Very long and acutely angled lateral lamella predisposes patients to iatrogenic injury during surgeries. The angle of the LLCP was wider in many diseases like Kallmann syndrome. The angle of LLCP varies significantly in males and females according to the positions of the skull base.[7] Type of olfactory fossa with lesser angles are expected to be safer when compared to other types of olfactory fossa with greater angles during surgical procedures.[8] The level of the ethmoidal roof is affected by the angle of the LLCP which plays an important role in surgeries of anterior skull base.[9]

Successful outcome of Endoscopic sinus surgeries with minimal complications can be achieved with prior knowledge of the endoscopic anatomy of anterior skull base and anatomical variations. Hence Radiological investigations and image-guided systems becomes an important tool which help the surgeons to perform safe and successful surgeries.[10]

With regard to less data available in North Karnataka region, this study was undertaken to determine the morphometry of olfactory fossa in both genders which would help the neurosurgeons and endoscopic surgeons to assess the LLCP during various skull base and endoscopic sinus surgeries.

Aim

The aim of this study was to establish the morphometry and sexual dimorphism of

Lateral lamellae of cribriform plate in North Karnataka region.

Objectives

a) To determine the sexual dimorphism of thickness of LLCP on both sides.

b) To determine the sexual dimorphism of angle of LLCP on both sides.

## Materials and Method

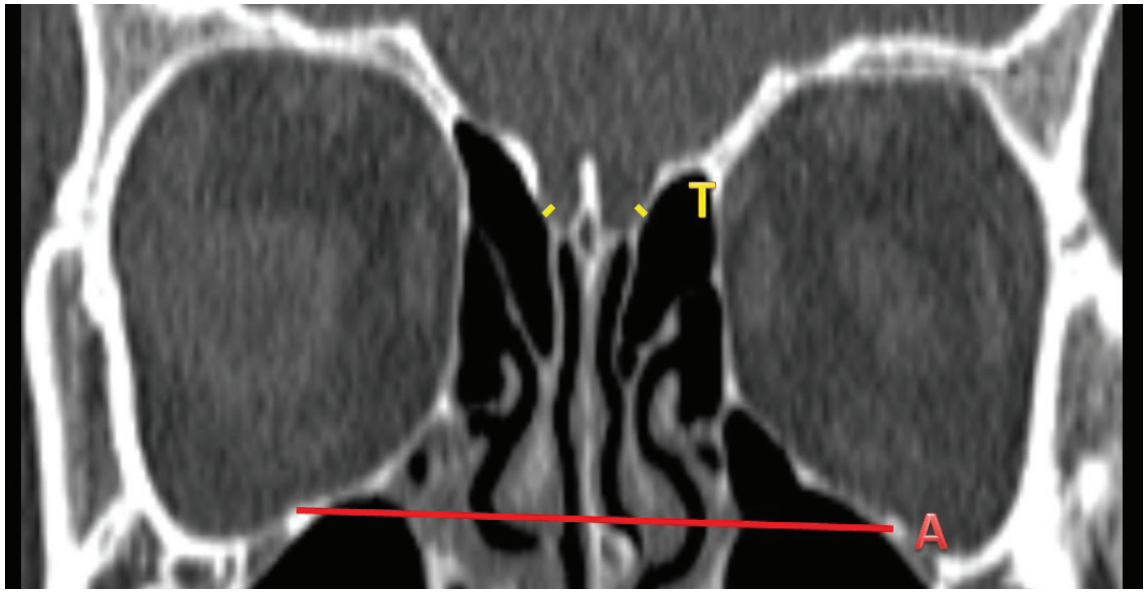
Six hundred and forty four Multidetector Computed Tomographic (MDCT) scans of the patients from all the districts of North Karnataka region of Karnataka state, India were collected after institutional ethics committee clearance. This prospective hospital based radiological study was carried out from April 2018 to April 2021.

Normal Paranasal MDCT scans of patients above the age of 16 years belonging to both genders were included in the study.

MDCT scans of Patients below the age of 16 years and MDCT scans of patients with nasal or paranasal trauma, congenital abnormalities of face, tumours or conditions involving bone destruction and surgeries were excluded from the study. While taking the MDCT of paranasal sinuses, patients were informed and instructed about the procedure before obtaining informed written consent. Axial MDCT images of 3mm thickness were taken from CT scanner (Siemens Somatom) by using bone window.

The thickness of LLCP was measured in both the sides in both genders in direct coronal scan showing the maximum depth of the olfactory fossa at the centre of infraorbital foramen as shown in figure 1.

FIGURE 1: coronal scan showing the thickness of LLCP at the centre of the infraorbital foramen

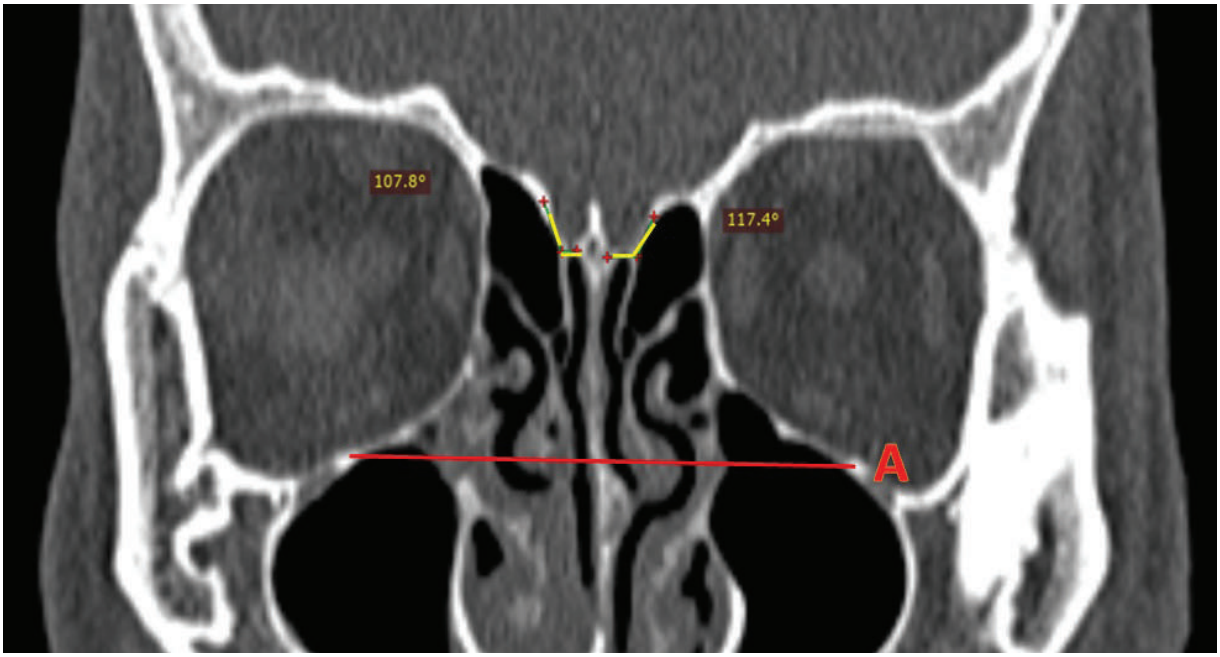



A=line at the level of infraorbital foramen

T=thickness of LLCP

The angle at which LLCP joins the cribriform plate was measured in the direct coronal scan showing the maximum depth of the olfactory fossa at the centre of infraorbital foramen as shown in figure 2.

**FIGURE 2: Coronal scan showing the angle of LLCP with cribriform plate at the centre of infraorbital foramen.**




A=line at the level of infraorbital foramen

Yellow lines showing the measurement of angle of LLCP with cribriform plate of ethmoid bone on right and left sides.

Comparative calculation of the thickness and angulation of LLCP was done on both the sides in both genders by using RadiAnt DICOM Viewer.

**Statistics**

Comparison of the thickness and angulation of LLCP on both the sides in both genders will be calculated by using Student 't' (unpaired) test. The data will be considered statistically significant if p is less than 0.05.

## Results and Discussion

The present study was carried out to determine the morphometry of the LLCP as there were less data in the North Karnataka region.

Angle of LLCP:

The angle of LLCP on both the sides in males and in females are shown in table 1 and 2 respectively.

**TABLE 1: Comparison of thickness and angulation of LLCP in both sides in males in MDCT scans.**

| Males n*=395 | Right side Mean±SD† | Left side Mean±SD† | P‡ value | Significance |
|---|---|---|---|---|
| Thickness of LLCP‖ | 1.26±0.66mm | 1.23±0.52mm | 0.47 | Not significant |
| Angulation of LLCP‖ | 106.25˚±20.35˚ | 109.38˚±16.81˚ | 0.018 | Significant |

*(n)=number,†(SD)=standard deviation, ‡(p)=significance value and ‖(LLCP)=Lateral lamella of cribriform plate.

Statistically significant difference (p=0.018) was observed in the angle of LLCP between two sides (right side angle=106.25˚ & left side angle=109.38˚) in male patients when compared to female patients.

**TABLE 2: Comparison of thickness and angulation of LLCP in both sides in females in MDCT scans.**

| Females n*=249 | Right side Mean±SD† | Left side Mean±SD† | P‡ value | Significance |
|---|---|---|---|---|
| Thickness of LLCP‖ | 1.21±0.56mm | 1.22±0.53mm | 0.8379 | Not significant |
| Angulation of OF** | 109.61˚±19.50˚ | 109.99˚±18.63˚ | 0.8241 | Not significant |

*(n)=number, †(SD)=standard deviation, †(p)=significance value, ‖(LLCP)=Lateral lamella of cribriform plate and ** (OF)= Olfactory fossa.

In females, there was no statistical difference in the angle of LLCP between both the sides as shown in table 2.

Comparison of mean angle of LLCP and its p value of both the sides in both genders are shown in table 3 and 4.

**TABLE 3: Comparison of thickness and angulation of LLCP in both sides in both genders in MDCT scans**

| n*=644 | | Males (n*=395) Mean±SD† | Females (n*=249) Mean±SD† | P‡value | Significance |
|---|---|---|---|---|---|
| Thickness of LLCP‖ | Right Side | 1.26±0.66mm | 1.21±0.56mm | 0.3219 | Not significant |
| | Left Side | 1.23±0.52mm | 1.22±0.53mm | 0.8136 | Not significant |
| Angulation of OF** | Right Side | 106.25˚±20.35˚ | 109.61˚±19.50˚ | 0.03852 | Significant |
| | Left Side | 109.38˚±16.81˚ | 109.99˚±18.63˚ | 0.6674 | Not significant |

*(n)=number, †(SD)=standard deviation, ‡(p)=significance value, ‖(LLCP)=Lateral lamella of cribriform plate and ** (OF)= Olfactory fossa.

Significant difference (p=0.03) in the angle of LLCP was also observed on the right side (mean angle in male patients=106.25˚ and mean angle in female patients =109.61˚) when compared between males and female patients as shown in table 3.

**Table 4: Comparison of thickness and angulation of LLCP in both genders in MDCT scans**

| n*=644 | Males (n*=395) Mean±SD† | Females (n*=249) Mean±SD† | P‡ value | Significance |
|---|---|---|---|---|
| Thickness of LLCP‖ | 1.21±0.56mm | 1.22±0.53mm | 0.8379 | Not significant |
| Angulation of OF** | 109.61˚±19.50˚ | 109.99˚±18.63˚ | 0.8241 | Not significant |

*(n)=number, †(SD)=standard deviation, ‡(p)=significance value, ‖(LLCP)=Lateral lamella of cribriform plate and ** (OF)= Olfactory fossa.

A study done by Luigi maione revealed highly significant wider angles of LLCP in Kallmann syndrome when compared to non congenital hypogonadotropic hypogonadism patients among 37 Kallmann syndrome patients in France.[11]

Significant differences was observed between lateral lamella angulation in men and women in a study done by S.Elwany in 300 patients in Egypt.[2]

Christian Güldner observed that the Patients with Keros type III olfactory fossa having an angle of LLCP with 107˚ are safer when compared with keros type I and type II olfactory fossa with 116˚ and 131˚ respectively on 141 patients.[8]

The angle of the lateral lamella was 63.1 ±17.8˚ anteriorly and 39.1 ±17.9˚ posteriorly ($p < 0.05$) in a study done by lucas kus.[7]

Tony Jacob observed that there was no significant difference in the angulation of the LLCP on left and right sides ($p > 0.05$).[12]

Thickness of LLCP:

The thickness of LLCP on both the sides in males and in females are shown in table 1 and 2 respectively. Comparison of mean thickness of LLCP and its p value of both the sides in both genders are shown in table 3 and 4. There were no significant differences observed in the thickness of LLCP between both the sides and in between the genders.

Keast A has revealed that the thickness of LLCP can vary from 0.2 to 0.05 mm.[13]

STRENGTHS AND LIMITATIONS OF THE STUDY:

This study will help neurosurgeons and endoscopic surgeons to assess LLCP during various skull base and endoscopic sinus surgeries as there were less data in North Karnataka region.

Only adult patients were included in the present study. This study was carried out only in patients of North Karnataka region during the study period.

## Conclusion

The thin LLCP offers less resistance to perforation which can lead to complications during various surgical procedures. Statistically significant difference ($p=0.018$) was observed in the angle of LLCP between two sides (angle=106.25˚ on right side & angle =109.38˚ on left side) in male patients. In females, there was no such difference. Significant difference ($p=0.03$) in the angle of LLCP was also observed on the right side (angle= 106.25˚ in male patients and angle = 109.61˚ in female patients) when compared between males and female patients.

The thickness of LLCP was not significant when compared between two sides in males and females. Hence, the morphometry of LLCP provides assessement in skull base surgeries.

**Ethical Clearance:** Ethical clearance was taken from BLDE (deemed to be university) Shri B M patil Medical college, hospital and research centre, Vijayapura, Institutional ethical committee and SNMC –Institutional ethics committee on human subjects research, Bagalkot.

**Source of Funding –** Self

**Conflict of Interest** –Nil

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# Effectiveness of Pisang Raja Peel Extract (*Musa Paradisiaca L*) on Bacterial Growth of *Porphyromonas Gingivalis* as the Cause of *Periodontitis*

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## Abstract

**Background.** Periodontal disease is a chronic inflammatory infection that causes damage to the tissue supporting the teeth. The most common periodontal diseases are gingivitis and periodontitis. Periodontitis is a complex, localized chronic inflammatory disease characterized by the destruction of connective tissue, periodontal ligaments, and bone supporting teeth. This periodontal disease is caused by plaque bacteria on the surface of the teeth, where the plaque is a thin layer of biofilm containing a collection of pathogenic microorganisms such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia* and *Fusobacterium nucleatum* which are soft deposits. *Porphyromonas gingivalis* is a gram-negative, black pigmented, rod-shaped anaerobic bacteria. Porphyromonas gingivalis is one of the dominant bacteria in chronic periodontitis which is found in subgingival plaques. Indonesia is a country with a tropical climate which makes it rich in biodiversity, which can be used as antibacterial agents. Plantain (*Musa Paradisiaca L*) is a fruit that is easy to get, but the skin is rarely used. The Pisang Raja peel (*Musa Paradisaca L*) contains chemical compounds saponins, tannins, flavonoids, and alkaloids which have antimicrobial properties. **Objective**: To determine the effectiveness of Pisang Raja peel (*Musa Paradisiaca L*) extract against the inhibition of Porphyromonas gingivalis bacteria as a cause of periodontitis. **Methods**: Using laboratory experimental methods, the test was carried out in the laboratory with the form of research in the form of Posttest Only Control Design and sampling by random sampling through 4 treatments and 6 repetitions. Statistical test was One Way Anova. **Results**: Based on the oneway Anova test, the concentration group of 16%, 32%, and 64% and the control group obtained a p-value of ($p < 0.01$) which means that there is a significant difference. Conclusion: Based on the results of this study, it is shown that the effectiveness of Pisang Raja peel extracts concentrations with concentration 16%, 32%, and 64% in inhibiting Porphyromonas gingivalis bacteria and control group obtained *p-value (p<0,01)* means there was significant correlation. **Conclusion**: Based on this research that there is effective correlation Pisang Raja peel extract with concentration 16%, 32%, dan 64% can inhibit the growth *Porphyromonas gingivalis*.

***Keywords****: Periodontitis, Porphyromonas gingivalis, Pisang Raja Peel (Musa Paradisiaca L)*

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## Introduction

Most people still do not make oral health problems their top priority. However, as we know, teeth and

mouth are "gateways" for the entry of bacteria so that they can interfere with the health of other organs. According to Riskesdas 2007 and 2013, the percentage of the population having dental and mouth problems increased from 23.2% to 25.9%. Of the population with dental and oral health problems, the proportion of the population receiving medical care increased from 29.7% in 2007 to 31.1% in 2013.[1]

Periodontal disease is a chronic inflammatory infection that causes damage to the supporting tissues of the teeth, characterized by loss of connective tissue adhesions and resorption of alveolar bone. The most common periodontal diseases are gingivitis and periodontitis. Periodontitis is a complex, localized chronic inflammatory disease characterized by the destruction of connective tissue, periodontal ligaments, and bone supporting teeth. This periodontal disease is caused by plaque bacteria on the surface of the teeth, where plaque is a thin layer of biofilm which contains a collection of pathogenic microorganisms such as Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Prevotella intermedia, Tannerella forsythia and Fusobacterium nucleatum which are soft deposits.[2], [3],[4], [5]

Porphyromonas gingivalis is a gram-negative, rod-shaped, black pigmented bacteria found in subgingival plaques. Porphyromonas gingivalis produces several virulences, namely fimbria, capsules, polysaccharides, lipopolysaccharides and hemolysis which are pathogenic in the oral cavity. Porphyromonas gingivalis is a bacterium that is often associated with the pathogenesis of periodontitis. In the subgingival plaques of chronic periodontitis patients, 85% of these bacteria were found. As an opportunistic bacterium that triggers periodontitis, Porphromonas gingivalis expresses various virulence factors, such as fimbriae, capsules, lipopolysaccharide (LPS), membrane proteins and membrane vesicles. Gingipain and lipopolysaccharide (LPS) were used to invade the periodontium tissue. [6],[7]

Indonesia is a country with a tropical climate which makes it has a lot of biodiversity, which can be used as antibacterial. One of them is the plantain banana with the scientific name Musa Paradisaca L which has anti-inflammatory, antioxidant and antibacterial properties.8 Several studies that have been conducted using plantain peels state that plantain peels contain saponins, alkaloids, tannins, quinones and flavonoids. which has activity as an antimicrobial. 9 Based on this description, the authors are interested in conducting research on the Effectiveness of Banana Peel Extract (Musa paradisiaca L) on Inhibition of Porphyromonas gingivalis bacteria as a cause of periodontitis.

## Methods

The research design used is True Experiment in the laboratory, namely testing is carried out in the form of research in the form of Posttest Only Control Design. This research was conducted at the Phytochemical Laboratory of the Faculty of Marine and Fisheries Sciences and the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University. This research was conducted from February-April 2021 to completion. The samples in this study were plantain peel extracts with various concentrations, namely 16%, 32%, and 64% which were tested on cultured Porphyromonas gingivalis.

A sample of plantain peel was weighed as much as 300 grams and extracted using the maceration method, soaked using 96% ethanol solvent for 24 hours with occasional stirring every 1 hour. The maceration results were filtered using a vacuum filter to extract the filtrate to obtain the ethanol maserate of plantain peels. The ethanol maserate was then concentrated using a rotary evaporator to obtain a thick extract of banana peels. Then the dilution was carried out using DMSO (Dimethyl sulfoxide) which aims to produce a concentration of 16%, 32% and 64%.

Mueller Hinton Agar (MHA) is placed on a petri dish, then the bottom of the petri dish is divided according to the number of paper disks. Before testing the inhibitory power, then making a suspension of Porphyromonas gingivalis bacteria by first using a loop needle that was previously heated above a methylated

lamp to pick up bacteria, then the bacteria on the loop needle was inserted into a test tube containing 0.85% NaCl. Then the solution is homogenized. Then enter the bacteria on the loop needle into the MC solution. Farland 0.5 to equalize the turbidity and capacity standard for the number of bacteria that will be taken.

Inhibition test on bacteria using agar disc diffusion method. In this test, replication was carried out 6 times. A total of 6 pieces of petri dishes containing MHA medium were inoculated with Porphyromonas gingivalis bacteria. Soaked the paper disk at each concentration and then put it on each petri dish. Then incubate all the petri dishes containing bacteria and banana peel extract for 1x24 hours with a temperature of 37°C. In each test group with 6 repetitions. The width of the clear zone formed is calculated using a digital digital term. The clear zone formed is a zone of inhibition of the growth of the bacterium Porphyromonas gingivalis.

## Result

The study was conducted by measuring the inhibition zone of plantain peel extract with a concentration of 16%, 32%, 64% and Chlorexidine 0.2% against the growth of Porphyromonas gingivalis bacteria. After conducting research to measure the inhibition of growth of the Porphyromonas gingivalis bacteria using a 16%, 32%, 64% concentration of Banana Banana Skin (Musa Paradisiaca L) extract solution, and a positive control of 0.2% chlorexidine with 6 replications, the results were obtained. research can be seen in table 1.

**Table 1. Diameter of Inhibitory Power Zone of Banana Banana Skin (Musa Paradisiaca L) Concentrations of 16%, 32%, 64% and Chlorexidine 0.2% on the Growth of Porphyromonas gingivalis Bacteria**

| | Banana Banana Skin Extract (Musa Paradisiaca L) | | | | | | Control (mm) | |
|---|---|---|---|---|---|---|---|---|
| Replication | Concentration 16% | Mean ± SD | Concentration 32% | Mean ± SD | Concentration 64% | Mean ± SD | K+ | Mean ± SD |
| 1. | 12 | 13,76± 0,47 | 13,6 | 15,51± 1,40 | 15,1 | 17,11 ± 1,61 | 14,2 | 16,00± 1,82 |
| 2. | 13,3 | | 14,2 | | 15,4 | | 13,5 | |
| 3. | 13,5 | | 16,2 | | 17,1 | | 16,6 | |
| 4. | 14,1 | | 17,1 | | 19,2 | | 18,4 | |
| 5. | 14,2 | | 15,3 | | 18,3 | | 16,9 | |
| 6. | 15,5 | | 16,7 | | 17,6 | | 16,4 | |

Table 1 shows that the inhibition zone has been formed in a solution of banana skin extract (Musa paradisiaca L) at a concentration of 16%, 32%, 64% and a positive control solution of 0.2% chlorexidine. The measurement results in the table above show that the diameter of the bacterial inhibitory zone in the banana peel extract solution (Musa paradisiaca L), the concentration of 16 %% at replication 1 is 12 mm, replication 2 is 13.3 mm, replication 3 is 13.5 mm, replication 4 is 14.1 mm, replication 5 is 14.2 mm, replication 6 is 15.5 mm. Inhibition zone of bacteria

in plantain peel extract solution (Musa paradisiaca l) concentration 32% at replication 1 is 13.6 mm, replication 2 is 14.2 mm, replication 3 is 16.2 mm, replication 4 is 17.1 mm , replication 5 is 15.3 mm, replication 6 is 16.7 mm. Then in the zone of bacterial inhibition in plantain (Musa paradisiaca l) peel extract solution, the concentration of 64% at replication 1 is 15.1 mm, replication 2 is 15.4 mm, replication 3 is 17.1 mm, replication 4 is 19, 2 mm, replication 5 is 18.3 mm, replication 6 is 17.6 mm.

In the control solution, a positive control solution of 0.2% chlorexidine was used which showed an inhibitory power of 14.2 mm in replication 1. in replication 2 it was 13.5 mm, in replication 3 it was 16.6 mm, then in replication 4 it was 18.4 mm, then in replication 5 it was 16.9 mm and in replication 6 it was 16.4 mm. The biggest zone of bacterial inhibition is in a solution of banana skin extract (Musa paradisiaca L) with a concentration of 64% in replication 4, which is 19.2 mm, while the lowest zone of inhibition is in a solution of banana skin extract (Musa Paradisiaca L) with a concentration of 16 % for replication 1, which is 12 mm.

**Tabel 2. Uji Normalitas**

| Type of solution | | Mean ± SD | p-value Shapirol wilk |
|---|---|---|---|
| Banana Peels (Musa Paradisiaca L) extract solution | Concentration 16% | 13,76± 0,47 | 0.913 |
| | Concentration 32% | 15,51± 1,40 | 0.620 |
| | Concentration 64% | 17,11 ± 1,61 | 0.660 |
| Positive Control | *chlorexidine 0,2%* | 16,00± 1,82 | 0.578 |

Note: Normality Test; Shapirol Wilk test: p> 0.05, normal data distribution

* Anova One-way test: p <0.01: significant

Based on the results of the Shapirol Wilk normality test, the overall solution of both the banana skin extract solution (Musa Paradisiaca L) with a concentration of 16%, 32%, 64% and a positive control of 0.2% chlorexidine showed a p-value> 0.05 so it can be concluded that all normally distributed data.

**Tabel 3. Uji One Way Anova**

| Group | Comparation | Mean Difference | Std. Error | p-value/ sig. | p-value |
|---|---|---|---|---|---|
| Banana Peels (Musa Paradisiaca L) extract solution 16% | Banana Peels (Musa Paradisiaca L) extract solution 32% | -1.75000 | 0,876 | 0.060 | 0.009 |
| | Banana Peels (Musa Paradisiaca L) extract solution 64% | -3.35000 | 0,876 | 0.001* | |
| | Chlorexidine 0,2% | -2.23333 | 0,876 | 0.019* | |
| Banana Peels (Musa Paradisiaca L) extract solution 32% | Banana Peels (Musa Paradisiaca L) extract solution 64% | -1.60000 | 0,876 | 0.083 | |
| | Chlorexidine 0,2% | -0.48333 | 0,876 | 0.588 | |
| Banana Peels (Musa Paradisiaca L) extract solution 64% | Chlorexidine 0,2% | 1.11667 | 0,876 | 0.217 | |
| *. The mean difference is significant at the 0.05 level., *Post Hoc test: Low Significant Difference (LSD) test; p<0.05: significant | | | | | |

Based on the results of the One Way ANOVA statistical test carried out, it showed a p-value of 0.009 ($p < 0.05$) or a p-value less than 0.05, meaning that there was a significant difference between the zones of inhibition of bacterial growth using a solution of bark extract. Banana (Musa Paradisiaca L) concentrations of 16%, 32%, 64% and Chlorexidine 0.2% positive control against the growth of Porphyromonas gingivalis bacteria.

## Discussion

This study has proven that banana peel extract has an effective inhibitory effect in inhibiting the growth of Porphyromonas gingivalis bacteria based on the results of statistical effect tests that have been carried out. This plantain peel extract can inhibit bacteria because of its phytochemical content in the form of effective antibacterial compounds. This is consistent with the research that tested the phytochemical compounds contained in plantain peels and identified the presence of flavonoids and saponins. The mechanism of flavonoids as antibacterials is to inhibit the synthesis of nucleic acids, quercetin, mostly due to inhibition of DNA gyrase. Sophora flavone G and (-) epigallocatechin gallate have been suggested to inhibit cytoplasmic membrane function, whereas licochalcones A and C can inhibit energy metabolism. [10]

The study was conducted in Nigeria by examining the ratio of plantain between the peel extract and its fruit extract against ten types of gram positive and negative bacteria. They obtained the results that most of the bacteria had more effect on the plantain peel extract than the fruit extract. This is due to the higher percentage of hydrocarbons, monoterpenes and oxygenated monoterpenes as potential antibacterial components in plantain peels.[11]

Ahmed (2016) states that plantain peels contain chemical compounds contained in plantain peel extract which can inhibit gram-negative and gram-positive bacteria. The chemical compounds found in banana peels are flavonoids and tannins which have biological and pharmacological properties. Plantain peels contain flavonoids and tannins which are important phytochemicals with various properties including, antibacterial, anticancer, anti-inflammatory, and antioxidant activity.[12]

Davis and Stout (1971) determined that the inhibition zone criteria showed that the antibacterial strength of 20 mm or more means very strong, 10-20 mm means strong, 5-10 mm means moderate and 5 mm or less means weak. In this study, the inhibition zone results were obtained with a concentration of 16%, 32% and 64% in mm (millimeters), namely, 13.76 mm, 15.51 mm, and 17.11 mm. Results obtained from concentrations of 16%, 32%, and 64% all indicate that above 10mm means that the strength of the antibacterial power is strong. The results of this study are consistent with research conducted at Sam Ratulangi University, Manado, Faculty of Dentistry regarding the inhibitory ability of brown algae against porphyromonas gingivalis, which found that the higher the concentration of the extract, the greater the resulting inhibition.[13],[14]

However, the anti-bacterial activity of plantain peel extract concentrations of 16% and 32% were still lower than chlorhexidine which was used as a positive control. Chlorhexidine is a mouthwash that can reduce plaque formation, inhibit plaque growth and prevent periodontal disease. This is due to the nature of Chlorhexidine itself, which is a bactericid and bacteriostatic against various kinds of bacteria. The mechanism of action of chlorhexidine is effective in inhibiting growth and killing gram-positive and gram-negative bacteria, depending on the concentration used. Chlorhexidine molecules have a positive charge (cations) and most of the bacterial molecular charges are negative (anions). This causes the strong adhesion of chlorhexidine to the bacterial cell membrane. Chlorhexidine will cause changes in the permeability of the bacterial cell membrane, causing the release of cell cytoplasm and cell components with low molecular weight from inside the cell to penetrate the cell membrane, causing bacterial death.[15]

## Conclusion

Plantain peel extract (Musa paradisiaca L) concentrations of 16%, 32% and 64% were effective in inhibiting the inhibition of Porphyromonas gingivalis bacteria and there were significant differences between the three concentrations in inhibiting Porphyromonas gingivalis bacteria ($p < 0.05$). However, the concentration of 64% and chlorexidine 0.2% was more effective in inhibiting Porphyromonas gingivalis bacteria.

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**Ethical Considerations:** Ethical clearance was obtained from Universitas Muslim Indonesia, Makassar; with number" 085/A.1/KEPK-UMI/III/2021. Just before the interview, written (or thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia, Makassar guidelines.

**Conflicts of Interest**: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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# Measurement of Maximal Oxygen Uptake (VO2Max) as a Cardiorespiratory Physiological Fitness Parameter Using Sensor Technology-Based Device Development

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## Abstract

**Background**: This study aims to measure the maximal oxygen uptake (VO2Max) as a cardiorespiratory physiological fitness parameter using the development of a sensor technology-based tool.

**Methods:** This study uses a research and development design (Research And Development). Validation is carried out by competent experts. In this study, the researchers asked for validation from three experts, namely IT (electronics) experts, test and measurement experts and Sports Physiology experts (maximum oxygen volume). A total of 19 male and 11 female adults participated in this study. The usage trial was carried out using the expert validity method with an assessment using a questionnaire, test and retest. The data analysis technique used is r-correlation

**Result:** Based on expert validation tests, the reliability, practicality, and effectiveness of the development of test instruments based on sensor technology can be used to measure VO2Max and provide accurate and effective results.

**Conclusion:**The development of a test instrument based on sensor technology can provide accurate and effective results for measuring maximal oxygen uptake (VO2Max) as a cardiorespiratory physiological fitness parameter. This tool provides advances for monitoring VO2Max routinely in daily life, especially for carrying out training programs for athletes without the need for trained personnel and special equipment and expensive costs.

***Keyword:*** *VO2Max,Cardiorespiratory,Sensor Tecnology*

## Introduction

Maximaloxygenuptake (VO2Max) isveryimportant in delivering O2 tomuscles during sustained physical exercise which is considered a standard of cardiorespiratory physiological fitness and is also a health parameter[1,2]. In addition, cardiorespiratory fitness is known to correlate with physiological factors such as body composition and blood pressure, as well as psychological factors such as depression[3]. High VO2Max is inversely proportional to the risk of cardiovascular disease and even death which reaches 17 million people worldwide[4,5].

The most commonly used VO2Max measurement instrument with incremental running tests on a treadmill

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or other equipment such as a cycle ergometer[6,7]. In conducting measurement tests, most of the trainers complained about the difficulty of measuring VO2Max because it requires trained personnel and special equipment as well as expensive costs which may not be practical to repeat measurement tests to monitor Vo2Max on a regular basis so it is very difficult to implement in the Training program.

Alternative solutions need to be sought to overcome these problems. Recently, sports devices combined with sensor technology have gained benefits as an effective tool for assessing physical activity in the general population[8].This is driven by advances in technology and lower costs. Sensor technology is widely used to measure heart rate and measure temperature that is inserted into personal devices such as smartphones and digital watches[3,8–11].However, until now the effectiveness of sensor technology has not been tested to measure VO2Max in the fields of sports and health.

To answer this question, it is necessary to directly measure the maximal oxygen uptake (VO2Max) as a cardiorespiratory physiological fitness parameter using the development of sensor technology-based tools.

## Methods and Materials

This study uses a research and development design (Research And Development). Called research-based development. After the product design is made, then ask for validation from a competent expert.Validation is carried out by competent experts aiming to find out the weaknesses and strengths of the product. In this study, the researcher asked for validation from three experts, namely: (1) IT (electronics) expert, (2) test and measurement expert (3) Sports Physiologist (maximum oxygen volume).Then the resulting product was tested for use in the field, as many as 19 men and 11 adult women participated in this study. The usage trial was carried out using the expert validity method with an assessment using a questionnaire, test and retest. The data analysis technique used is r-correlation.

## Result and Discussion

The results of the research used in this literature review are as follows:

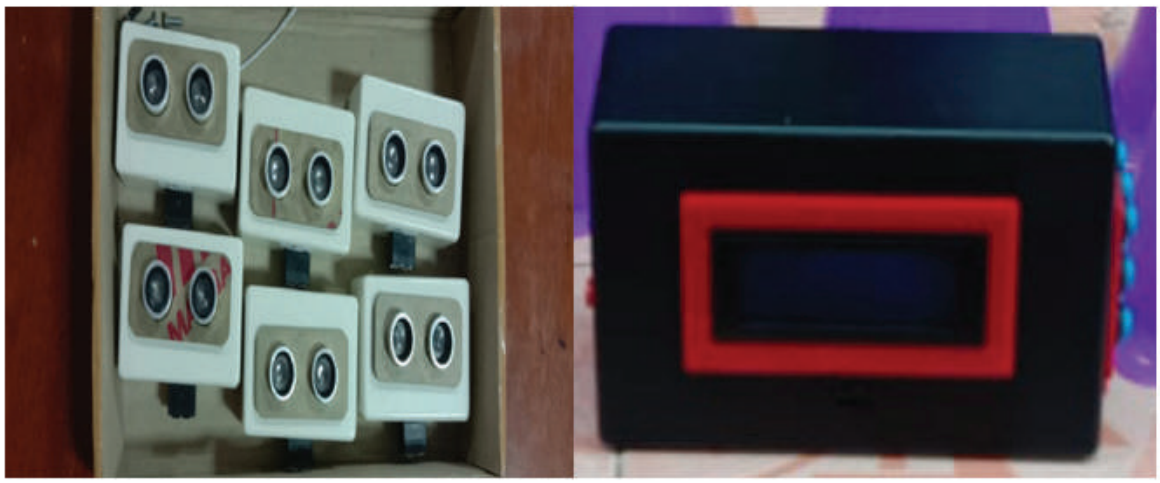

**Figure 1. Sensors and Data Storage LEDs Used in the Tool**



**Figure 2. Results of the Development of VO2Max Measurement Tools Based on Sensor Technology Expert**

**Table 1. Questionnaire scores for the development of -based VO2Max measurement toolssensor technology by experts**

| Expert | Aspect | Score | Relative Frequency |
|---|---|---|---|
| Media | Suitability | 11 | 15% |
| | Accuracy | 28 | 30% |
| | Conveniences | 19 | 25% |
| | Practicality | 26 | 30% |
| | Amount | 84 | 100% |
| Test and Measurement | Suitability | 12 | 35% |
| | Accuracy | 20 | 25% |
| | Conveniences | 18 | 25% |
| | Practicality | 16 | 15% |
| | Amount | 66 | 100% |

***Cont...*** **Table 1. Questionnaire scores for the development of -based VO2Max measurement toolssensor technology by experts**

| | | | |
|---|---|---|---|
| Sports Physiology Material | Suitability | 21 | 30% |
| | Accuracy | 16 | 20% |
| | Conveniences | 18 | 20% |
| | Practicality | 22 | 30% |
| | Amount | 77 | 100% |

Expert Validity Test

**Table 2. Percentage and Eligibility Level of Experts**

| Expert | Presentase | Eligibility Level |
|---|---|---|
| Media | 84% | Very good |
| Test and Measurement | 66% | Good |
| Sports Physiology Material | 77% | Good |

Based on the assessment by the three experts, the results of the development of the VO2Max test instrument based on sensor technology can be used to measure VO2Max.

**Reliability**

**Table 3. Descriptive Analysis of Reliability Test**

| Day | n | Maximal Oxygen Uptake (VO2Max) | | |
|---|---|---|---|---|
| | | $\bar{x}$±SD | Minimum | Maximum |
| The First Day | 30 | 30,49±5,17 | 23,60 | 45,50 |
| The Second Day | 30 | 32,65±4,56 | 27,20 | 45,50 |

Based on the results of the correlation coefficient of the reliability test, the tool developed as a VO2Max test instrument based on sensor technology is said to be reliable and consistent in retrieving VO2Max data.

**Practicality**

**Table 5. Results of Practitioner's Percentage from Expert Assessment**

| Expert | Percentage | Eligibility Level |
|---|---|---|
| Media | 86,7% | Very good |
| Test and measurement | 60,0% | Enough |
| Sports Physiology Material | 73,3% | Good |

Based on the results of the validation by the three experts, it was found that the tool developed by the VO2Max instrument based on sensor technology had a "Good" level of practicality.

**Effectiveness**

**Table 6. Effectiveness Percentage Results from Expert Assessment**

| Expert | Percentage | Eligibility Level |
|---|---|---|
| Media | 73% | Enough |
| Test and measurement | 72% | Enough |
| Sports Physiology Material | 70% | Enough |

Based on the results of the validity of the three experts, it was found that the tool developed by the VO2Max instrument based on sensor technology has an "Enough" level of effectiveness.

## Discussion

Cardiorespiratory physiological fitness level can be measured by estimation of VO2Max[12].In this research, we use sensor technology based VO2Max instrument development to estimate VO2Max estimation.

In the validity test by media experts, it was found that the feasibility level was included in the very good category, validity test by test and measurement experts obtained a level of feasibility that is included in the good category, then the validity test by the exercise physiology material expert is included in the good category. The results of the correlation coefficient of the reliability test of the tool developed as a VO2Max test instrument based on sensor technology obtained results on the first and second days with a high correlation so that it can be said to be reliable and consistent in retrieving VO2Max data. Based on the results of the validation by the three experts, the results of the development of a VO2Max measurement tool based on sensor technology have a level of practicality with a feasibility level included in the good category, and based on the results of the validation by the three experts, the results of the development of a VO2Max measurement tool based on sensor technology have a level of effectiveness with a feasibility level included in enough category. The results of the expert validity test prove that the development of a VO2Max test instrument based on sensor technology can be used to measure VO2Max. this is confirmed by research[13]that the optical heart rate (OHR) device in the form of sensor technology worn on the wrist can accurately estimate heart rate,

energy expenditure. In addition, research[14]reported that machine learning analyzes of sensors provided significant advances in the assessment of energy expenditure and aerobic fitness.

During aerobic exercise, VO2Max is very important to determine and track a person's cardiorespiratory physiological fitness[14,15].The higher a person's VO2Max, the better the level of cardiorespiratory fitness, so it has the potential to increase performance in the world of sports[16–18]. The ability to predict VO2Max with the development of a test instrument based on sensor technology has the potential to provide an opportunity to obtain valuable information about cardiorespiratory fitness more effectively. The finding in this study is that the development of sensor technology-based test instruments can be used routinely in everyday life, especially to undergo training programs for athletes without the need for trained officers and special equipment and expensive costs. Therefore, the development of sensor technology-based test instruments can be used in the future to measure the level of cardiorespiratory fitness.

## Conclusion

The development of a test instrument based on sensor technology can provide accurate and effective results for the measurement of maximal oxygen uptake (VO2Max) as a cardiorespiratory physiological fitness parameter. This new technology provides advances for monitoring VO2Max routinely in everyday life, especially for carrying out training programs for athletes without the need for trained personnel and special equipment and expensive costs.

**Conflict of Interest:** The authors declare no conflict of interest

**Funding:**This research uses private funds

**Ethical Clearance:**Taken from ethical committe.

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# Alpha-Mangostin and Gamma-Mangostin Isolated from Mangosteen (*Garcinia mangostana* L.) as Promising Candidates against SARS-CoV-2: A Bioinformatics Approach

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## Abstract

The world is endangered by the COVID-19 pandemic caused by SARS-CoV-2, people are dying in thousands every day, and without an actual treatment, it seems that bringing this global health problem to a quit is impossible. Natural products have been in constant use since ancient times and are proven by time to be effective. Medicinal plants from Indonesia may lead to the discovery of the novel drugs. Mangosteen or *Garcinia mangostana* L. is a native tropical fruit from Southeast Asia and is known to contain bioactive compounds. Interestingly, the main xanthone derivatives are alpha-mangostin and gamma-mangostin, these compounds have a variety of pharmacological activities such as antiviral activity. In summary, this study showed potential pharmacological benefits of alpha-mangostin and gamma-mangostin isolated from mangosteen against SARS-CoV-2. Thus, mangosteen exhibits as a valuable plant and a candidate for future drug development to fight SARS-CoV-2. However, further trials, such as *in vitro* and *in vivo* evaluation, are needed to prove the validity of these findings.

***Keywords:*** *Alpha-mangostin, COVID-19, Gamma-mangostin, Garcinia mangostana L., SARS-CoV-2.*

## Introduction

Indonesia is covered by many vegetations, including tropical rain forests. In addition, Indonesia is one of the top five countries in the world that has high plant diversity, including approximately 6,000 medicinal plants[1,2,3,4,5]. Consequently, Indonesia is rich in medicinal plants used by its population in curing many diseases. On the other hand, there has been around 92 million people globally who have been infected by SARS-CoV-2 (the causative agent of COVID-19) and more than 2 million deaths as the fast result of this pandemic. In Indonesia, there are more than one million cases and more than 25,000 deaths. Data was retrieved from Johns Hopkins University online website that tracks COVID-19 cases in real-time[6].

Mangosteen or *Garcinia mangostana* L. is appertain to the family of Clusiaceae and genus *Garcinia*. *Garcinia* is a large genus which consists of around 400 species originated from East India and Southeast Asia, including Indonesia[7]. Pratiwi *et al*. stated that the mangosteen production centers in Java are Blitar, Purwakarta, Bogor, Banyuwangi, Subang, Ciamis, Sukabumi, Cilacap, Purworejo, and Banjarnegara[8]. Moreover, based on the morphological and cytological studies, it can be suggested that mangosteen originates from Southeast Asia. As a matter of fact, mangosteen is a plant that has been used as traditional medicine for hundreds of years worldwide[9].

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Mangosteen contains bioactive compounds such as xanthones, tannins, and some vitamins. In fact, mangosteen's pericarp has many important benefits for health. The main compounds in the content of mangosteen's pericarp are xanthones; such as alpha-mangostin, gamma-mangostin, beta-mangostin, and so on. The main xanthone derivative, such as alpha-mangostin and gamma-mangostin, have a variety of pharmacological activities such as antiviral activity[10,11,12].

## Materials and Methods

### Data retrieval

We extracted phytocomponents of mangosteen from PubChem, an open chemistry database at the National Institutes of Health (NIH), USA. We revealed the Canonical SMILES of alpha-mangostin and gamma-mangostin and submitted them to the SwissADME web server for further analysis.

**Table 1. Alpha-mangostin and gamma-mangostin revealed from the PubChem database.**

| Compounds | Formula | Molecular Weight | IUPAC Name | Canonical SMILES |
|---|---|---|---|---|
| Alpha-mangostin | C24H26O6 | 410.46 g/mol | 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one | CC(=CCC1=C(C2=C(C=C1O)OC3=C(C2=O)C(=C(C(=C3)O)OC)CC=C(C)C)O)C |
| Gamma-mangostin | C23H24O6 | 396.43 g/mol | 1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl) xanthen-9-one | CC(=CCC1=C(C2=C(C=C1O)OC3=C(C2=O)C(=C(C(=C3)O)O)CC=C(C)C)O)C |

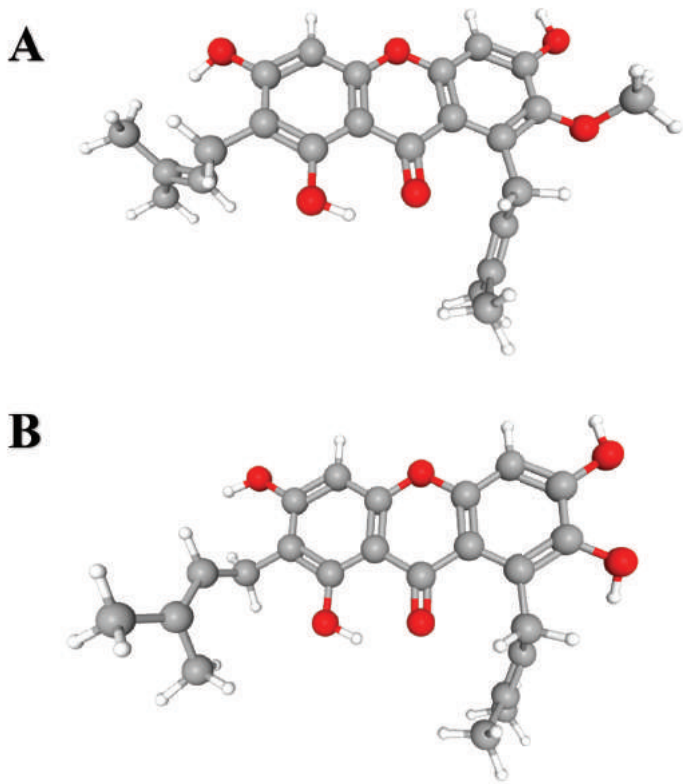


**Figure 1. Chemical structures of alpha-mangostin (A) and gamma-mangostin (B) isolated from mangosteen.**

Pharmacokinetics and drug-likeness predictions

In the present study, we predicted the pharmacokinetic properties and druglike nature of the phytocomponents using the SwissADME web server and identified gastrointestinal absorption prediction for oral drug probability[13] and Lipinski parameter for the drug-likeness prediction based on Lipinski *et al*.[14]

**Biological activity prediction**

We performed PASS (Prediction of Activity Spectra for Substances) web resource as a strong potential tool to predict the biological activity. This web resource estimates the predicted activity spectrum of a compound as probable activity (Pa)[13].

## Results and Discussion

We successfully revealed pharmacokinetics, drug-likeness, biological activity predictions of alpha-mangostin and gamma-mangostin from mangosteen as presented in Table 2 and Figure 2. In addition, phytochemical screening, based on ethnomedicinal data, is considered as an effective approach for the discovery of new therapeutic agents. The major bioactive secondary metabolites of mangosteen are xanthone derivatives. The major constituents from the xanthone fraction in mangosteen were found to be alpha-mangostin and gamma-mangostin. More than 60 other xanthones were isolated from its different plant parts, including 3-isomangostin, β-mangostin, gartanin, mangostanin, 1-isomangostin, garcinone B, 9-hydroxycalabaxanthone, mangostanol, mangostinone demethylcalabaxanthone, 8-deoxygartanin, and garcinone D[1,10]. The majority of investigations are focused on the extraction and structure elucidation of xanthones from the pericarp of mangosteen. Recently, the presence of these compounds in the stem, seed, and heartwood was reported by many researchers[2,15].

**Table 2. Pharmacokinetics, drug-likeness, biological activity predictions of alpha-mangostin and gamma-mangostin.**

| Compound | Pharmacokinetics Prediction (Gastrointestinal Absorption) | Drug-likeness Prediction (Lipinski) | Antiviral Activity Prediction |
|---|---|---|---|
| Alpha-mangostin | High | Yes | Herpesvirus (0.423) and rhinovirus (0.390) |
| Gamma-mangostin | High | Yes | Herpesvirus (0.453), rhinovirus (0.393), picornavirus (0.311), influenza (0.267), cytomegalovirus or CMV (0.244), hepatitis B (0.235), poxvirus (0.231), and HIV (0.191) |

Mangosteen is an important medicinal plant in traditional medication system. Studies of mangosteen's pharmacological properties has started since the 1990's. Mangosteen is an important medicinal plant in the family of Clusiaceae. In the recent history, this plant is reported for its various medicinal properties. In Asia, the pericarp of mangosteen is used as antimicrobial, antiparasitic agents, and for wound healing. The pericarp decoction of mangosteen is administered to relieve gonorrhea and diarrhea.

Mangosteen stem bark and leaves are recognized to have anti-inflammatory properties for many skin disorders. In the Philippines, leaves and bark is adopted as a medication for diarrhea and various urinary problems. In Thai traditional medicine, the pericarp is used as the medication of skin infections and wounds. In addition, mangosteen root stew is used by women to treat menstrual disorders. Moreover, mangosteen has also been used for medical purposes in Caribbean and Latin America, for example as a digestive aid in Brazil[16]. Traditional medicinal properties of mangosteen are employed for hemorrhoids, tuberculosis, mycosis, fever, abdominal pain, leucorrhoea, and convulsants[10].

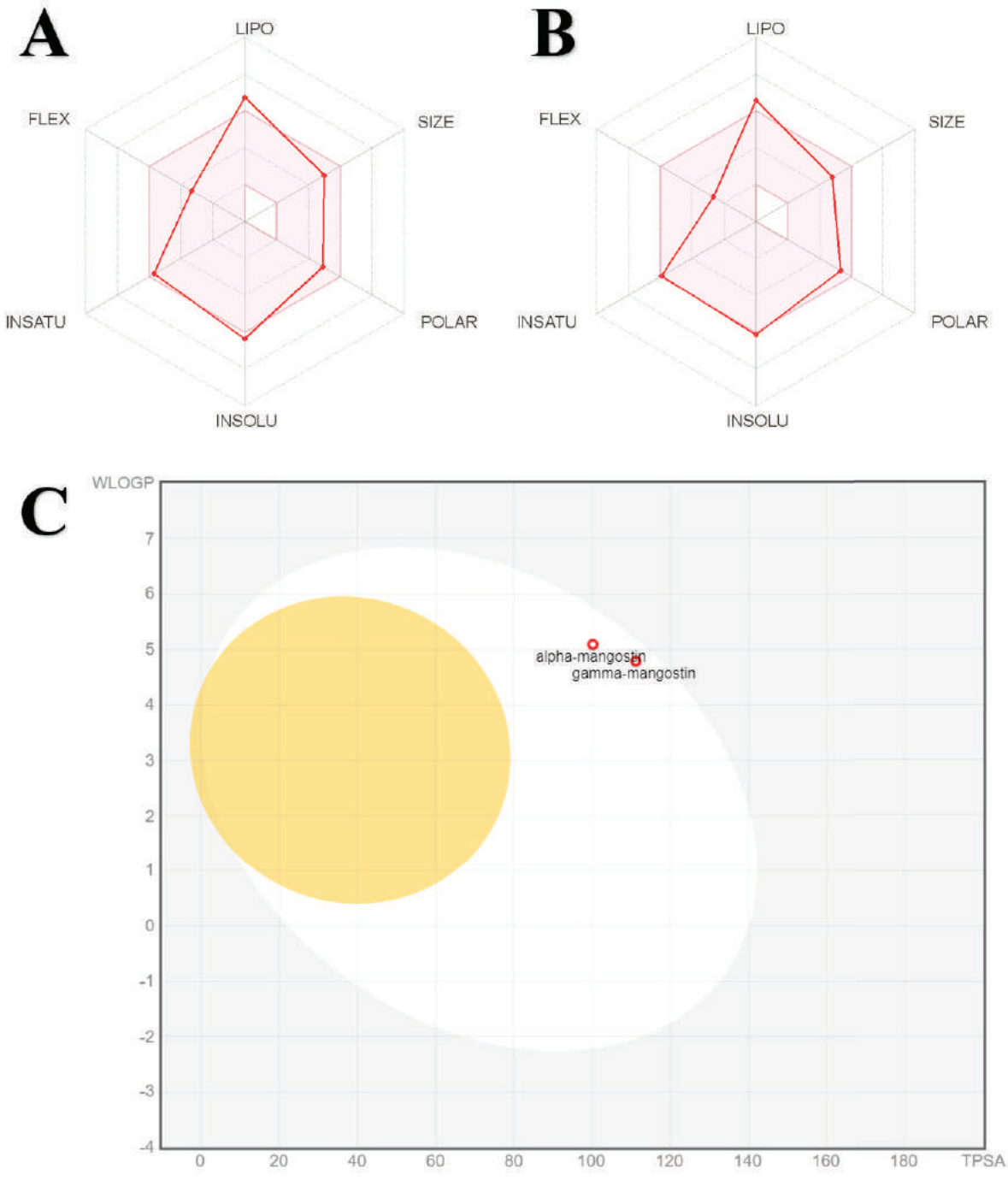


**Figure 2. Radar-like representation of the drug-likeness of alpha-mangostin (A) and gamma-mangostin (B) predicted by SwissADME web server. BOILED-Egg plot to globally estimate their gastrointestinal absorption and brain penetration, two major ADME behaviors impacting pharmacokinetics (C).**

In addition, alpha-mangostin and gamma-mangostin from mangosteen inhibited HIV-1 with $IC_{50}$ values of 5.1 and 4.8 μM, respectively[17]. Vlietinck *et al*. discovered the role of α-mangostin as a non-competitive inhibitor of HIV-1 protease by inhibiting the HIV virus replication cycle[18]. Patil *et al*. performed *in vitro* and *in vivo* studies, and revealed that α-mangostin, a xanthonoid from *Garcinia mangostana*, is a promising natural antiviral compound against chikungunya virus[19]. Moreover, a study by Tarasuk *et al*. stated that alpha-mangostin inhibits both dengue virus production and cytokine/chemokine expression[20]. In line with this, Sugiyanto *et al*. and Yongpitakwattana *et al*. demonstrated the inhibitory effect of alpha-mangostin to dengue virus replication and cytokines expression in human peripheral blood mononuclear cells and dendritic cells. In addition, gamma-mangostin reported to inhibit hepatitis C virus and SARS-CoV-2[21,22].

Bioinformatics provide more efficient target discovery and validation approaches, thus helps ensure that more drug candidates are successful during the approval process, making it more cost-effective[23]. Notably, the work of Lipinski *et al*. analyzed orally active constituents to describe physicochemical ranges for high probability opportunities as an oral drug. This called Rule-of-five delineated the relationship between pharmacokinetics and physicochemical parameters. Lipinski's rule of 5 helps in distinguishing drug-like and non-drug like molecules. It predicts a high probability of success or failure due to drug-likeness for molecules complying with 2 or more of the following rules, such as molecular mass less than 500 Dalton, high lipophilicity, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, molar refractivity should be between 40-130[14].

In the present study, an attempt was made to investigate a more extensive pharmacological appearance of phytoconstituents by the application of PASS web resources. The proposed *in silico* method extends further to generate novel bioactivities of selected phytochemical leads, related side-effects, and their mechanisms. In addition, the recent version of PASS predicts approximately 3750 pharmacological activities, specific toxicities, biochemical mechanisms of action, and metabolic terms on the basis of the structural formula of drug-like substances with average fidelity ~95%[24]. This might be further validated through *in vitro* as well as *in vivo* trials. In line with this, the present study revealed the use of PASS in exploring hidden pharmacological potential of alpha-mangostin and gamma-mangostin as an antiviral (Figure 3).

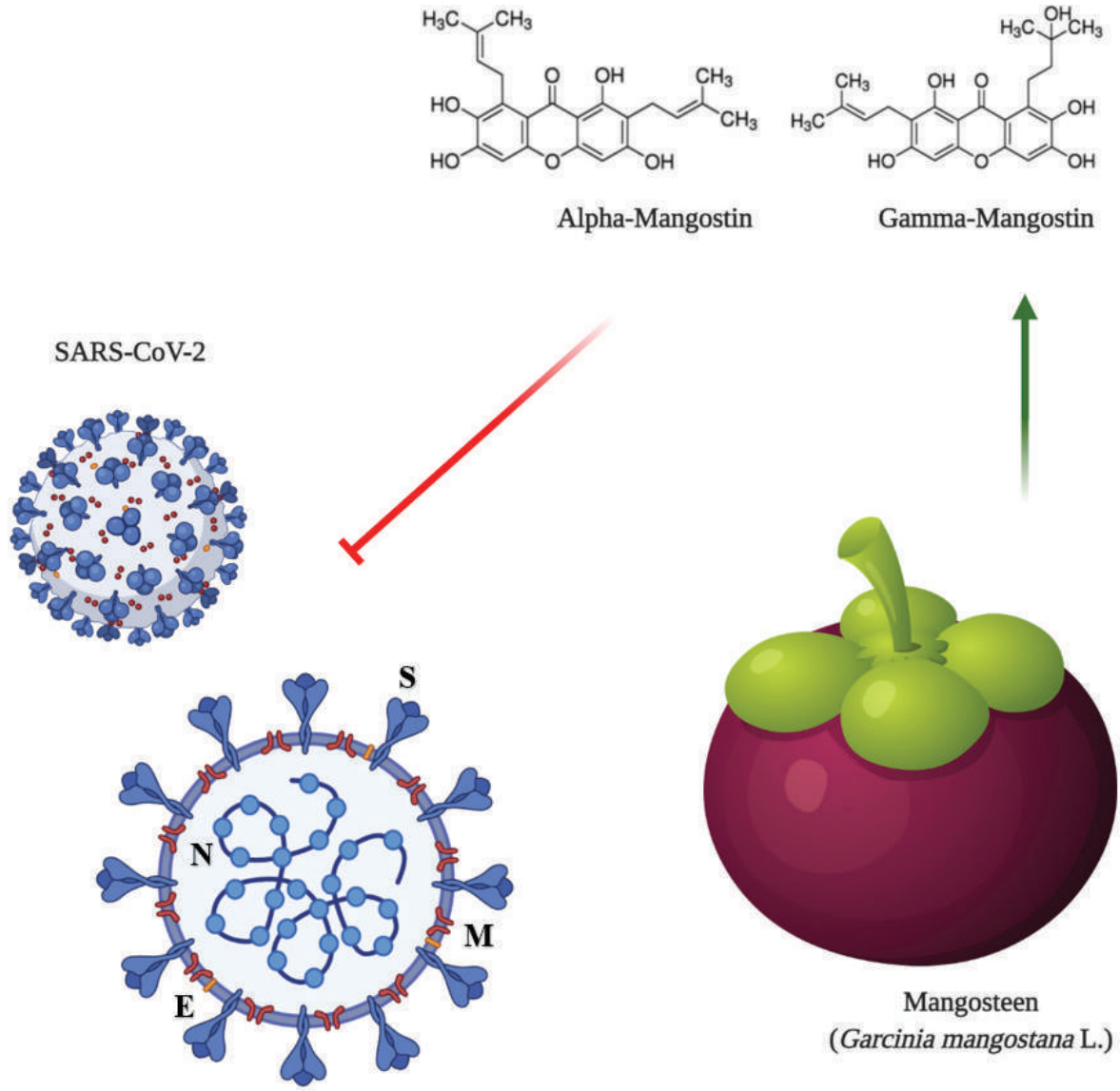


**Figure 3. Alpha-mangostin and gamma-mangostin isolated from mangosteen as a promising candidate against SARS-CoV-2. E: Envelope protein, M: Membrane protein, N: Nucleocapsid phosphoprotein, and S: Spike protein. This figure created in BioRender.**

## Conclusion

In summary, this study showed the potential pharmacological benefits of alpha-mangostin and gamma-mangostin isolated from mangosteen against SARS-CoV-2. Thus, mangosteen exhibits as a valuable plant and establishes as a candidate for future drug development to fight SARS-CoV-2. However, further trials, such as *in vitro* and *in vivo* evaluation, are needed to prove the validity of these findings.

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

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**Ethical Approval:** No ethical approval needed.

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# Prevalence of Musculoskeletal Disorder in Wrist and Fingers amomg Amateur Piano Players in Vellore

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## Abstract

**Background:** Now a days the number of students studying piano in tertiary institution is being increasing. The piano player is similar to athletic activity to play the notes accurately. Posture plays a vital role in piano playing. Musicians are prone to injuries due to the nature of musical practice, inappropriate body postures and potentially harmful playing techniques. Majority of amateur players are prone to injuries, the main objective of the study was to find out the prevalence of musculoskeletal discomfort among piano players in three different wrist styles in piano playing. **Methodology:** A total 150 samples of age 8 to 15 years of both gender from Kingsway music academy. who use to play piano minimum 30 mins with minimum 6 months of learning experience were recruited for the study, initially their level of pain and discomfort in hand and wrist was measured using Cornell hand discomfort questionnaire **Conclusion:** The statistical analysis shows that there is pain in fourth and fifth finger in all wrist position and also weight playing method has significant pain among three wrist position .This also study concludes that correct positioning during piano playing can minimize the playing related musculoskeletal disorder.

***Keywords:*** *Amateur piano player, Posture, Playing related musculoskeletal disorder, Wrist style*

## Introduction

Piano is a classical and fascinating instrument. In 18th century Bartolomeo Cristofori invented first generation of piano. By twentieth century piano was highly developed in its quality both in mechanism and sound. Piano is one of the most common musical instrument learned now a days the number of students who study music in tertiary institution is been increasing

The piano has much heavier mechanical action of the hammers[1]. Piano also has the capacity to accommodate difficult and complex music. The touch of each key weights around 52g for present day acoustic piano. Piano needs a application of greater music effort to stabilize the finger joints, when the upper limb exerts force near between 48.26 to57.28 kg per minute or 168.9 to 201.4 within minutes to play[1]. Piano playing is similar to athletic activity due to high demands in practice and the requirements to play musical notes accurately[2]. Posture plays a vital role in piano, correct balance and posture may achieve tension free playing. Biomechanically piano playing requires certain posture which has to be followed to avoid playing related musculoskeletal disorder.

There are two main kinds of motor skills in common use[1]. The traditional playing method which is further classified into high wrist playing method and neutral wrist playing method. Second the weight

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playing method[1].

The first is the traditional playing methodology, while adopting high wrist method player hold their wrist area at higher level than the piano. In neutral wrist position the players hold their wrist at the same level of piano, on parallel the players who adopt weight playing methodology hold their wrist mostly rotated while playing.

Musician are prone to injuries due to the nature of musical practice, inappropriate body posture, potentially harmful techniques. Injuries that occur in musician are usually termed as playing related musculoskeletal disorder. Symptoms of playing related musculoskeletal disorder include pain, numbness, swelling, tingling .The occurrence of playing related musculoskeletal disorder is almost unavoidable when a given motion is highly repetitive combined with the prolonged use of body segments and without a proper understanding of the anatomical limits and motion range of the human body. Playing related musculoskeletal disorders was first recorded since 19th century ,Robert and Clara Schuman were the first injured pianist, both of them studied under same teacher Frederic weick[3].Generally all level of musician from beginners to professional players are suspected to have playing related musculoskeletal disorder, but most of the players are not aware of the playing related musculoskeletal.

Pianists are among the group at high risk for playing related musculoskeletal disorder. Faulty piano techniques are one such reason for the cause of playing related musculoskeletal disorder. The prevalence of playing related musculoskeletal disorder 23%to 93%[2]. The root of playing related musculoskeletal disorder starts when the student begin to learn piano, now days due to multi students with one teacher results in lack of supervisions for proper use of body posture.

Factors that contribute to the incidence of playing related musculoskeletal disorder are biomechanical inefficiency of posture and massive amount of repetitive and sustained movement of hand wrist and fingers can cause stress and pain. Playing related musculoskeletal disorder can also occur due to inefficient of motor skills. Generally pianist report playing related musculoskeletal disorder occurring at the wrist due to faulty piano playing methodology. Pianist should pay attention to any degree of pain condition in hands that affects playing because the symptoms might be the first sign of developing playing related musculoskeletal disorder. Some of the playing related musculoskeletal disorder were sever enough to threaten and even end musician career and some players even developed physical handicaps that impact all aspect of musician. The main objective of the study was most common musical instrument learned by children is piano off late. There are different styles in playing piano. Many studies show the prevalence of musculoskeletal pain in pianist ,but narrowing down the study to particular region will increase the study scope to one area. It can help in understanding the musculoskeletal discomfort in three styles of wrist position and associated risk factors. So that children who start their passion can be given awareness ergonomically. The study will also promote the awareness of playing related musculoskeletal disorder into the curriculum of piano pedagogical program

## Materials and Method

It is a cross sectional study conducted in the year (2019-2020) at Kingsway music academy Vellore, Tamil Nadu, India. Totally 150 participants, both gender of age group 8-15 years were selected, Students who learn piano in tertiary institution for minimum 6 months, parallelly who practice 30 mins- 45mins a day were recruited for the study . Professional players, students who underwent any orthopedic surgery in upper limb, Students who learn other instrument along with piano , were excluded for the study . Participants were explained about the procedure and informed consent was obtained . To assess their level of pain Cornell Hand Discomfort Questionnaire was used. The participants were explained about the components in Cornell Hand Discomfort Questionnaire, then they were asked to fill The questionnaire consists of 6

shaded figure to analyze the pain in their fingers and wrist and it includes five items about how often they experience pain and three items about symptomology of pain and three items interference of pain in their daily activities , Higher score represent the greater level of pain in particular region .

## Results and Discussion

According to table I out of 150 participants 2.7% were 8 years of age, 6% were 9 years of age ,22% were 10 years of age, 12.7% were 11 years of age, 8.7% were 12 years of age.8.7% were 13 years of age,13% were 14 years of age, 25% were 15 years of age,1 % were 16 years of age.

According to table II 61.3% were male and 38.7 % were female

According to table III total 5 participants reported in high wrist method and the intensity of pain in Area A is 12.3%Area B is 38.7%Area C 32.3% Area D 0.0%Area E 10.3%Area F 6.5%.

According to table IV total 66 participants reported in neutral wrist method and the intensity of pain of Area A 15.5%is Area B is 40.8%Area C 10.5%Area D 2.3%Area E 12.7%Area F 18.1%.

According to table V total 79 participants reported in weight playing method and the intensity of pain in Area A 9.9% Area B 39.9%Area C 11.2%Area D 2.5% Area E 7.3%Area F 29.2%.

According to table VI the intensity of pain in High Wrist 38.1% Neutral Wrist 21.3% and weight playing 58.8%

**TABLE I: AGE FREQUENCY**

| | | Frequency | Percentage |
|---|---|---|---|
| Age | 8 | 4 | 2.7 |
| | 9 | 9 | 6.0 |
| | 10 | 33 | 22.0 |
| | 11 | 19 | 12.7 |
| | 12 | 13 | 8.7 |
| | 13 | 13 | 8.7 |
| | 14 | 20 | 13.3 |
| | 15 | 38 | 25.3 |
| | 16 | 1 | .7 |

**TABLE II: GENDER FREQUENCY**

| | | Frequency | Percentage |
|---|---|---|---|
| Gender | Female | 58 | 38.7 |
| | Male | 92 | 61.3 |
| | Total | 150 | 100.0 |

**TABLE III: PREVALENCE OF PAIN IN HIGH WRIST METHOD**

| High Wrist | Mean | INTENSITY OF PAIN % |
|---|---|---|
| Area A | 1.9000 | 12.3% |
| Area B | 6.0000 | 38.7% |
| Area C | 5.0000 | 32.3% |
| Area D | 0.0000 | 0.0% |
| Area E | 1.6000 | 10.3% |
| Area F | 1.0000 | 6.5% |

**TABLE IV: PREVALENCE OF PAIN IN NEUTRAL WRIST METHOD**

| Neutral | Mean | INTENSITY OF PAIN % |
|---|---|---|
| Area A | 2.2955 | 15.5% |
| Area B | 6.0530 | 40.8% |
| Area C | 1.5530 | 10.5% |
| Area D | 0.3409 | 2.3% |
| Area E | 1.8864 | 12.7% |
| Area F | 2.6894 | 18.1% |

**TABLE V: PREVALENCE OF PAIN IN WEIGHT PLAYING METHOD**

| Weight Playing | Mean | INTENSITY OF PAIN % |
|---|---|---|
| Area A | 1.8987 | 9.9% |
| Area B | 7.6203 | 39.9% |
| Area C | 2.1329 | 11.2% |
| Area D | 0.4747 | 2.5% |
| Area E | 1.3987 | 7.3% |
| Area F | 5.5759 | 29.2% |

**.TABLE VI: PREVALENCE OF PAIN IN THREE WIRST METHOD**

| Method of Playing | Mean | INTENSITY OF PAIN % |
|---|---|---|
| High Wrist | 2.5833 | 38.1% |
| Neutral | 2.4697 | 21.3% |
| Weight Playing | 3.1835 | 58.8% |

## Discussion

The children's in this 20th century era are keen to get involved in and eager to acquire skills in recreational activities such as sports,martial arts, dancing and music. The most of the children's are passionate about their recreational activities, few of them are intending to make their passion as their profession from their young age of the life.

With an objective of finding about the prevalence of musculoskeletal discomfort among the amateur young pianist, 150 subjects were selected between the age group of 8 to 15 years, both the male and female genders,

Out of 150 participants, 5 Participants opted high wrist method, 66 participants opted neutral wrist method and 79 participants opted weight playing method. From the above results analyze that In high wrist method the level of pain is higher in Area B (38.7%) and Area C(32.3%).In neutral wrist method the level of pain is recorded high in Area B (40.8%) and Area A (15.5%) .In weight playing method also reported high intensity of pain in Area B(39.90%) and Area F (29.20%).In comparing all three wrist position the participants reported a specific pain in Area B (4th and 5th finger) which has the maximum intensity of pain. The players who adopted High wrist method opted (38.10%) of pain, the players who opted neutral wrist method reported (21.3. %) of pain and players who opted weight playing method reported (58.80%) of pain which is significantly higher among three wrist method

Across the globe many children's are attracted towards learning the musical instruments and it becoming their passionate recreational activity. The piano an acoustical musical instrument from a French origin is attracted and learned by many children's across the globe. When the keys in the piano is pressed the hammer incorporated in the piano strikes the string and produces the sound of musical note, when the key pressed strongly with the fingers, the velocity of hammer striking the string increase and it produces more sound of the musical note.

The all recreational activities are prone for musculoskeletal injuries, the musculoskeletal injuries among musicians are known as Playing Related Musculoskeletal Disorder's (PRMDs). The PRMDs among pianist are pain, muscle fatigue, tendinitis, tenosynovitis, bursitis,nerve entrapment such as carpal tunnel syndrome,numbness and pins & needles.

The PRMDs are common among experienced pianist and amateur pianist, the amateur young pianist are more prone for the PRMDs. The cause for the PRMDs among the amateur young pianist is due to repetitive motion, sustaining the posture for long period of time, long practice hours, lack of frequent rest breaks, wrong body mechanics.

The pianist commonly assumed three wrist postures while playing piano they are high wrist posture,neutral wrist posture and weight on piano. In the high wrist posture the wrist will be high to the keyboard, in the neutral wrist posture the wrist

will correspond to the level of keyboard, in weight on keyboard the weight of the fingers will be on the keyboard.

The posture of fingers can be flat or rounded and the motion can be horizontal or vertical. The posture of the elbow can either flexed or extended but preferably 30 to 40 degree of flexion will be maintained, the shoulders will be either elevated or depressed while playing piano.

While playing piano, the posture which assumed for long time and the repetitive motion acts as precursor for the PRMDs. The fingers and hand region comes first and more vulnerable for PRMDs, the elbow and shoulder regions comes second and third vulnerable region for PRMDs.

The wrist and hand are commonly affected in amateur young pianist, the extrinsic muscular and neuro-vascular structures for the wrist and the hand orginates from the elbow and forearm. The elbow joint and forearm influences the wrist mechanics, in the anatomical position the forearm along with hand will be in a fully supinated position, in the neutral position the forearm will be in mid-pronation position, in the functional position forearm will be in a pronated position, concamitantly wrist will also be pronated,volar surface of the hand will be down, the concomitant wrist motion along with forearm is produced by the ligamentous structures of the wrist.

While playing piano, the forearm will be in functional position, in a fully pronated position, in this position the radius twist along the shaft of ulna with an concamintant arthrokinematic changes in superior and inferior radio-ulnar joints, this arthrokinematical changes will have a effect on the volar & dorsal ligamentous complex of carpals and induces arthrokinematic changes in proximal and distal carpal rows. In this situation when wrist is flexed the proximal row slides dorsally and distal row slides vollarly, with some contribution to radial and ulna deviation if added to it, reverse of this occurs in wrist extension. The thumb which is an king of the hand plays an key role while playing piano, major motion occurs on the 1st carpo-metacarpal joint, 1st metacarpal slides on the trapezium, the proximal and distal joints too contribute for manipulation of the keys in keyboard. The 2nd to 5th Meta Carpal Joints flexes & extends and abducts & adducts, the interphalengeal joints flexes and extends while playing piano.

When the wrist and forearm is not in physiological neutral position, the musculoskeletal and neurovascular structures of the hand which transversing the wrist are prone to injuries. Maintaining the forearm and the wrist in a functional pronated position for a long time and performing a repetitive motion in this position causes pain and lose of grip strength of the hand.

The all three wrist positions which used while playing the piano is the functional position fully pronated position, physiologically it is not a neutral position, the neutral wrist position which mentioned in types of wrist position used in playing the piano is not a true neutral position. When using the wrist and hand for playing piano in these position will affect the normal alignment changes in forearm and wrist osseous structures, the change in the alignment of osseous structures can cause mechanical disadvantageous situation to musculo-tendinous structures and makes it prone for injury. The alignment changes in carpal bones due to the pronated position will interfere with the carpal tunnel and prone to produce carpal tunnel syndrome. The repetitive motions of fingers are prone to cause ishaemia in the intinsic hand muscle, cause fatigue, pain and mysofascial trigger points.

Biomechanically the position which assumed while playing piano by pianist is the functional position,fully pronated position, sustaining this position for long periods of hours or performing repetitive motions is vulnerable to cause PMRDs. In the amateur young piano players and students, there will be more flexibility in muscles and laxity in the joints, the young children's are more prone to get PRMDs with the functional position of the wrist and forearm and the PRMDs as consequence of it is inevitable.

This PRMDs had made few young children's to leave their passionate recreational activity from learning it and continuing

## Conclusion

This study concludes that there is a prevalence of specific pain in Fourth and Fifth finger among all three wrist position. This study also concludes that weight playing methodology has significant increase in the level of pain among three wrist position.. The PRMDs among young pianist can be minimized and avoided by appropriate flexibility, strengthening and endurance programs and by guiding them with proper body mechanics, importants of rest breaks, proper nourishment and hydration. These measures will make them to continue their passionate recreational activity through out their life.

**Limitations**

Sample size is less.

Limited age group was included.

Specific geographical area was investigated.

**Recommendations**

Motion Capture Analysis camera can be used.

Interference with large sample.

Research can be conducted in depth interview with players who experienced injury.

Intervention can be given and analysed .

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*Original Article*

# Estimateof Biological Activity of Parsleyextract on the Isolated Pathogenic Bacteria in Baghdad City

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## Abstract

This research investigates the most common urinary tract infection bacteria and their susceptibility to antibiotic disc and parsley (Aquas and methanol) extracts. The urinary tract microbial were isolated from patients of urinary tract infection (UTI) pregnant women patients from Baghdad Hospital. The pathogenic organisms were collected, cultured and identified. 50 microbial isolate wasset as, *E. coli 56* (46.66%), *Staphylococcus aureus19* (15.8%). *Proteus mirabilis* 6 (5%) and *Klebsiella pneumonia* 3 (2.5%). The pathogenic bacterial showed various zone of sensitivity to different antibiotics. Within the parsley Antimicrobial activity ranged from 3 to 20 mm. Antibacterial activity of parsley extract (5-22) mm on UTI isolated pregnant women and methanol extracts was between (3-15) mm. Therefore, Parsley extract had a pharmacological to treat all of 50 isolates that were identify from patients. In conclusion, the findings suggested that different extractsmay content as a broad-spectrum bactericidal factor, to control the emergent pathogens any way of their drug resistance mechanismsto treat urinary tract infections.

***Key word:*** *parsley, Petroselinumcrispum, UTI infection, water, methanol, inhibition zone.*

## Introduction

Urinary infections, are the most common form of infection in community practice. Annually, Among the 150 million of people worldwide have been invistigated with urinary tract infection [(1).] Managing urinary tract infection requires investigation,possible site for a responsible infection and pathogen that can include both the upper or the lower urine tract system[(2).] Lower urinary tract infections (cystitis) are characterized by a spectrum of dysuria, urgency, recurrence andSuprapubic pain [(3).]

The causes of urinary tract infection, by (sex and age)beforehand use of antibiotics and pollution inside or out of the hospital not to remind that they differ from environment to other. The microorganisms that at most cause urinary tract infection are G-negative intestinal microbial, essentially*Escherichia coli*, which are a lot offstudied microorganisms, follow by another G-negative bacteria like*Klebsiella*, *Enterobactersp,Acinetobactersp,Proteus sp,* The *Pseudomonas S.*[(4).] This problem was increased bacterial resistance. So in the last decade studies on natural remedies for the development of alternative drugs, whether natural or synthetic plant extracts have also been a valuable source of natural products to maintain health Human, and therefore in Iraq, increased plant compounds used for medical purposes as well as the world WHO Health Plants Ceremony is the best source for a variety of medications [(5).]

Medicinal plants has become diffuse and has enriched massive different biological effect and the combination of native, EuropeanandAfrican cultures [(6).] A number of researchers around the world have investigated the antiactivity properties of extracts plant[(7).]Parsley is a medicinal plant containing many proven medicinal properties including antioxidants,

anti-diabetic, analgesic, cramps, immunity, anticoagulant, ulcer, laxative, estrogen, diuretic, antihypertensive for fungus[8]. The beneficial effects of zucumonumcrispum on the digestive system claimed in ethnic medicine from various states, have been demonstrated by the mechanisms of spasm, analgesia, gastro protective, anti-secret and laxative in modern scientific investigations [9]Many researchers indicates that in several plants there are multi compound such as peptides unsaturated with long chain alkaloidal (aldehydes, phenols, and ethanol, chloroform, water, butanol and methanol are soluble compounds)[10]. Furthermore, the activity, of plant crispum on urinary tract inflammation, has been demonstrated through diuretic activity. The antiseptic property, can be (urinary tract) due to antimicrobial activity.Therefore, The purpose of this study areisolation, identification of microbial urinary tract infection with testing the sensitivity of the microbial organism to some antibiotic disc and biological activities of (parsley extract plants) of medicinal uses in Iraq.

**Material and Methods:-**

**Sample collection:-** A total 50 urine samples were collected from pregnant women infected Urinary Tract Infections from Baghdad hospital. The samples were collected an aseptically in sterile container from period April-August, 2019.

**Diagnosis bacteria:-** All isolated with selective and differential media were identified depend on morphological, Gram stain and classical method biochemical analysis, used for identification of bacteria including: IMVIC test.

**Preparation plant:-** For this work, plants parsley dried and ground to powdered of plant materials were used for extraction with different solvents (methanol and watery) were obtained from market in Baghdad City. The plants were cleaned with tap water then were process with (distilled water). The plant dried in room temperature, grounded to powdered, and stored in room temperature at 25°C until uses

**Extract preparation:-**

A total of 100 gm. of the leave parsley powder was steeped in (200 ml) of different solvent (ethanol, and watery) till 2 days, and filtered out oflayer muslin cloth and centrifuged at 3000×g for 15 minutes. The ethanol and methanol extracts were concentration with a Rotator evaporator at 40°C. After that, the extract was kept aseptically in sterile vials at 4°C until use. [11].

**Antibacterial Activity of extracts by diffusion method:-**

Antibacterial activity of parsley extract ( watery and methanol ) were evaluated by determine inhibition zone (mm) diameter and compared with standard antibiotic disc, disc diffusion method was used by sterile filter paper disc (diameter : 6 mm) which impregnated with extract in a known volume (20 µl) and appropriate concentration of the extract(500, 1000, 1500 µg/ml) and placed on a plate of inoculated nutrient agar , so used discs containing gentamicin (10 µg/disc) as positive controls and discs containing sterile water ( 20 µg/disc ) as a negative control in the study. The incubation period (1 day), evaluated the antimicrobial activity of every concentration by measuring inhibition zone with (mm). All methods were performed in deplicate and the values ofmeanwere taken.

**Susceptibility for antibiotic disc:**

The resistance of microbial isolates to different antibiotics was estimated by using the diffusion diskmethod on Mueller-Hinton agar plates (Bauer *et al*.,1966)[12], The antibiotics disc includethe: amoxicillin (25 µg), Trimethoprim (10µg), Penicillin (10 U), Ofloxacin (5 µg), gentamicin (10 µg), Nitrofurantoin (100 µg). These antibiotic disks were submitted on culture agar plates and incubated at 37 C for 24 hrs. Then determine and measured Inhibition zone (mm) and recorded as sensitive and Resistance according to (CLSI) guidelines (CLSI, 2010)[13].

## Statistical Analysis

Statistically was carried out using statistically software (SPSS version 10). The comparisons between groups were done using P <0.05 was considered as statistical significant.

## Results

Petroselinumcrispumis a herbal plant it's used in traditional medicine for the treatment of UTI. For there, its precise role has not been investigated through a clinical study. Thus, our study is the first clinical trial study trying to explore the role of (Petroselinumcrispum) parsley in the treatment of pregnant women at urinary tract infection. Urinary tract infection, are caused by many microorganisms including gram positive like *Staphylococcus* and gram negative such as *Ecoli* and *pseudomonas and protues*. A total of 50 bacterial isolate were collected from clinical sample of UTI patients from Baghdad hospital isolates as 43 positive culture while 7 as negative culture were isolated from 50 UTI sample.

Distribution of bacterial population in pregnant women with UTI was explained in the (table e 1.) which include four strain of organism. *Escherichia coli* (*E. coli*) was the most common organism isolated accounting for (n= 17, 34%) and the second highest organism was *Klebsiella* (n=11; 22%) followed by *Staphylococcus* (n=7; 14%) and *Proteus* (n=7; 16%).

**Table 1: Distribution of bacterial population from urine sample according to (Number and percentage).**

| Organism isolated | Number (N0.) | Percentage (%) |
|---|---|---|
| Escherichia coli | 17 | 34 |
| Proteus mirabilis | 7 | 16 |
| Klebsiella pneumonia | 11 | 22 |
| Staphylococcus aureus | 7 | 14 |
| No growth | 7 | 14 |
| Total | 50 | 34 |

Table 2shownAll thepathogenic isolates, represented differences in different biochemical characteristics, all the bacterialisolates were a negative result for motility excepted *Escherichia coli and Proteus mirabilis*as well as (+ve) positive (catalase and citrate) so urease (+ve) positive for all except *E. coli*, while (-ve) negative oxidase for all bacterial isolate. The results observed that there is an agreement with the previous studies. Gram-negative non-sporulatingrods that are oxidase negative and catalase-positive.

**Table 2: Result of biochemical test of bacterial isolates.**

| Bacterial isolates | | | Biochemical test | | |
|---|---|---|---|---|---|
| | Citrate | Urease | Oxidase | Catalase | Motility |
| Escherichia coli | + | _ | _ | + | + |
| Klebsiellapneumoniae | + | + | _ | + | _ |
| Staphylococcus aureus | + | + | _ | + | _ |
| Proteus mirabilis | + | + | _ | + | + |

The table (3),observed that *E. coli* were found sensitive to Nitrofurantoin, Gentamicin, Ofloxacin (I.Z = 16, 20, 18) mm respectively. While this bacteria was resistance to the other antibiotics. *Staphylococcus aureus* were found sensitive toAmoxicillin, Gentamicin, Ofloxacin with inhibition zoon (5, 10, 5) mm respectively. While this bacteria was resistance to the other antibiotics. While the*Klebsiellapneumoniae* were found sensitive toGentamicin, Ofloxacin with inhibition zoon (22, 18) mm respectively. While this bacteria was resistance to the other antibiotics. And*Proteus mirabilis* were found sensitive toTrimethoprim,Gentamicin with inhibition zoon (7, 10) mm respectively. While this bacteria was resistance to the other antibiotics.

**Table 3: Antimicrobial susceptibility test against isolated pathogens.**

| Antibiotics | Organism typeName of Organism | | | |
|---|---|---|---|---|
| | Klebsiella | E. coli | S.aureus | Proteus irabilis |
| Amoxicillin | - | - | - | - |
| Trimethoprim | - | 16 | - | 7 |
| Gentamicin | 22 | 20 | 10 | 10 |
| Ofloxacin | 18 | 18 | 5 | - |
| Nitrofurantoin | - | - | - | - |
| Pencilline | - | - | - | - |

The result in table (4)there was, a significant deference among all concentrations of watery extract toward each bacterial isolates, in the watery extract the results, showed appearance that aqueous extracts of Parsleywas strong activity at high concentrations (1500µg/ ml) against all isolates while the weakest activity at low concentrations ( 500µg/ ml ) on *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus mirabilis,*theinhibition zones were increased with the increasing of concentrations of the extract treatment. The best concentration was at 100% against *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus*

*mirabilis*with inhibition zones of (22, 19.5, 12, 13) mm respectivelywhen compare with positive control.

**Table 4: The effects of watery extract of parsley on growth of bacterial isolates.**

| Bacteria Spp | Concentrations of watery extracts | | | Positive control |
|---|---|---|---|---|
| | 500 | 1000 | 1500 | Gentamycin |
| Escherichia coli | 12 | 15 | 22 | 20 |
| Klebsiella pneumonia | 11 | 16 | 19.5 | 22 |
| Staphylococcus aureus | 6 | 8 | 12 | 10 |
| Proteus mirabilis | 7 | 8 | 13 | 10 |

Table (5)showed that, a significant deference among all concentration, of Methanol extract toward each bacterial isolates.In the Methanolextract the results, showed that methanol extracts of parsleystrong activity at high concentrations (1500µg/ ml) against *E coli, K. pneumoniae*, *Staphylococcus aureus and Proteus mirabilis*with inhibition zones of (15, 11.5, 9.5, 8) mm respectively.Whilst the inhibition zone in both concentration (500, 1000µg/ ml) f o r *E coli,* was (5,10 mm) followed by *K. pneumonia* in (500, 1000µg/ ml) was (6mm, 9mm) inhibition zoon. And w e a k e r activity against was *Staphylococcus aureus (6mm, 8mm) and Proteus mirabilis was* (3mm, 6mm).

**Table 5: The effects of methanol extracts of parsleystems on the growth of bacterial isolates**

| Bacteria Spp | Concentrations of Methanol extracts | | | Positive control |
|---|---|---|---|---|
| | 500 | 1000 | 1500 | Gentamycin |
| Escherichia coli | 5 | 10 | 15 | 20 |
| Klebsiella pneumonia | 6 | 9 | 11.5 | 22 |
| Staphylococcus aureus | 6 | 8 | 9.5 | 10 |
| Proteus mirabilis | 3 | 6 | 8 | 10 |

## Discussion

-In this paper (50) isolates.Include*E. coli* was most predominant,uropathogen with (34%), followed by (22%) *Klebsiellasp, Staphylococcus aureus* (14%), *Staphylococcus sp*. and *Proteusmirabil*(14. %) These result agree with Rana et al [14] who found that *E. coli* 43% followed by *Klebsiella pneumonia*14.1%, *Pseudomonas aeruginosa* and *Proteus mirabilis* 9.4 %, *Staphylococcus aureus* 7.8%, *Morganellamorganii*

6.2%.

Result on antibiotics susceptibility is different, by clinical isolates has become a major factor in drug choice and success of treatment. Similarly other studies, was found gram negative bacteria isolate of UTI were multi drug resistant to Ampicillin, Amoxicillin, Ceftizoxime, Cefepime, Tetracyclin. and Nitrofuration[15].

Result of this study present that (*Petroselinumcrispum*) Parsley extracts had effect on both G positive (+ve) and G negative (-ve) bacteria isolated from pregnant women suffering of urinary tract infection. Except *staph. aureus* which showed no activity at concentrations (500µg/ ml), because the extracts of *parsley* are broad spectrum in their activities, The methanol extract weaker activity against*Staph.aureus*(500µg/ ml) (I.Z = 7 mm and*protuesmerabeles*(500µg/ ml) (I.Z =5) mm, while highly activity against *E.coli , k. pneumonia.*. The results of this study were agree with the results conducted byresearches[16-17]they areshowed ethanolic extracts are inhibited the different species of G positive and G negative bacteria with inhibition of 8 from 11 bacteria species. Therefore parsley leave extract could be use as one method because most of plant are safe with little side effectand a wide range of antibiotic resistant microorganisms parsley plants have been used for the treatment in traditional medicine of urinary tract disease[18].

## Conclusions

Our results in the current research conclude the aqueous and methanol extracts of parsley showed a significant antibacterial activity but the watery extract is more effective than methanol extract. Therefore it can be used against urine bacterial infections. All isolates have sensitivity to Gentamycin, so Gentamycin possess higher efficacy whilst Ofloxacin has lower efficacy except *Staph aureus.*

**Recommendation** In future study identification the chemical nature componrnt of parsleyand study effect of each component on oral pathogenic bacterial growth and other organisms.

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**Ethical Clearance:** None

**Conflict of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/ or publication of this article.

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# Psychological Autopsy: A Lead to the Truth Untold

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## Abstract

Psychological autopsy is an important technique of investigation of an alleged suicidal case. The fundamental of *psychological autopsy* is based on the ultra-careful collection of records that help reconstruct the psychosocial domain of individuals. This paper gives insight into understanding of autopsy types and their uses. Relevance of psychological autopsy in forensic practice is highlighted such as validity, admissibility, scope and application in court of law for administration of justice.

***Key words:*** *Autopsy, Psychological autopsy, collection of evidence, Court law, Justice*

## Introduction

Autopsy, as the name suggests is made up of two words, " auto" and "opis" ,meaning self and examination respectively[1]. It is the detailed examination of external as well as internal body organs so as to get an answer to questions including the cause, mode and manner of the death [2]. Each organ is examined so as to find the answers to the mysteries left behind the dead. Autopsy due to its admissibility in the court continues to be the most important landmark in investigation. Autopsy should be done in a proper manner so as to corroborate the evidences found during investigation, so as to enable justice being rendered to the dead [3]**.** Conduction of autopsy aims at answering the following questions:

1. Cause of death
2. Mode of death
3. Post mortem interval
4. Crime scene reconstruction
5. Identification of the corpse
6. In cases involving newly born babies, still birth
7. Life style of deceased

Autopsy could be divided into six types based on the reason of its conduction. It is categorized into medical autopsy, anatomical autopsy, psychological autopsy, virtual autopsy, medico-legal autopsy and post-mortem examination. Medical autopsy is performed on the patient who dies during the course of his treatment in a hospital, generally performed by a pathologist. The main aim of medical autopsy is to know the effects of various treatments provided to the patient during his life time. Anatomical autopsy is generally done by medical students, so as to understand the structure of a normal human being. It is performed on the body's left unclaimed. Post mortem examination is done generally on the external surfaces of the corpse. Body fluids are collected during the procedure. Virtual autopsy being non destructive in nature involves the use of radiological techniques, such as X-rays, ultrasound , CT-scan so as to know

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about the cause of death , without having any ethical hindrance. Medicolegal autopsy is where suspicion of a foul play occurs. It is done so as to find out the cause of death, as per the request of Investigating Officer or SDM.

**Psychological Autopsy Definition**

Psychological autopsy also known as psychiatric autopsy [4] is conducted to know about the cause of unequivocal deaths. The cases covered under psychiatric autopsy are the ones whose reason for death could not had been clarified. Psychological autopsy as the name suggest , looks into knowing about the psychology of the deceased, immediate prior to the incident as well as of long back in time.[5]

**Applied Aspect Of Psychological Autopsy**

Cavanagh-et-al suggested suicidal cases as the ones that involve a consideration of psychological autopsy so as to know the prior condition[6]. Psychological autopsy enables us to know the about the death being actually suicidal in nature or not. In the simplest terms, psychological autopsy tells about the mental state of the deceased.

A questionnaire is prepared for the relatives, friends and family of the deceased, along with searching for information that could enhance the information regarding the mental state of the victim at the time of crime commission. Siddamsetty-et-al found that more than 20% of case being brought to coroner have unclear mode of death[4]. The cases having suspicious circumstances, where a conclusion could not have been found psychological autopsy plays an important role. It enables to reduce the ambiguity and provide justice to the deceased.

**Methodology Used For Psychological Autopsy**

1. Interviewing the close inmates and family members of the deceased.

2. Gathering all the personal information about the deceased. Both primary and secondary information about him is to be collected.

3. Preparation of a questionnaire to found an answer to during the investigation.

Psychological autopsy gives an insight about the reason of unclear by knowing the mental state of the victim based the certain pre- decided factors, prominently know as suicide risk factors, as the autopsy is carried out to know about the death being a suicide or not. The areas taken into consideration includes, the medical status , occurrence any mental disorder[7], history of previous suicide attempts, any help taken priory from mental health professionals, personal life issues, ill-physical health, previous family history of suicide, the lethality rate of the method used , general stress busting pattern, lifestyle of the victim, change in lifestyle and the way in which the informants reacted to death .Interview is conducted based on the areas defined above , so as to know about the relevant information regarding the victim enabling the investigator to reconstruct the probable sequence of events , based on responses gathered from the questionnaire.

**Implementations of Psychological Autopsy**

Implementation of psychological autopsy in the following cases:

1. UNDERSTANDING PERSONALITY: collection of personal information from the family members and close inmates about deceased could enable us to know the probable cause of death. In cases where murder in self defense is argued for, psychological autopsy could act as an corroborative evidence, telling us about the personality of the victim.

2. TO UNDERSTAND MOTIVE: Suicidal notes obtained on the scene of occurrence enables us to know about the intention behind the act, that if whether the act committed was in impulsive action or pre-planned one.

3. CRIMINAL CASE: In the cases involving parents, or, spouse, as the culprit could be solved by

psychological autopsy. Shashi Tharoor was booked for Sunanda Pushkar death case[8] in which he was accused for abetment of suicide was confirmed through psychological autopsy. Dexter case, having steven lake as the main culprit[9] in all the 4 murders, later committed suicide. The case was solved with the help of psychological autopsy.

4. LIFE INSURANCE: In the cases involving suicide, the burden to prove it as one, lies on the arms of the company so as to make them free from debt they have to pay to the deceased. Psychological autopsy is performed in such cases enabling us to know about the mental status of the victim prior to suicide.

5. CARE IN THE INSTITUTION: In the cases involving suicide having depression as the reason, the state of other inmates is also to be assessed so as to reduce chances of any future incident.

6. UNDERSTANDING SUICIDE: one of the most important implementation of psychological autopsy is to know about the reason of suicide. Various studies have been conducted on the validity of psychological autopsy in suicidal cases. Reasons ranging from mental illness to social pressure and illness related suicides. Based on the answers provided to the questionnaire result could be found out. Ortigo KM-et-al and Isometsa ET suggested as one of the major reason for suicide as found by psychological analysis[10] [5]is personality disorder.

The ethical considerations though come into play while considering psychological autopsy. A prior form is signed to maintain the right for dignity and if infringement is felt the process is stopped there and then.

**Validity**

Psychological autopsy is the major key to find out the reason for the death in cases involving suicide. But its validity is still under question due to an undefined set of rules and regulations to be followed during the procedure. The next concern relative to it's validity lies in the incompetency of the personals conducting the autopsy, might be having low forensic expertise and so can miss out important case related facts.

**Admissibility**

Considering the admissibility of psychological autopsy is of significant value in the cases where progression through other evidences is not possible. Generally serves as corroborative evidence, providing a sensible lead for investigation, acting as a golden standard in investigation.

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# Diplopia as the Initial Manifestation of Cerebral Vasculitis in a Patient with Systemic Lupus Erythematosus: Diagnostic Approach and Challenges

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## Abstract

The term Neuropsychiatric systemic lupus erythematosus (NPSLE) encompasses all neurologic and or psychologic symptoms arising in patients with SLE after other causes have been ruled out. Cerebral vasculitis as a manifestation of NPSLE is a rare case with a very broad spectrum of clinical presentation and severity. We report a case of a female with cerebral vasculitis presenting with diplopia as its early manifestation. Diagnosing cerebral vasculitis remains challenging given that biopsy as the gold standard is an invasive procedure and may not be available in many settings. A high suspicion of diagnosis requires clinicians' judgement in reviewing combination of detailed history taking, physical examination, neuroimaging, and other available supporting modalities.

***Keywords:*** *Cerebral vasculitis, diplopia, nerve VI palsy, systemic lupus erythematosus*

## Introduction

The term Neuropsychiatric systemic lupus erythematosus (NPSLE) encompasses all neurologic and psychologic symptoms arising in patients with SLE after other causes have been ruled out. Due to its broad features, it is crucial to promptly determine whether any neurologic or psychologic complaint arising during the course of the disease is a primary disorder, secondary to increased SLE disease activity, or to other systemic manifestations which is not related to SLE through thorough exclusion process [1]. The prevalence of NPSLE varies widely ranging between 37-95% depending on the diagnostic criteria used, study design, and other baseline characteristics[2].

Double vision, or diplopia, is a disturbance of visual focus which can occur as a result of either ophthalmologic or neurologic disorder with many possible aetiologies ranging from mild diseases such as refractive error or dry eyes to the lethal ones namely intracranial injuries [3]. With regards to that, it is vital to localize the underlying pathology of diplopia in a timely manner, particularly in SLE patients, to confirm the diagnosis and establish whether it is a manifestation of NPSLE or not. Patients with NPSLE generally have poorer prognosis, therefore, an early diagnosis and adequate treatment is required to prevent further life-threatening complications [4]. In this report, we illustrate the diagnostic steps of NPSLE manifesting as cerebral vasculitis with diplopia as the early complaint.

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## Case Report

A 24-year old Indonesian female was admitted to the emergency department with sudden complaint of shortness of breath since two days before admission. She also complained of cough since 1 week before admission and joint pain in her elbows and knees. There was not any fever. Two days ago, she started to experience double vision. She had been diagnosed with SLE since 4 years ago and routinely takes mycophenolate sodium 180 mg BID and methylprednisolone 4 mg QD. She had a history of being diagnosed with pneumonitis lupus one year ago and have completed six sessions of cyclophosphamide chemotherapy in which she achieved complete clinical response.

On physical examination, her general appearance was ill with severe dyspnoea, blood pressure 110/80 mmHg, respiratory rate 28 times per minute, heart rate 112 times per minute, and body temperature $37.1^0$ C. Rhonchi was found in middle side of right hemithorax. Regarding the presence of double vision, the first approach is to promptly determine whether it is a monocular or binocular diplopia. During the eye examination, the patient claimed that the double vision resolved when either eye was covered establishing a binocular type diplopia. The most common cause for binocular diplopia are neurologic. We continued to perform neurologic examination to the patient covering motoric, sensory, reflexes, and cranial nerves function. It was found that the motoric, sensory, and reflexes were normal. However, during the cranial nerves examination, the patient failed to move her left eye's gaze to lateral side indicating that she may have cranial nerve VI palsy.

Blood test showed haemoglobin level 6.4 g/dL, leukocytes 2740 cells/mm3, neutrophil 77.3%, lymphocytes 18%, random plasma glucose 102 mg/dL, BUN 31 mg/dL, creatinine serum 1.04 mg/dL, CRP 30.49 mg/dL, potassium 4.7 mmol/L, ESR 20, C3 45 mg/dL, C4 13 mg/dL, and blood gas analysis as follows: pH 7,51, $pCO_2$ 24,9 mmHg, $pO_2$ 139 mmHg, $HCO_3$ 20 mmol/l, BE -3,2 mmol/l, $SO_2$ 99,4%. The chest x-ray showed infiltrates in right paracardial. She was initially assessed with suspected case of pneumonitis lupus with bacterial pneumonia as its differential diagnosis, and suspected case of NPSLE manifesting as cranial nerve VI palsy. Consequently, she was planned to have sputum and blood culture examination to determine the true cause of the pneumonia; and brain CT scan to evaluate the cranial neuropathy. She was also planned to have lipid profile and electrocardiography to screen possible microvascular risk factors. Meanwhile, she was treated with oxygenation support, intravenous methylprednisolone 1 mg/kg body weight, levofloxacin 750 mg intravenous QD as empirical antibiotics for possible infection, and blood transfusion.

The CT scan of the brain with contrast conducted on the second day of treatment discovered: multiple small hypodense lesion in right and left basal ganglia and calcification of left basal ganglia suggesting a vasculitis with lacunar infarction in basal ganglia. The blood test done earlier showed normal lipid profile (total cholesterol 141mg/dL, low density lipoprotein 79mg/dL, high density lipoprotein 28mg/dL, and triglycerides 132mg/dL); the electrocardiography only recognized a sinus tachycardia; while the sputum and blood culture examination required at least 5-7 days of processing. A diagnosis of cerebral vasculitis as a manifestation of NPSLE was added. In the time she was diagnosed with cerebral vasculitis, a blood examination to inspect the presence of antiphospholipid (aPL) antibodies was also performed in order to rule out the possibility of coexisting antiphospholipid syndrome (APS). The result showed normal aCL (aCL IgG 3 and aCL IgM <2), normal LA (47,4 seconds), and negative IgG and IGM of anti-$\beta_2$glycoprotein-1.

On the grounds that there was a possible bacterial infection existing due to pneumonia in this patient, determining the management brought a dilemmatic situation as both pneumonitis lupus and NPSLE requires high dose of glucocorticoids and cyclophosphamide

which are strong immunosuppressants. Considering the minimal clinical features of infection and sepsis found in this patient, it was decided to increase the methylprednisolone dose to a pulse of 500 mg daily for 3 consecutive days with close monitoring. By the time the sputum and blood culture revealed no signs of bacteria on the sixth day of treatment, the patient was immediately treated with cyclophosphamide. Nonetheless, the cough and dyspnoea worsened and her condition was deteriorating with signs of oxygen desaturation. She refused to be treated with ventilation support and passed away due to respiratory failure on the 8th day of treatment.

## Discussion

Cerebral vasculitis as one of NPSLE's manifestation is rare of which prevalence fell below 7% in post-mortem studies [5]. It is defined as inflammation occurring in cerebral blood vessels with very diverse early clinical manifestations and may resemble common neurology symptoms such as loss of consciousness, headaches, seizures, stroke, and optic or cranial neuropathies. A case-series report conducted in a health centre in Portugal discovered that during 10 years of observation, there were only 4 cases of cerebral vasculitis in SLE patients of which each has different initial manifestation, covering: cognitive dysfunction, lower limb monoparesis, seizure, and diplopia [6]. Brain vessel biopsy is its diagnostic gold standard, however, it is an invasive procedure and may not be available in many settings. It requires complex technique, particularly when affected site is difficult to access resulting in varying sensitivity rates [7,8]. As a result, high suspicion of the disease is usually made by experts' clinical judgement in the field after reviewing detailed history-taking, physical examination, neuroimaging, and other supporting modalities. Given the unpredictable course of the disease, the diagnostic process may pose as challenges for clinicians, especially those in low-resource settings.

When this patient was discovered with diplopia, it is crucial to quickly determine the underlying cause. The first step toward a patient with diplopia is to ascertain whether it is a monocular or binocular type by asking the patient to close their eyes alternately. Monocular diplopia is generally a result of ophthalmologic disorders while binocular diplopia is due to neurologic problems. In monocular diplopia, the double vision disappears when the affected eye is covered but returns when the normal eye is covered. While in binocular diplopia, the double vision disappears when either eye is covered. When the examination revealed a binocular type, the differential diagnosis narrows down to neurologic causes, therefore, a thorough neurologic physical examination was conducted [3,9,10].

After the examination showed a cranial nerve VI (abducens nerve) palsy, this case drifted us to a whole new diagnostic algorithm to follow. With its long course between lower pons and the eye, the abducens nerve is very much susceptible to injuries. The past publications have established that immediate neuroimaging examination is not recommended due to its lack of diagnosis benefit and cost-effectiveness. The evaluation of nerve VI palsy begins with excluding history of trauma or physical injuries. After trauma has been excluded, it is advisable that we conduct evaluation of vasculopathy risk factors (i.e. diabetes, hypertension, hyperlipidaemia, and coronary artery disease) as microvascular ischemic disease is a frequent underlying cause of cranial nerve VI palsy. Most cases of cranial nerve VI palsy due to vasculopathies ameliorate within 3-6 months after treatment, however, if improvement was not observed within the timeframe or symptoms worsen, neuroimaging examination is then advised. Conversely, patients without microvascular risk factors are suggested to have immediate neuroimaging examination to evaluate other possible aetiologies. MRI is more preferred to CT scan due to its higher sensitivity. Brain CT scan is hardly representative because it generally can only display large ischemic

infarcts, however, in some cases it may show brain calcifications within old ischemia, similar to what we discovered in this case [11,12].

This patient was planned to have brain CT scan because history of trauma and microvascular risk factors have been excluded, and emergency MRI examination was not available at the time. After the CT scan result supported a diagnosis of cerebral vasculitis, the patient was treated accordingly.

The therapy of NPSLE depends on the underlying mechanism whether it is inflammatory or ischaemic/thrombotic. The management of NPSLE with inflammatory process includes high dose of intravenous glucocorticoids in combination with monthly cyclophosphamide therapy, while NPSLE with the latter mechanism requires anticoagulant and antithrombotic if aPL antibodies are present. It is not uncommon that both mechanism may even coexist in the same patient. When the inexistence of aPL antibodies were proven by normal results of aCL, LA, and anti-$\beta_2$glycoprotein-1, it was clear that the patient did not require additional anticoagulant or antithrombotic treatment. Meanwhile, this patient had pneumonia either due to lupus pneumonitis or infection as a comorbid of which exact aetiology was still being evaluated. The mainstay of pneumonitis lupus treatment is similar to NPSLE consisting of high dose of steroids followed by cyclophosphamide. It is important to note that administration of high dose steroid in combination with cyclophosphamide may suppress immune system thus requiring extra cautious in patients with suspected infections [13,14].

This patient was treated with pulse dose of methylprednisolone for three days followed by administration of cyclophosphamide after the culture result indicates no sign of bacterial infection. Nonetheless, at the same time the patient's condition has deteriorated and she eventually died of respiratory failure due to severe lupus pneumonitis. Cerebral vasculitis in SLE is generally a reversible case, however, pneumonitis lupus has poor prognosis with mortality rate as high as 50% [6,15].

## Conclusion

In summary, an SLE patient with diplopia was unexpectedly discovered to have cerebral vasculitis through thorough history taking, physical examination, laboratory, and neuroimaging examination. Cerebral vasculitis as manifestation of NPSLE is a rare case with very broad clinical features and prognosis, thus requiring good clinical judgement and experience in the field. Therefore, it is important that clinicians are able to conduct diagnostic investigation by processing all subjective and objective findings to arrive at a safe clinical-decision that benefits the patients, such as deciding the examinations that need to be taken (taking cost-effectiveness and patients' convenience into consideration), therapy to give, and even decision to immediately refer when diagnostic modalities are limited.

### Patient Informed Consent

Patient consent was obtained from patient and family for writing this case report.

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# The Levels of Salivary IgA and Lactoferrin and Some Salivary Parameters in Waterpipe Smokers and Cigarette Smokers

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## Abstract

**Background:** Waterpipe and cigarette are two types of tobacco consumption, their smoking is associated with many adverse effects it has a detrimental effect on saliva and causes a reduction in salivary pH and flow rate in addition to their controversial effect on salivary IgA and lactoferrin. **Materials and Methods**: unstimulated saliva was collected from 84 subjects in the early morning between 8-10 am. Subjects were divided equally into three group'swaterpipe smokers, cigarette smokers, and non-smokers all of the participants are adult male aged between 25-60 years, Sandwich ELISA technique was used for detection and estimation the level of salivary IgA and lactoferrin. **Result:** IgA and Lactoferrin results showed significantly($p \leq 0.01$), higher concentration in cigarette and waterpipe smokers group than non-smokers Results also showed that Salivary flow rate(SFR) and pH was higher in the non-smoker's group followed by waterpipe and cigarette smokers groups with a highly significant difference ($p \leq 0.01$), The correlation between Lactoferrin and salivary IgA also showed a significant ($p \leq 0.01$),moderate positive correlation in the cigarette group only. **Conclusions**:Smokingincrease Salivary IgA and lactoferrin concentrations while it reduces SFR and pH of saliva, a significant positive correlation was founded between lactoferrin and salivary IgA only in cigarette smokers

***Keywords****: salivary IgA, lactoferrin, salivary parameters waterpipe smokers*

## Introduction

Tobacco is a product obtained from the leaves of an annually-grown herbaceous plantknown as Nicotiana tabacum, there are twotypes of tobacco consuming either smoking like waterpipe, cigarette, and pipeand non-smoking chewing tobacco like kheni [1, 2].

Globally, there are about one billion men and 175 million women ages older than 15 are smoker currently, tobacco contains more than 7000 lethal chemicals compounds, including at least 70 known carcinogens compound that can damage nearly every organ in the human body [3],includecarbon monoxide, cadmium, cobalt, and polycyclicaromatic hydrocarbons [4]. A cigarette is the most favored product of tobacco used by about (82%) of tobacco consumers [5].

Another type of tobacco consumption is waterpipe which use has recently increased in popularity, and modern-day waterpipe smokers involve young teens, university students, and even high-school students [6]. Tobaccohas an adverse effect on saliva which consists of 99 %water while the remaining 1 % is composed of organic and inorganic molecules [7]. Saliva has important functions in the oral cavity which include maintaining a moist oral mucosa that is less susceptible to abrasion, neutralization of acids or bases, protecting against demineralization [8], it protects the oral mucosa from dryness and food debris, and it acts as alubricant during the process of mastication, it provides the fluid in which solid food

dissolves and distributesit to the locations of the taste buds (9).

Saliva also helps in the process of formation of plaque and protects the enamel of teeth at the same time [10, 11, 12]. Tobacco causes asignificant reduction in salivary pH and flows ratewith increased duration ofexposureand this increases the probability of more caries exposure [13, 14, 15]. As well as it hasmany adverse effects on both innate and adaptiveimmune responses (16).One of their implication was salivary IgA which is a dimeric (dIgA) composed of two IgA monomeric, secretory components (SC) and J (joining) chains [17]. sIgA neutralizing toxins and enzymes, preventing the binding of pathogens to mucosal surfaces and facilitating their removal in the mucosal layer [18], studies found that high-intensity caries is associated with increased levels of salivary sIgA which is combined with specific epitopes of cariogenic bacteria, resulting in a locally specific immune response [19].

Tobacco showed a controversial effect on salivary IgA levels, some revealed higher s-IgA in smokers in comparison to non-tobacco consumersmay be due to irritation of tobacco to mucosa so IgA defends these membranes from multitudes of soluble antigens [20, 21, 22]. While others studies showedthat the levels of IgA decrease in smokers when compared with non-tobacco users [23]. Another salivary parameter affected by tobacco is Lactoferrin (Lf) whichis an iron-binding protein.

LF is present in several mucosal secretions such astearsand saliva [24] Lactoferrin is a multi-functional compound due to its high affinity to ferric iron, which deprives microbes of the free iron required for their growth and its tendency to bind with microbial and target host cell surfaces. [25, 26]. Tobacco showed a controversial effect on salivary lactoferrin,whether an increased or decreased level [27, 28].

## Materials and Methods

The subjects who participated in this study were 84 divided equally into three groups two smokers groups (waterpipe smokers, cigarette smokers) and one non-smokers group), all subject were adult male between 25 and 60 years old with a duration of smoking more than 5 years. Subjects with systemic diseases, such as diabetes mellitus, chronic heart disease, and acquired immunodeficiency syndrome or those who took antibiotics, within the last 3 months; or reported having periodontal treatment during the last 6 months had been excluded.

Unstimulated saliva was collected from subjects in the early morning between 8-10 am. Before collecting the saliva; the subjects were asked to avoid eating or drinking for three hours,the subjects were asked to spit saliva into the sterilized cups that possess graduationsin order to determine SFR (29). The pH was measured directly using a pH Meter to prevent any degeneration of the sample.

Determination of salivary Lactoferrin and salivary IgA was conducted by using sandwich ELISA technology, the ethical approval had been obtained from the College of Dentistry- University of Baghdad,

## Statistical Analysis

Data were analyzed using SPSS (statistical package of social science) software version 25. In this study the following statistics were used:

**Descriptive statistics:** including means, medians, standard deviations, standard errors, mean ranks, minimum and maximum values, and statistical tables and figures.

**Inferential statistics:** including:

**a) Kruskal-Wallis H test:** to compare the measured variables among the groups.

**b) Mann-Whitney U test:** to test any statistically significant difference between every two groups.

**c) Spearman's rank correlation coefficient test (r):** to test the relation between the measured variables in each group.

## Results

**Table 1: The differences between, waterpipe smokers, cigarette smokers, and non-smokers groups considering salivary IgA, lactoferrin, and other salivary parameters.**

| Variables | Groups | Descriptive statistics | | Group difference | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Median | Mean Rank | KWH test | p-value | Groups | | Mann-Whitney U test | p-value |
| Salivary IgA (µg /ml) | Control | 69.90 | 20.875 | 36.181 | 0.000 | Control | Waterpipe | 132.5 | 0.000 |
| | Waterpipe | 153.15 | 47.518 | | | | Cigarette | 46 | 0.000 |
| | Cigarette | 173.45 | 59.107 | | | Waterpipe | Cigarette | 273 | 0.051 |
| Salivary Lactoferrin (µg /ml) | Control | 0.87 | 28.589 | 15.103 | 0.001 | Control | Waterpipe | 202 | 0.002 |
| | Waterpipe | 1.09 | 45.571 | | | | Cigarette | 192.5 | 0.001 |
| | Cigarette | 1.25 | 53.339 | | | Waterpipe | Cigarette | 288 | 0.088 |
| SFR | Control | 0.90 | 61.250 | 31.641 | 0.000 | Control | Waterpipe | 185 | 0.001 |
| | Waterpipe | 0.70 | 41.089 | | | | Cigarette | 74 | 0.000 |
| | Cigarette | 0.50 | 25.161 | | | Waterpipe | Cigarette | 224.5 | 0.005 |
| pH | Control | 7.00 | 60.571 | 47.553 | 0.000 | Control | Waterpipe | 255 | 0.022 |
| | Waterpipe | 6.90 | 49.357 | | | | Cigarette | 23 | 0.000 |
| | Cigarette | 6.60 | 17.571 | | | Waterpipe | Cigarette | 63 | 0.000 |

*SFR= salivary flow rate, P≤0.05 Significant, P>0.05 Non-significant*

Table 1 showed that IgA results in (µg /ml) had thehigher median values in cigarette smokers group (173.45) and waterpipe smokers group (153.15) followed by non-smokers (69.9) with a highly significant difference (p≤0.01), Mann-Whitney test clarified that even the median value of cigarette group was higher than waterpipe group but statistically there was no significant differences (P>0.05) between them.

Also, lactoferrin results in (µg /ml) revealed that the higher median values were in the cigarette smokers group (1.25) and waterpipe smokers group (1.09) followed by non-smokers (0.87) with a highly significant difference (p≤0.01), the median value of

cigarette group was higher than waterpipe group but statistically, there were no significant differences (P>0.05) between them,as clarified by Mann-Whitney test.

Whereas Salivary FR results presented that median values of the non-smoker's group (0.9)have a higher value followed by the waterpipe smokers group (0.7) and cigarette smokers group (0.5) with a statistically highly significant difference( p≤0.01)

finally, Salivary pH results showed that median values of the non-smoker's group (7.0)have a higher value followed by the waterpipe smokers group (6.9) and cigarette smokers group (6.6)with a statistically highly significant difference( p≤0.01)

**Table 2: The correlation of salivary lactoferrin with salivary IgA**

| Variables | | Groups | | |
|---|---|---|---|---|
| | | Control | Waterpipe | Cigarette |
| s-IgA | r | 0.218 | 0.192 | 0.580 |
| | P | 0.266 | 0.327 | 0.001 |

*P≤0.05 Significant, P>0.05 Non significant*

Table:2 showed that the correlation of Lactoferrin with IgA was weakly positive in the non-smokers and waterpipe groups with non-significant statistical differences (P>0.05), while there was a moderate positive correlation in the cigarette smokers group with a highly significant statistical difference (p≤0.01).

## Discussion

The results of the present study revealed that tobacco smoking in general increase salivary IgA as the median values of smokers groups was higher than non-smokers with a highly significant difference, as smokers have an IgA level that is about two-fold of that of non-smokers, this may be due to the stimulation effect of smoking on the mucosal immune system that drives to increase production of the s-IgA which protects these membranes against soluble antigens by preventing their adhesion to the surface of mucosal cells [21]. These results agree with previous research on IgA which stated that salivary IgA concentrations were significantly higher in tobacco smokers [20, 30] and disagree with the previous study which showed lower levels of IgA in smokers in comparison with the non-tobacco consumer [31], and other study said that there is no significant difference in the concentration of IgA between waterpipe smokers and non-smokers [32].

Many previous studies showed that waterpipe smoke and cigarette smoke contains many similar toxicants such as carbon monoxide, tar, and nicotine [33, 34], and this may explain why their IgA and lactoferrin levels were so close in this study. Lactoferrin results also showed that the smoker's group had highly significant median values in comparison with non-smokers groups this agree with other studies which found that both active and passive smoking has been associated with an increase in lactoferrin concentrations in human secretions [35, 36].

The results also showed that the median of SFR of non-smokers group had a higher level followed by the waterpipe smokers group and then cigarette smokers group as smoking diminished SFR, and this agrees with the results of previous researches which demonstrated that long-termconsumption of any type of tobacco is one of the risk factors for reducing SFR

[37, 38, 39]. Another study proved that smoking for a long duration causes a reduction in the secretion of saliva and changes their quality from serous to thick [40]. While it disagrees with a study that said that SFR does not affect bythe consumption of tobacco [41].

The present study also revealed that pH median was higher in control groups followed by waterpipe smokers group and then cigarette smokers group and this in consent with former studies [42, 43, 44] as the reduction of salivary pH of smokers may be due to the impairment of the salivary mechanism defense bytobacco usage which may eventually result in multiple mucosaland dental diseases [41] and also decreasing of bicarbonate secretion with decreasing in SFR may lead to alter salivary pH and turn it acidic that resulted in improvement in the growth of aciduric bacteria and creating uninhabitable conditions for the protective oral bacteria. This leads to a shift in the oral environmental balance to favor the growth of cariogenic bacteria which produce acid from sugar and further reduces the salivary pH [45, 13].

On the other hand, The correlation between salivary lactoferrin and IgA revealed a significant positive correlation in the cigarette smokers group, this could be due to the potential association between them against cariogenic bacteria, as in one previous study which showed a potential association between LF and s-IgA in a patient infected with human immunodeficiency virus [46], or could be due to their response to irritation caused by cigarette smoking, while there was a non-significant positive correlation between them in waterpipe smokers and non-smokers groups, this positive correlation agrees with the previous study done by Ide et al in 2016 demonstrated that even with a non-significant correlation between them, increase or decrease in value of one was associated with a slight tendency for the other to also be increase or decrease, respectively [47].

## Conclusions

Waterpipe smoking and cigarette smoking diminished SFR and pH of saliva significantly,while salivary IgA and Lactoferrin concentrations were increased in the tobacco smokers groups, and a significant positive association was found between salivary IgA and lactoferrin levels on cigarette smokers only

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

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# Incidence of Abruption and Placenta Previa in Pregnant with Previous Caesarian Section

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## Abstract

Caesarean section is a surgical procedure in which the incision made through a women's abdomen (laparotomy) and uterus (hysterectomy) to deliver one or more babies. In each previous c/s the risk of placenta previa and abruption increase. The aim of this study is to find out the associated between the previous caesarean section and placenta previa and abruption in this pregnancy. A retrospective study was carried out during the period between 1st July 2018 and 20th august 2019 in Al-Zahraa Teaching Hospital. During the period of study, 119 pregnant women with history of previous scar presented to the hospital for vaginal delivery or c/s, 70 of these patients diagnosed that they have placenta previa and as have placenta abruption by ultrasound and confirmed during delivery. Another 49 patients were without complication. Pregnant women with APH due to other causes were excluded. Data were collected through detailed history. Official agreements and verbal consent of all women were taken. Statistical analysis performed by using the statistical package for social sciences and appropriate statistical test were applied.

***Keywords****:Caesarean section, placenta previa, abruption, pregnants and vaginal delivery*

## Introduction

Recent revised classificationof placenta previa, it classifies into true placenta previa when the placenta tissue covers the internal cervical, and low-lying placenta when the placental lies within 2cm of the cervical but does not cover it(1).

### Incidence of Placenta Previa

Placenta previa complicates approximately 3-5 per 1000 pregnancies in the world (2). The incidence is significantly more at 20 weeks about 5% and then it starts diminish until it reaches 0.5% at weeks 36 and above (3).

### Placenta Abruption

Defined as premature separation from the uterus of a normally implanted placenta after 20 weeks gestational age and before the delivery of fetus(1).

### Incidence of Placenta Abruption

The incidence rate of placental abruption 0.5% or 1 in 200 deliveries (10), of all antepartum hemorrhage, about one-third can be due to placental abruption,40% - 60% of placental abruption occur prior to 37 weeks gestational age(1).

The recurrence rate of placental abruption was higher after severe than mild PA, after severe abruption there was two-fold recurrence riskwhereas after mild, there was no risk for recurrence (11) .

### Classification of placental abruption

It classifies into three types:

### 1-Revealed hemorrhage

The hemorrhage occurs from the lower part of placenta and blood escapes through the cervical. In this type, the major hemorrhage is apparent externally.

**2-Concealed hemorrhage**

In this type, the blood accumulates between the placenta and the uterine wall.

**3-Concealed and revealed of mixed hemorrhage**

In this type, the hemorrhage occurs close to the placenta and is both concealed and revealed [12].

**Clinical feature**

Sign and symptom depend on the type of placental abruption, patient may be asymptomatic, or may present with painful vaginal bleeding, or may present with sign of shock (in concealed bleeding)[12, 10 and 14] .

**Management of placental abruption**

The management depend on the severity of abruption, fetal status and the gestational age.

**Immediate delivery**

Depend on the severity and whether the fetus alive or dead. If the fetus alive, caesarean section delivery has been shown to have a better outcome than vaginal delivery.

If the fetus is dead (20%) of case, vaginal delivery should be trained after maternal resuscitation [16], [15].

**Methodology**

**The study setting**

The data collected from the obstetrical department and labor room in al-Zahraa teaching hospital in Al-kut city. Al-kut is the center of wasit governorate.

**The study design**

A cross-sectional design was conducted to achieve the aim and objectives of this study. The collected cases were women with previous caesarean section admitted to the labor room, at total of (119) pregnant ladies were included during the period of study, (70) of ladies were with placenta previa or abruption, were (49) women without complication.

**Sampling**

**Case definition**

The cases were taken from patients whose age were between (18-45) years, who are pregnant in between 28-41 weeks of gestation presented with APH due to P.P and P.A

**Inclusion criteria**

Pregnant aged between (18-45) years, 20-41weeks of gestational age, who had second births with previous caesarean section presented with APH due to P.P and A.P females that were not having placenta previa or abruption were selection as control.

**Exclusion criteria**

Patients with no information on maternal demographics and behaviors during pregnancy patient without previous scar, APH not due to P.P or P.A.

**Method used for diagnosis**

The patient included in this study were diagnosed to have low lying placenta in their first trimester confirmed by performing transvaginal ultra sound using vaginal probe. In the third trimester, transvaginal u/s used to diagnose placenta previa.We depend mainly on the clinical presentation for the diagnosis of the placental abruption rather than transvaginal u/s.

**Period of the study**

The period of the collection of the data, was extended from 1 July 2018 -20 august 2019. The rest of time was for data analysis and writing up the thesis.

**Collection of the data**

Data were obtained from pregnant women attending the study setting for management through direct interview by the investigator himself.

In addition to collecting the basic demographic details questions were asked about the medical history, social history, gynecological history and

obstetric history to confirm the presence or absence of related risk factor.

The database included the socio-demographic information on the patients age, number of C/S, number of antenatal care visit interval between subsequent pregnancy, number of miscarriages, socio-economic status and fetal outcome.

## Data Analysis

By using the statistical package for social sciences (SPSS) software for windows, version 23, IBM, USA, data of 119 pregnant women with previous c section were entered and analyzed.

Chi square test was used to assess the significance of differences in frequencies of each category.

P value equal or less than 0.05 was considered significant.

## Result

In this study, 119 patient with history of previous scar presented to the labor room during the period of study,51(42.9%) of them were without PP OR PA, whereas,68(57.2%) of them presented with complication, 49(41.2%) of them were have placenta previa and 19(16%) were have placental abruption . The patient age was ranged between 18-45, 14.3% of patients aged between 18-20, 37% aged between 21-25, 23% aged between 26-30, 16.8% aged between 31-35, 7.6% aged between 36-40 and 8% aged >41. Increased maternal age >21 no associated with increase the number of placentae previa and abruption as p-value was 0.296 (not significant). Table(1) show the gestational age of the patients.

**TABLE 1: Gestational Age**

| | | Frequency | Percent |
|---|---|---|---|
| Valid | 2500 | 1 | .8 |
| | 30.00 | 1 | .8 |
| | 31.00 | 1 | .8 |
| | 34.00 | 3 | 2.5 |
| | 35.00 | 2 | 1.7 |
| | 36.00 | 23 | 19.3 |
| | 37.00 | 26 | 21.8 |
| | 38.00 | 30 | 25.2 |
| | 39.00 | 22 | 18.5 |
| | 40.00 | 7 | 5.9 |
| | 41.00 | 3 | 2.5 |
| | Total | 119 | 100.0 |

Increase the number of caesarean sectionsare associated with increase the risk of PP or PA, 39.1% of patients with previous one scar have placenta previa, whereas 14.5% were have placental abruption, 75% with previous two scar were have placenta previa and 25% were have placental abruptionas p-value was 0.000 (significant). TABLE (2) show the number of caesarean sections of the patients.

**TABLE 2: Number of Caesarean Section**

| | | Frequency | Percent |
|---|---|---|---|
| 1 | | 110 | 92.4 |
| | 2.00 | 8 | 6.7s |
| | 4.00 | 1 | .8 |
| | Total | 119 | 100.0 |

There is not association between the interval between subsequent pregnancy and the risk of placenta previa and abruption in next pregnancy as p-value was 0.5 (not significant). TABLE (3)show the interval between subsequent pregnancy calculated in months.

**TABLE 3: Interval Between Subsequent Pregnancy in Months**

| | | Frequency | Percent |
|---|---|---|---|
| Valid | 200 | 2 | 1.7 |
| | 4.00 | 2 | 1.7 |
| | 6.00 | 9 | 7.6 |
| | 7.00 | 3 | 2.5 |
| | 8.00 | 11 | 9.2 |
| | 9.00 | 7 | 5.9 |
| | 11.00 | 5 | 4.2 |
| | 12.00 | 38 | 31.9 |
| | 18.00 | 5 | 4.2 |
| | 24.00 | 21 | 17.6 |
| | 30.00 | 1 | .8 |
| | 36.00 | 10 | 8.4 |
| | 42.00 | 1 | .8 |
| | 48.00 | 2 | 1.7 |
| | 60.00 | 2 | 1.7 |
| | Total | 119 | 100.0 |

Poor antenatal care visits are associated with increase the riskas p-value was 0.01(significant). TABLE (4) show the number of antenatal care visits.

**TABLE 4: Number of Antenatal Care Visits**

| | | Frequency | Percent |
|---|---|---|---|
| Valid | 0 | 23 | 19.3 |
| | < 4 | 61 | 51.3 |
| | >= 4 | 35 | 29.4 |
| | Total | 119 | 100.0 |

The socioeconomic status of the patient has association with the risk of complicationsas p-value was 0.015 (significant). table (5) show the socioeconomic status of the patients.

**TABLE 5: Socioeconomic Status**

| | | Frequency | Percent |
|---|---|---|---|
| Valid | low | 9 | 7.6 |
| | medium | 94 | 79.0 |
| | high | 16 | 13.4 |
| | Total | 119 | 100.0 |

**TABLE 6: Patient Character**

| | | Frequency | Percent |
|---|---|---|---|
| Valid | normal | 51 | 42.9 |
| | Placenta previa | 49 | 41.2 |
| | Placental abruption | 19 | 16.0 |
| | Total | 119 | 100.0 |

There is significant association with the history of miscarriage and the occurrence of PP or PA in next pregnancy as p-value was 0.005 (significant).

**Ethical Consideration :** The study protocol was approved by the department of gynecology and obstetrics, medical college, Wasit university.

Agreement of the hospital administration office was obtained.Verbal consent of all women was obtained prior to participation.

**Conclusions:** The risk of placenta previa and placental abruption increased in the subsequent pregnancy after c/s delivery at first and, the risk increases with increase the number of c/s scars.

Poor antenatal care visit and socioeconomic status are associated with increase the risk of PP and AP.

Increased maternal age associated with increase the risk for PP and PA.

Patient with history of abortion have risk for develop PP and PA.

**Recommendation**

1-decrease the number of elective c/s if there is no medical indication.

2-provide good antenatal care center to identify the pregnant women with risk factor and when to intervene.

3-patient with history of previous PP OR PA should be followed carefully on routine ultrasound.

**Older than 40 years -2%**

1-previous c/s birth: there is a strong associated between previous c/s and risk of subsequent development of placenta previa (18).

2-multiparity: women who had previous pregnancies have excess risk of PP (5).

3-multiple gestation: it occurs due to the larger surface of placenta or due to the risk factor that led to the development of multiple gestation as: increase mother age, family history(5).

4-dilatation and curettage: damage of endometrium or the myometrium may be factor in implantation of placenta in the lower uterine segment. (19).

5-prior PP: mothers with PP have a ten-fold risk of recurrence at subsequent pregnancies(5).

**Conflict of Interest – Nil**

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**Ethical Clearance – Not required**

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# Levels of Suicidal Ideations and Intents among the Inmates in the Correctional Institutions in Baghdad City - Iraq

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## Abstract

**Background**: Suicidal ideations concern thinking about suicide or an uncommon and inappropriate preoccupation with suicide. The range of suicidal ideation and intent differs significantly from brief thoughts to wide thoughts, to comprehensive planning, and role-playing.

**Objectives:** To explore inmates' levels of suicidal ideation and intent in the prisons of Baghdad City and to find out the relationship between these levels and some demographic characteristics of those inmates.

**Methodology:** A descriptive analytic design was used to guide this study which was conducted from October 15th, 2015 to the June 10th, 2016. A non-probability sample of 100 inmates in Baghdad correctional institutions was recruited. The study instrument is a questionnaire consisted of nine demographic characteristics and 19 items represent Beck suicidal ideations and intents scale. Data were analysed by using descriptive statistical measures of frequency, percent, and distribution; and inferential analysis (Chi-squire).

**Results:** The study results displayed that more than half of the inmates are from twenties and thirties decades (58.0%), 68.0% having elementary and secondary school, half of them are with six to 15 years imprisonment, and 69.0% are married. Three quarters have moderate and high levels of suicidal ideations and intents. The study also finds that the more the period of imprisonment and the less of income the inmates have the higher the levels of suicidal ideation they have.

**Recommendations:** There must be a good awareness of suicide by being well-trained to identify the behavioral and verbal signs that point out to early successful suicide prevention.

***Keyword:*** *Correction Institutions, Inmates, Intents, Suicidal Ideation*

## Introduction

Suicidal ideation is any self-reported thoughts of engaging in suicide-related behavior[5] and to have suicidal intent is to have suicide or deliberate self-killing as one's purpose[6]. A suicide attempt may result in no injuries, injuries, or death[7]. Intent refers to the aim, purpose, or goal of the behavior rather than the behavior itself[8]. Suicide and other forms of suicidal behaviors such as suicidal ideation and direct nonfatal self-harm, present substantial difficulties in correctional settings[16]. Suicidal thoughts are predictors and precursors of suicide in prisons[9]. As a set, inmates have greater suicide rates than their community counterparts[10] and there is some indication that rates are growing even in places where the numbers of inmates are declining[11]. Accordingly, pre-trial convicts have a suicide attempt rate of about 7.5 times, and sentenced prisoners have a rate of nearly six times the rate of males out of jail in the general population[12]. In a rare prospective study, Lekka and

his colleagues (2006) found that 17.9% of inmates with suicide ideation attempted suicide within 12 months, whereas none of the inmates without ideation attempted suicide[13]. There is also an evidence that people who have attempted suicide in the past are more likely to have current suicidal thoughts[1].

There are very few studies examining the prevalence of suicidal ideation and intents in inmate populations and none of these have focused on inmates of correctional institutions in Baghdad City.

This study aims to (1) explore the levels of suicidal ideation and intent among the inmates in the correctional institutions of Baghdad City, and (2) find out the relationship between the levels of suicidal ideation and intents and inmates' demographic characteristics.

## Methodology

A descriptive analytic study was conducted from October 15th, 2015 to June 10th. A formal consent was issued by the Ministry of Justice to facilitate data collection from the correctional institutions. A purposive sample of 100 inmates (70 male inmates and 30 females) who were selected from two correction institutions of Baghdad City. A questionnaire was built to measure the levels of suicidal ideation and intents. This questionnaire consists of nine demographic characteristics of the inmates: gender, age, level of education, marital status, residency, period of imprisonment, period to elapse, occupation, and income; and 19 items represent BSI for Beck suicidal ideations and intents scale[4] which supposed to assess these levels among the inmates in correction institutions. Each item ranged from zero as "there is not"; one as "somehow there is; two as "there is"; and four as "strongly there is". The total score ranged from zero to 57 for the total suicidal ideation and intents scale. The total items scores were measured scored and finally rated on 3-level rating scale. Three levels were determined by applying quartile descriptive analysis; low level is with cut-off point ranged between 14 and 22; moderate level is with cut-off point ranged between 23 and 28; high level is with cut-off point ranged between 29 and 54.

## Data Analyses/Statistics

Data were analyzed using descriptive statistical measures of distribution, cross-tabulation, frequency, percent; and an inferential data analysis (Chi-square).

## Results

**Table 1. Inmates' demographic characteristics**

| **Gender** | | | **Place of Residency** | | |
|---|---|---|---|---|---|
| Gender | f | % | Place | f | % |
| Male | 70 | 70.0% | Rural | 90 | 90.0% |
| Female | 30 | 30.0% | Urban | 10 | 10.0% |
| Total | 100 | 100.0% | Total | 100 | 100.0% |
| **Age** | | | **Level of Education** | | |

***Cont....*** **Table 1. Inmates' demographic characteristics**

| year | f | % | Level | f | % |
|---|---|---|---|---|---|
| 20-29 | 19 | 19.0% | Illiterate | 10 | 10.0% |
| 30-39 | 39 | 39.0% | Elementary | 22 | 22.0% |
| 40-49 | 29 | 29.0% | Secondary | 42 | 42.0% |
| ≥50 | 133 | 13.0% | College and more | 26 | 26.0% |
| Total | 100 | 100.0% | Total | 100 | 100.0% |
| **Period of imprisonment** | | | **Period to elapse** | | |
| Year | f | % | Year | f | % |
| ≥5 | 18 | 18.0% | > 1 | 22 | 22.0% |
| 6-10 | 32 | 32.0% | 1-5 | 30 | 30.0% |
| 11-15 | 18 | 18.0% | 6-9 | 16 | 16.0% |
| 16-20 | 20 | 20.0% | 10-14 | 22 | 22.0% |
| ≥21 | 12 | 12.0% | ≥15 | 10 | 10.0% |
| Total | 100 | 100.0% | Total | 100 | 100.0% |
| **Occupation before imprisonment** | | | **Income** | | |
| Occupation | f | % | Income | f | % |
| Jobless | 70 | 70.0% | Adequate | 30 | 30.0% |
| Employee | 2 | 2.0% | Almost adequate | 31 | 31.0% |
| Self-employer | 28 | 28.0% | Inadequate | 39 | 39.0% |
| Total | 100 | 100.0% | Total | 100 | 100.0% |

| **Marital status** | | |
|---|---|---|
| Status | f | % |
| Unmarried | 23 | 23.0% |
| Married | 69 | 69.0% |
| Divorced | 7 | 7.0% |
| Widowed | 1 | 1.0% |
| Total | 100 | 100.0% |

Seventy percent of the inmates are males; more than a half age twenties and thirties; 90% live in country; half are imprisoned for 10-years and less; more than a half having five years to elapsed; 66% are of education of elementary and secondary; 70% are jobless; 70% having almost adequate and inadequate income; and 69% of the inmates are married.

Table 2. Distribution in levels of suicidal ideation and intents

| | Levels of Suicidal Ideation and intents | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Low | | Moderate | | High | | Total | |
| No. of inmates | f | % | f | % | f | % | f | % |
| | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |

More than two-fifth (41.0%) have high level; 31.0% have moderate level; and 28.0% have low level of suicidal ideation.

**Table 3. Levels of inmates' suicidal ideation according to the gender, age, period of imprisonment and period elapsed**

| **Variables** | | **Levels of Suicidal Ideation and intents** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Low** | | **Moderate** | | **High** | | **Total** | |
| | **f** | **%** | **f** | **%** | **f** | **%** | **f** | **%** | |
| Gender | Male | 22 | 22.0% | 18 | 18.0% | 30 | 30.0% | 70 | 70.0% |
| | Female | 6 | 6.0% | 13 | 13.0% | 11 | 11.0% | 30 | 30.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Age | 20-29 | 6 | 6.0% | 6 | 6.0% | 7 | 7.0% | 19 | 19.0% |
| | 30-39 | 10 | 10.0% | 13 | 13.0% | 16 | 16.0% | 39 | 39.0% |
| | 40-49 | 9 | 9.0% | 7 | 7.0% | 13 | 13.0% | 29 | 29.0% |
| | 50≤ | 3 | 3.0% | 5 | 5.0% | 5 | 5.0% | 13 | 13.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Period of Imprisonment | ≤5 | 4 | 4.0% | 9 | 9.0% | 5 | 5.0% | 18 | 18.0% |
| | 6-10 | 11 | 11.0% | 6 | 6.0% | 15 | 15.0% | 32 | 32.0% |
| | 11-15 | 4 | 4.0% | 7 | 7.0% | 7 | 7.0% | 18 | 18.0% |
| | 16-20 | 4 | 4.0% | 5 | 5.0% | 11 | 11.0% | 20 | 20.0% |
| | ≥21 | 5 | 5.0% | 4 | 4.0% | 3 | 3.0% | 12 | 12.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Period Elapsed | ≤1 | 7 | 7.0% | 5 | 5.0% | 10 | 10.0% | 22 | 22.0% |
| | 2-5 | 10 | 10.0% | 12 | 12.0% | 8 | 8.0% | 30 | 30.0% |
| | 6-9 | 2 | 2.0% | 3 | 3.0% | 11 | 11.0% | 16 | 16.0% |
| | 10-13 | 6 | 6.0% | 7 | 7.0% | 9 | 9.0% | 22 | 22.0% |
| | ≥14 | 3 | 3.0% | 4 | 4.0% | 3 | 3.0% | 10 | 10.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |

Less than a third (30.0%) of male inmates and 11.0% of female inmates have high level of suicidal ideation. This table also reveals that 16.0% of thirties decade have high level of ideation and only 3.0% of the age of 50 and more have low level of suicidal ideation.

Regarding the period of imprisonment, 15.0% of the inmates with period of imprisonment of 6-10-years and 3.0% of inmates of period of 21-years and longer have high level of suicidal ideation and intents.

According to the period to elapse, 11.0% of the inmates with 6-10-years period to elapse have high level of suicidal ideation and only 3.0% of the inmates with the period of 14-years and more have low level of ideation.

**Table 4. Inmates' levels of suicidal ideation and intents according to their age**

| Variables | | Levels of Suicidal Ideation and intents | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Low | | Moderate | | High | | Total | |
| f | | % | f | % | f | % | f | % | |
| Marital status | Unmarried | 6 | 6.0% | 9 | 9.0% | 8 | 8.0% | 23 | 23.0% |
| | Married | 20 | 20.0% | 21 | 21.0% | 28 | 28.0% | 69 | 69.0% |
| | Divorced | 2 | 2.0% | 1 | 1.0% | 4 | 4.0% | 7 | 7.0% |
| | Widowed | 0 | 0.0% | 0 | 0.0% | 1 | 1.0% | 1 | 1.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Occupation | Jobless | 20 | 20.0% | 18 | 18.0% | 32 | 32.0% | 70 | 70.0% |
| | Employee | 8 | 8.0% | 12 | 12.0% | 8 | 8.0% | 28 | 28.0% |
| | Self-employee | 0 | 0.0% | 1 | 1.0% | 1 | 1.0% | 2 | 2.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Monthly Income | Adequate | 11 | 11.0% | 11 | 11.0% | 8 | 8.0% | 30 | 30.0% |
| | Almost adequate | 3 | 3.0% | 12 | 12.0% | 16 | 16.0% | 31 | 31.0% |
| | Inadequate | 14 | 14.0% | 8 | 8.0% | 17 | 17.0% | 39 | 39.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Level of Education | Illiterate | 2 | 2.0% | 4 | 4.0% | 4 | 4.0% | 10 | 10.0% |
| | Read & write | 1 | 1.0% | 0 | 0.0% | 2 | 2.0% | 3 | 3.0% |
| | Primary | 4 | 4.0% | 9 | 9.0% | 6 | 6.0% | 19 | 19.0% |
| | Secondary | 12 | 12.0% | 14 | 14.0% | 16 | 16.0% | 42 | 42.0% |
| | College | 13 | 13.0% | 6 | 6.0% | 7 | 7.0% | 26 | 26.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |
| Residency | Rural | 25 | 25.0% | 27 | 27.0% | 38 | 38.0% | 90 | 90.0% |
| | Urban | 3 | 3.0% | 4 | 4.0% | 3 | 3.0% | 10 | 10.0% |
| | Total | 28 | 28.0% | 31 | 31.0% | 41 | 41.0% | 100 | 100.0% |

Married inmates have the highest level of suicidal ideation and intents (28.0%) and 1.0% of widowed inmates have high level of ideation.

Concerning inmates' occupation, 32.0% of jobless inmates have high level of suicidal ideation and only 1.0%of self-employed inmates have high level of suicidal ideation.

Furthermore, 17.0% of inmates with inadequate income and 16.0% of inmates with almost adequate income have high level of suicidal ideation and intents.

Moreover, 16.0% secondary school level of education have high level of ideation and only 1.0% with read and write level of education have low level of ideation.

Finally, 38.0% of inmates living in country have high level of suicidal ideation and intents and only 3.0% of urban inmates have high level of suicidal ideation.

| Table 5. Association between prisoners' suicidal ideation and intents and demographic characteristics | | | | |
|---|---|---|---|---|
| ×2 | | Suicidal Ideation and intent | | |
| | | df | p-value | |
| Variables | Age | 1.32 | 3 | 0.97 |
| | Gender | 3.27 | 1 | 0.19 |
| | Period of Imprisonment | 11.58 | 4 | 0.02 |
| | Period to elapse | 9.03 | 4 | 0.34 |
| | Marital Status | 3.07 | 3 | 0.80 |
| | Occupation | 4.18 | 2 | 0.38 |
| | Monthly Income | 10.09 | 2 | 0.03 |
| | Level of Education | 8.67 | 4 | 0.37 |
| | Residency | 6.34 | 1 | 0.73 |
| There is a significant relationship between period of imprisonment and monthly income regarding the levels of suicidal ideation (x2= 11.58, p-value= 0.02) (x2= 10.09, p-value= 0.03) respectively. | | | | |

## Discussion

Different periods of imprisonment and periods to elapse reflect the dissimilarity of levels of criminality of convicted persons and do not related to other reasons[16]. High percentages of inmates who are jobless and with almost adequate and inadequate income indicate the logic interpretation to commit crimes of different intensities[13,17]. Regarding the levels of suicidal ideations and intents, the study reveals different levels; low, moderate, and high. These levels allocate randomly according to demographic characteristics which are considered as risk factors to the suicidal ideations and intents, for gender differences; 30.0% of the total male inmates have high level of suicidal ideation while only 11.0% of total female inmates have high level. This is because the majority of inmates are men[11,12] but women in prison are also at high risk of suicide[19]. Those who age thirties have high level of the total 41.0% with high level of suicidal ideation and intent[20]. Period of imprisonment greatly influence the level of ideation and intent and it is considerable risk factor for suicidal ideation. Long period might cause hopelessness within the inmates[21]. Many studies indicate that the more period of imprisonment to elapse the higher levels of suicidal ideation intent are, contradicts the present study which reveals that the long period to elapse does not give indicator to higher level of suicidal ideation and intent[12-13]. 28.0% of married inmates have high level of suicidal ideation. This is due to the majority of inmates are married. So, the percentage does not reflect the real prevalence of the suicidal ideation. Also, this high level of suicidal ideation among jobless inmates does not represent real fact of the prevalence of suicidal ideation and intent because the majority of inmates are jobless. The less income the inmates have, the higher level of suicidal and intent they have. This could be inmates with adequate income would ensure good life for his family and for his future after release from imprisonment. The high levels of suicidal ideation and intent among secondary school and rural residency do not represent the real

prevalence the majority of distribution of inmates is among these two variables.

**Recommendations:**

1. To prevent suicidal risk among the inmates the staff of correctional institutions should have a good awareness regarding suicide.

2. Well-trained staff can identify the behavioral and verbal signs that indicate early successful suicide prevention.

3. An appropriate suicide prevention program needs to let the staff engage.

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# Relationship between Vitamin D AND IL6 in Convalescent Healthcare Workers with Covid-19 in Baquba Hospitals in Diyala Province

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## Abstract

The outbreak of coronavirus disease 2019 (COVID-19) and pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a major concern globally. One hundred Convalescent HCWs patients with COVID-19 works in Diyala, Hospitals center, from October 2020 to March 2021 were included. We recruited 100 non-infected from healthy people and 100 PCR-confirmed infected HCWs. In this review, we have summarized and discussed recent immunological studies focusing on the response of the host immune system, cytokine storms such as IL6 have been discussed as part of immunopathology mechanisms in SARS-CoV-2 infection. This may help us understand patients' immune status with COVID-19, particularly those with severe clinical presentation, and form a basis for further immunotherapeutic investigations. This study calculate that there is the relation between IL6 and Vitamin D3 in convalescent patients, accounting for a range of previously described clinical predictors and, potentially directing future therapeutic strategies, including about Vitamin D.

***Keywords:*** *IL6 and Vitamin D3 in convalescent healthcare workers.*

## Introduction

The global pandemic of coronavirus disease 2019 (COVID-19), which began in Wuhan, China, in December 2019 (1). Has quickly spread to more than 58 countries(2). These viruses are enveloped positive-sense single-stranded RNA viruses sized 80–220 nm in diameter (3). The envelope bears crown-like, 20-nm in length spikes that look like the corona of the sun under electron microscopy, hence given its name coronavirus (4). The immune system is the best defence because it supports the body's natural capability to protect against pathogens (eg, viruses, bacteria, fungi, protozoan, and worms (5). An antiviral immune response is typically coordinated by IFN-type cytokines that activate cells and increase the response against these invading agents, triggered by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), Signaling downstream of these PRRs induces the activation of nuclear factor κB (NF-κB) to produce inflammatory cytokines and phosphorylation of interferon regulatory factors (6). Such as toll-like receptors (TLR), fundamental for pathogen recognition and activation of innate immunity (7). Type 7 of TLR (TLR7) – expressed on the surface of endosomes predominantly in the lungs, placenta, and spleen – might play a central role in COVID-19 (8). This receptor has been reported to quickly recognize single-stranded SARS-CoV-1 RNA, inducing the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-

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12 in plasmacytoid dendritic cells[9]. In Iraq, obtained COVID-19 primarily via people who have visited Iran [10]. Vasculitic processes essential organ damage in seriously ill patients, induced by the activation of inflammatory cascades, complement activation, and pro-inflammatory cytokines (i.e. interleukin (IL)-6) [11]. It has been proposed that ineffective early innate antiviral response followed by impaired adaptive immune responses and hyper inflammation may lead to micro thrombosis and tissue injury, resulting in ARDS, multiorgan failure, and death [12]. When produced Vitamin D3 in the skin through the action of UVB radiation reaching 7-dehydrocholesterol in the skin, followed by a thermal reaction, Vitamin D has been found to modulate macrophages' response, preventing them from releasing too many inflammatory cytokines and chemokines. Found that calcitriol (1,25 -dihydroxy vitamin D3) exerted a pronounced impact on the ACE2/Ang (1–7) MasR axis with enhanced expression of ACE2, Masr and Ang (1–7) generation [13], Another property of vitamin D relevant both to antibacterial and antiviral mechanisms are promoting autophagy. Autophagy is a fundamental biological process that maintains cellular homeostasis via intracellular membrane encapsulation of damaged organelles and misfolded proteins. Laboratory methods ready for the detection of IL6 include enzyme-linked immunosorbent assay (ELISA) [14].

## Materials and Method

### Collection of Sample

The present study was is taking place occurring in Baquba City, the center of Diyala province. The population of this study includes convalescent healthcare workers patients during the period from October 2020 to March 2021. It included 100 health workers patients previously diagnosed with coronavirus infection, their age range from (20-65 years) and 100 healthy humans as control, their age range from (20-65 years).

IL6 Detection

The IL-6-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on the microtiter plate. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is proportional to the IL-6 concentration. A calibration curve is plotted and IL-6 concentration in samples is determined by interpolation from the calibration curve.

**Table (1) show Laboratory diagnostic kits use in the study.**

| Diagnostic kits | Manufacture Company | Country of Origin |
|---|---|---|
| Interleukin-6 human ELISA(ELISA) kits | Demeditec Diagnostics GmbH | (Germany) |
| Elecsys Vitamin D total II | Roche COBAS E411 | Japan |

### Vitamin D detection

The total duration of the assay: 27 minutes

· 1st incubation: By incubating the sample (12 µL) with pretreatment reagents 1 and 2, bound 25 hydroxy vitamin D is released from the VDBP.

· 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the 25 hydroxy vitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24, 25 dihydroxy vitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite.

· 3rd incubation: After the addition of streptavidin-coated microparticles and 25 hydroxy vitamin D labeled with biotin, unbound ruthenium labeled VDBPs become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25 hydroxy vitamin D is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin.

· The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Pro Cell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

· Results are determined via a calibration curve which is an instrument specifically generated by 2 point calibration and a master curve provided via the cobas link.

**Statistical Analysis**

Using SPSS (package or social science) statistical program. The significant value was chosen at the level (p=0.05).

**Results**

**The immunity (IgM, IgG titer) against Covid -19 among control group**

One hundred healthy individuals were included in this study to detect immunity. The healthy individuals were randomly chosen from blood donors who were attended to the Central Blood Bank in Baquba and from healthy individuals who were attended to the Public Health Laboratory for pre-marriage medical checkups, and all of them were negative for Covid IgM, IgG titer. As shown in table (2).

**Table (2): The immunity against Covid-19 among control group.**

| | | IgM titer | IgG titer | IgM of control group | IgG of control group |
|---|---|---|---|---|---|
| N | Valid | 100 | 100 | 100 | 100 |
| | Missing | 0 | 0 | 0 | 0 |
| Mean | | .07813 | 263.15048 | .0433 | 4.4800 |
| Std. Error of Mean | | .015049 | 31.121927 | .00091 | .22144 |
| Median | | .04800 | 141.91000 | .0400 | 4.9500 |
| Mode | | .043 | 14.220 | .04 | 6.00 |
| Std. Deviation | | .150486 | 311.219270 | .00911 | 2.21442 |
| Variance | | .023 | 96857.434 | .000 | 4.904 |
| Skewness | | 8.339 | 1.695 | .520 | -.181- |
| Std. Error of Skewness | | .241 | .241 | .241 | .241 |
| Kurtosis | | 76.205 | 2.713 | -.100- | -1.064- |
| Std. Error of Kurtosis | | .478 | .478 | .478 | .478 |
| Minimum | | .032 | 4.780 | .03 | .50 |
| Maximum | | 1.472 | 1536.210 | .07 | 8.80 |
| Sum | | 7.813 | 26315.048 | 4.33 | 448.00 |

The immunity in convalescent healthcare workers according to age

The results showed there is a significant difference at p-value ($p< 0.05$) in the immunity in convalescent healthcare workers according to age. As shown in table (3).

**Table (3): The immunity in convalescent healthcare workers according to age**

| Age | | COV19 IgG titer/after 6 ths |
|---|---|---|
| (20-30) Years | Mean | 172.6334 |
| | N | 53 |
| | Std. Deviation | 236.28635 |
| (31-40) Years | Mean | 397.9152 |
| | N | 19 |
| | Std. Deviation | 303.28023 |
| (41-50) Years | Mean | 308.6800 |
| | N | 21 |
| | Std. Deviation | 334.00402 |
| (51-60) Years | Mean | 446.1157 |
| | N | 7 |
| | Std. Deviation | 538.77731 |
| Total | Mean | 263.1505 |
| | N | 100 |
| | Std. Deviation | 311.21927 |

Sig. P value after 6= 0.006

The immunity in convalescent healthcare workers according to gender

The study showed there is no significant difference at p-value ($p< 0.05$) in the immunity in convalescent healthcare workers according to gender. As shown in figure (1).

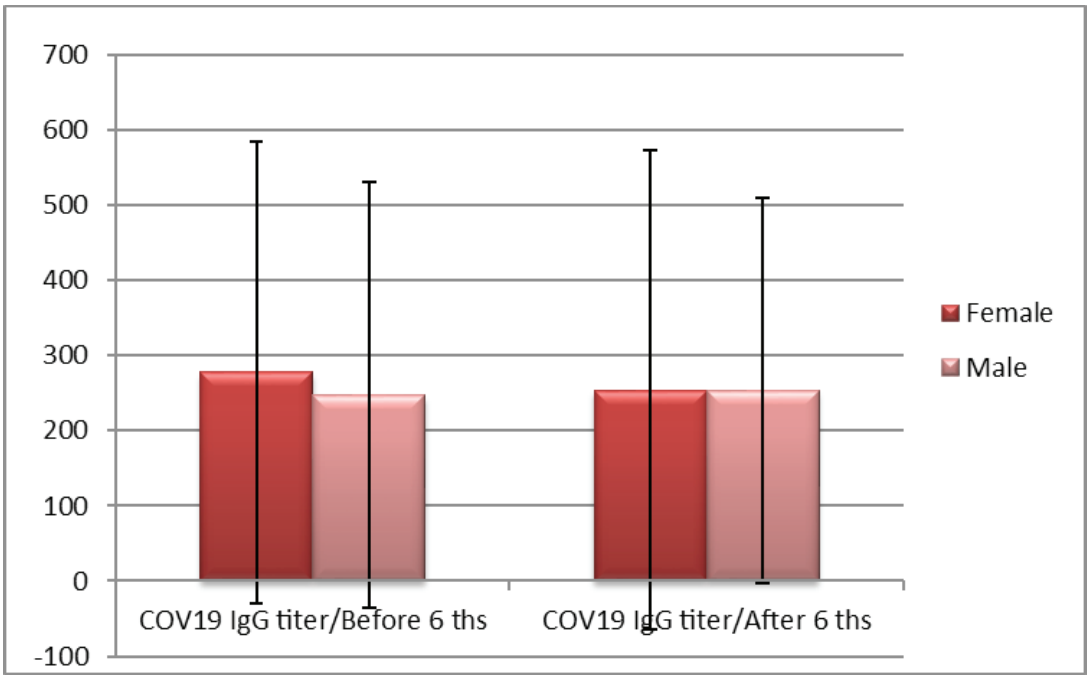


**Figure (1): The immunity in convalescent healthcare workers according to gender**

**Correlations study in convalescent healthcare workers were investigated with IL6.VIT D3 titers**

The present study shows the convalescent healthcare workers were investigated with IL6. VIT D3 titers there is the relation between IL6 and VITD3 at p-value ($p< 0.01$) as shown in table (4)

**Table (4): Correlations study in convalescent healthcare workers were investigated with IL6.VIT D3 titers.**

| | | IL6 | D3 Ng/mL |
|---|---|---|---|
| IL6 | Pearson Correlation | 1 | .282** |
| | Sig. (2-tailed) | | .004 |
| | N | 100 | 100 |
| D3 ng/mL | Pearson Correlation | .282** | 1 |
| | Sig. (2-tailed) | .004 | |
| | N | 100 | 100 |

## Discussion

One hundred healthy people were included in this study to detect Covid IgM and IgG titer. None of these people was positive for Covid (IgM or IgG) titer. These results are obviously lower than those obtained in another study in the closer surrounding countries in the Turkish population the IgG antibodies against SARS-CoV-2 in serum samples of all participants were detected by chemiluminescent microparticles immunoassay. The rate of seroprevalence was 2.7% among non-infected [15]. This variation in occurrence between studies may be due to different epidemiological trends of Coronavirus infection in

different countries, which may be due to environmental factors geographical factors, the difference in host genetic susceptibility, Sampling size, immune status and detection technique and/or various viral strains circulating in different parts of the world. Environmental factors such as high temperature and high relative humidity are the first and most important causes [16]. According to age the results of this study revealed that there was a significant increase at ($p < 0.05$) in the immunity in convalescent healthcare workers according to age with age .similar findings were reported by others [17].

This observation can be explained by the fact that they showed neutralizing antibodies IgG have a positive correlation with age. According to gender, the obtained results from this study revealed that the number of females infected with Covid 19 was higher than the number of males. While statistical analysis showed no significant differences at ($p< 0.05$) between males and females. Because the patients in this study were in the convalescent stage and the small size of the sample, these results disagree with [18,19]. This observation can be explained by the fact that they showed neutralizing antibodies IgG have a positive correlation with gender, Male sex is also associated with a greater risk of more severe COVID-19 outcomes. Also, this study showed a positive significant correlation between Interleukin6 and Vitamin D3 [20]. that IL6 elevation lies at the nexus between low vitamin D status and higher risk of Covid-19 infection, severe morbidity and death in these vulnerable populations. It further proposes that these IL6-mediated risks can be ameliorated by vitamin D supplementation which appears to inhibit IL6 expression. This result was reported also by [21].

## Conclusion

Positive relation between IL6 and Vitamin D3 has been calculated in convalescent patients, accounting for a range of previously described clinical predictors and, potentially directing future therapeutic strategies, including about Vitamin D.

**Conflict of Interest**: None

**Funding**: Self

**Ethical Clearance**: Not required

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# A Clinical Study of Parasitical Leishmaniasi in Al-Kut Province

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## Abstract

leishmaniasis is a potentially intense and skin disease. individuals with leishmaniasispossess one or different long lesions on the skin. We obtained the sample from the patients init at alzahra Teaching Hospital, a total of 190 cases of leishmaniasis have been registered. In this study were 190 individuals. 72 (38 %) females and 118 (62 %) males. The most infection with cutaneous leishmaniasis was in the male. The majority of patients were at the age of (1-15) years 63 (53.3%) in males while in females the highest proportion 39 (54.1%). Infection with cutaneous leishmaniasis had the highest percentage in February 44 (23%) when compared to other months ofinfection.Results observed for infection with cutaneous leishmaniasisin patientsuneducated had the highest percentage 116 (16%) when compared to educated of infection.Medical reference have to be knowing the truththat chronic diseaseactivities produceexpansion of the disease and riserange rate in the status that outlet to medical treatment is not easy mostly in poorly conditions of cutaneouschronic diseaselocations.

*Key words: leishmaniasis, disfiguring disease, educated, lesions, Kut hospital.*

## Introduction

leishmaniasis is arepresenting a great public health problem in Eastern Mediterranean Region,In spite ofthe great research conducted on Leishmaniadisease. [(1)]. Diversechronic disease, parasitological and clinical aspects confuse a defy for the management and control of the disease. The disease gets into humanswith the siting of an infected female sand fly, as well as can rarely be inherited by other such as blood transfusions and needle sharing[(2)]). Fresh studies hold shown the reactivation of various foci in China, Brazil and different country[(3,4)]**.** In addition, co infection with Leishmania and human immunodeficiency virus is attractive extra and extrafishy.

Although estimated to source the (ninth) largest disease encumbrance among people corruptive diseases, leishmaniasis is in generalcareless in discussions of equatorial[(5)]. They have a componentlife cycle. *Leishmaniaspp*can reproduction a great assortment of clinical symptoms in humans. All of these report,on these parasites is substantial to progress, invistegationof epidemiological research to backingdrug and vaccineexpansion.The WHA resolve and the adept committee permit highlighted the want to update the epidemiological index base in order to programsuitable approaches to the control of leishmaniasis[(6,7)]. The thoroughness of this mensuration be founded on the hardness of the incidence, intensity, period and death-rate data for a certain condition, as well as the underlying

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assumptions used in the calculations and results[8]. In parasiteinfecting, males are influenced more extremely and extraroughly than females[9]. Differentiateobviousspecies bias during infection and disease, several climactericfactors have to be taken into counting. The spreadof parasitic diseases is high in developing countries; however, in some countries, classical differences in sociocultural attitude between individual fromfemales and males are extra stated than in industrialized countries. Therefore, foreign factors such as mission, sharing, access to sources of infection, and motility strength falsely create sex bias during the disease. This study was aimed to investigate the epidemiological and statically status of leshmaniasis disease in Al-Kutecity, with factors influencing the outcome.

## Materials and Method

### Sampling and examination

Samples were collected in sterile containers. The species were examinedmacroscopically to detect color, smell, consistency, mucus and blood.

### location of Study

The study locationin the Iraqi provinces of Al-Kute. Thesurvey people was the patients with suspiciousclinical lesion who were referred to the Iraqi's clinics and health centre during for a period of one year of 2020.

## Result

The total numbers of leishmaniasis patients included in this study were 190 individual. it also illustrate male more sensitive to infection.The most infection with cutaneous leishmaniasis was 118(62%), more dominant than in females 72 (38 %) Table (1).

**Table (1): Distribution of cutaneous leishmaniasis patients according to sex.**

| Sex | Number | % |
|---|---|---|
| Male | 118 | %62 |
| Female | 72 | %38 |
| Total | 190 | %10 |

Table(2). showsthe distribution of cutaneous leishmaniasis patients according to age & se**x.** ages of patients studied as between 1 and 45 years. The plurality of patients were at age of 1-15 years, 39 (54.1%) female and 63 (53.3%) male.16-25years,18(25%)femaleand31(26.2%)male.(26-35)years,8(11.1%)femaleand21(17.7%) male.(36-45)years,7(9.7%)femaleand3(2.5%)male.

**Table (2): Distribution of leishmaniasis patients according to age & sex.**

| Groups | Male (%) | Female (%) | Total (%) |
|---|---|---|---|
| 1-15 | 63 (53.3) | 39 (54.1) | 102 (53.6) |
| 16-25 | 31(26.2) | 18 (25) | 49 (25.7) |
| 26-35 | 21(17.7) | 8 (11.1) | 29 (15.2) |
| 36-45 | 3 (2.5) | 7 (9.7) | 10 (5.2) |
| Total | 118 (62.1) | 72 (37.9) | 190 (100) |

Table (3) shows the number of cases with cutaneous leishmaniasis according to Educated and uneducated of infected person. the people with no formal education had the higher of of116 (61 %), and the infection with educated people was 74 (38 %) .

**Table (3): Educated and uneducated distribution males and females during 2019.**

| Educated | Uneducate | Total |
|---|---|---|
| 74(39) | 116(16) | 190(100) |

Table (4) shows the cases increased fromJanuary to March, reaching a high in the wintertime, and then declined to a minimum between April to Augest. The lowest numbers of cases in a month were reported for June and July 2020 was in (5(2.6%) and 3 (1.5). and the maximum number in February 2020 (44(23%) cases). followed byJanuary 43 (22 %), December 23(12 %), October 18 (9 %), November 16(8%),march 11(5.7%).

**Table (4): Distribution of cutaneous leishmaniasis patients according to months.**

| Months | Number (Male and Female) | % |
|---|---|---|
| January | 43 | 22 |
| February | 44 | 23 |
| March | 11 | 5.7 |
| April | 7 | 3.6 |
| May | 6 | 3 |
| June | 3 | 1.5 |
| July | 5 | 2.6 |
| August | 8 | 4 |
| September | 6 | 3 |
| October | 18 | 9 |
| November | 16 | 8 |
| December | 23 | 12 |
| Total | 190 | 100 |

## Discussion

In AL-Kute province the studycarried outin 2020 with different factors infected withleishmaniasis such as age, sex, sex with age, educated , uneducated and the month.

Tab (1) showed distribution of cutaneous leishmaniasis patients according to sex. The survey of this study display that occurrence of 190 cases leishmaniasis, the number of infected males were 118 (62%) while females were 72 (38%).probablybecause the male outlayextra time in outdoor activities in compare to the females.[10].

Table (2) showed distribution of cutaneous according to investigations with the relationship between age and sex. Was found that the highest incidence of males is in age (1-15) proportion 63 ( 53.3%),while in females the highest incidence is in ages (1-15) proportion 39 (54.1%), that's mean the age in males is more effective and movement as well as playing in places that may be contaminated insect sand fly , found more reservoir animals while females this age is found inside house ,especially in areas near river as well as non-resistance to insects because of their small age. this survey agreed with a study[11].

Table (3) showed were observed for infection with cutaneous leishmaniasis of patients infected in uneducated had the highest percentage 116 (16%) when compared to educated of infection.This result agrees withstudies that carried outin different Iraqi province.[12]

Table(4)showedthenumbersInfectionratesduring the months of the year, with the highest percentage of infection during February 44 (23%), while the lowest percentage was in a june 3 (1.5%) reported a disparity in parasitic infection rates *Leishmaniatropica*. The periodtime of incubationLeishmania is between (2–6 months). When the insects bites the host in October, cases would become visible, in January or February. This survey agreed with the result was done in Iran. [13,14]

## Conclusions

leishmaniasis affected a big number of people in Al-Kut affecting males being affected more than females, also leishmaniasiswith affecting in younger age groups mostly among residency, it was more prevalent in the population of uneducated becauseignorenceon of this disease and the presence of sandflies and vectors and lifestyle habits including sleeping outdoors, and most of happening in winter. Therefore the medical reference have to be knowing of the fact that chronic disease induce expansion of the disease,and increase incidence average in the situation that arriving to treatment, is difficult particularly in poorly conditions of cutaneous chronic disease locations.

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# Determinant Factors affecting Quality of Life of Children with HIV/AIDS

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## Abstract

HIV/AIDS causes physical problems, such as recurrent infections, growth disorders and malnutrition. HIV/AIDS also causes psychosocial problems, such as being shunned by friends and getting discriminatory behavior. All of the problems described earlier affect children's mental growth and development which will affect the quality of life of children with HIV/AIDS**.**

This research is a cross sectional study, conducted at the Intermediate Care Unit of Infection Disease of Children Outpatient Clinic in Dr. Soetomo General Hospital Surabaya and Voluntary Counselling and Testing Outpatient Clinic in Gresik General Hospital, Probolinggo General Hospital and Trenggalek General Hospital in November 2019-May 2020. Data collected was descriptively, then statistically analyzed by bivariate and multivariate analysis.

According to bivariate analysis, factors significantly related to quality of life of children with HIV/AIDS were age, monthly caregiver income, distance between diagnosis and therapy, immunological status, nutritional status and treatment adherence. Multivariate analysis showed that age (p=0,014, Odds Ratio [OR] 18,780, confidence interval [CI] 1,810-194,844) and treatment adherence (p=0,010, OR 7,823, CI 1,631-37,516) had a significant relationship with the quality of life of children with HIV/AIDS (p <0.05).

In conclusion, age, monthly caregiver income, distance between diagnosis and therapy, immunological status, nutritional status and treatment adherence are significantly related to the quality of life of children with HIV/AIDS. Age and treatment adherence have the most significant relation with the quality of life of children with HIV/AIDS .

***Keywords:*** *Determinant factors, HIV infection, quality of life*

## Introduction

Children with HIV/AIDS encounter some problems including physical, mental and social problems. Physically, children may experience recurrent infection, malnutrition, and growth disturbance. Children also may face mental problems, such as unconfident, fear, depression, anxiety, anger, desperation, social withdrawal or other emotions. Unsupportive social environments may give them discriminative behavior, excommunication, stigmatization, and rejection. Mental and social problems may cause distress and decrease of immune system that may worsen their condition.[1,2]

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Children's parents may also face problems in raising their children due to recurrent infection, growth disturbance, malnutrition, sadness, rejection, denial, guilt, anxiety, anger and other emotional feelings. Lack of affection given to children with HIV/AIDS may worsen children's condition.[1,4]

Previous research has analyzed several variables that may affect the quality of life of children with HIV/AIDS. Nevertheless, similar research is still hard to find in Indonesia. Therefore, this research was held to find determinant factors influencing the quality of life of children with HIV/AIDS so that intervention can be given to increase the quality of life.

## Materials and Method

This research used cross sectional study and was held in Dr. Soetomo General Hospital in Surabaya, Gresik General Hospital, Probolinggo General Hospital, and Trenggalek General Hospital from November, 2019 to May, 2020.

Sampling method used was a total sampling technique, including pediatric HIV/AIDS patients meeting certain criteria. The inclusion criteria were children that had been diagnosed with HIV/AIDS, had results of CD4 test in the last 6 months, were able to fill the PedsQL questionnaire and agreed to sign the informed consent form. The exclusion criteria were patients that had other chronic diseases, had no CD4 test results in the last 6 months, and only filled less than half of PedsQL questions.

The independent variables analyzed were age, sex, caretakers, caretakers' income, caretakers' education, caretakers' job, first diagnosis, duration between being diagnosed and getting therapy, immunodeficiency, nutritional status, stage when diagnosed, adherence to therapy, availability of medical center, care provider, facilities and supplies, occurrence of opportunistic infection when being diagnosed, and antiretroviral (ARV) category. The dependent variable is quality of life assessed using PedsQL.

Patients qualifying the criteria were listed. Information for consent was explained to the subject and caretakers and an informed consent form was signed by the caretakers. Data needed were collected. Subsequently, subjects were asked to fill the PedsQL questionnaire.

Data collected were descriptively analyzed and presented in the form of table and graph. Variables were analyzed statistically using Chi square. Variables proven significantly related to quality of life then analyzed using logistic regression tests and presented as Odds Ratio. Subsequently, variables significantly correlated to Quality of Life were analyzed using determination and calibration tests. Statistical analysis was done using SPSS.

## Results and Discussion

The sample size was 52.

The results of descriptive analysis are shown in Table 1 and 2.

**Table 1. The Descriptive Analysis of Characteristic of Children with HIV/AIDS**

| Characteristic | | Results | |
|---|---|---|---|
| | | n | % |
| Sex | Male | 28 | 53.8 |
| | Female | 24 | 46.2 |
| Age | 2-5 years old | 9 | 17.3 |
| | >5 years old | 43 | 82.7 |
| Address | Surabaya | 8 | 15.4 |
| | Outside Surabaya | 44 | 84.6 |
| PedsQL | Good | 31 | 59.6 |
| | Bad | 21 | 40.4 |

Most of the subjects were male, more than 5 years old, lived outside Surabaya, and had good quality of life according to the PedsQL questionnaire. This study has the advantage of being able to find out

quickly how the quality of life of children with HIV / AIDS because it is done at one time, this study also focuses not only on children living in a big city such as Surabaya but several other cities such as Gresik, Trenggalek, and Probolinggo.

**Table 2. Descriptive Analysis of Determinant Factors of Children with HIV/AIDS**

| Determinant factor | | Results | |
|---|---|---|---|
| | | n | % |
| Caretakers | Parents | 29 | 55.8 |
| | Not parents | 23 | 44.2 |
| | | | |
| Caretakers' education | Elementary school or not graduated | 26 | 50 |
| | Junior high school or higher | 26 | 50 |
| | | | |
| Caretakers' income | less than 1 million IDR | 31 | 59.6 |
| | 1-5 million IDR | 21 | 40.4 |
| | | | |
| Caretakers' job | Housewife or unemployed | 10 | 19.2 |
| | Employed | 42 | 80.8 |
| | | | |
| First diagnosis | Less than 2 years ago | 18 | 34.6 |
| | More than 2 years ago | 34 | 65.4 |
| | | | |
| Duration between diagnosis and therapy | Less than 6 months | 41 | 78.8 |
| | More than 6 months | 11 | 21.2 |
| | | | |
| Immunodeficiency | None to mild | 39 | 75 |
| | Moderate to severe | 13 | 25 |
| | | | |
| Nutritional status | Good | 31 | 59.6 |
| | Bad | 21 | 40.4 |
| | | | |
| Stage when diagnosed | 1-2 | 28 | 53.8 |
| | 3-4 | 24 | 46.2 |
| | | | |
| Adherence to therapy | Good | 38 | 73.1 |
| | Bad | 14 | 26.9 |
| | | | |
| Medical care provider | Available | 49 | 94.2 |
| | Unavailable | 3 | 5.8 |
| | | | |
| Medical centers | Available | 49 | 94.2 |
| | Unavailable | 3 | 5.8 |
| | | | |
| Medical facilities and supplies | Available | 49 | 94.2 |
| | Unavailable | 3 | 5.8 |
| | | | |
| Opportunistic infection when diagnosed | Occurred | 20 | 38.5 |
| | Not occurred | 32 | 61.5 |
| | | | |
| ARV category | First line | 50 | 96.2 |
| | Second line | 2 | 3.8 |

Most of the subjects were cared for by parents. Their caretakers were mostly employed and had under 1 million IDR per month. Most of them was first diagnosed more than 2 years ago and treated less than 6 months after being diagnosed, have none to mild immunodeficiency, good nutritional status, first diagnosed with stage 1-2, good adherence to therapy, access to medical care provider, medical center, facilities, and supplies, and get first line of ARV therapy. Most of them have no opportunistic infection when diagnosed.

The results of bivariate analysis using Chi Square are shown in Table 3. Variables correlated to the quality of life have p value less than 0.05.

**Table 3. The Results of Bivariate Analysis between the Determinant Factors and Quality of Life of Children with HIV/AIDS**

| Determinant factor | | PedsQL | | P value | Odd ratio (CI 95%) |
|---|---|---|---|---|---|
| | | Good | Bad | n | |
| Sex | Male | 17 (54.8) | 11 (52.3) | 0.862 | 0.906 (0.298-2.751) |
| | Female | 14 (45.1) | 10 (47.6) | | |
| | | | | | |
| Age | 2-5 years old | 1 (3.2) | 8 (38) | 0.001* | 18.462 (2.090-164.046) |
| | >5 years old | 30 (96.7) | 13 (61.9) | | |
| | | | | | |
| Domicile | Surabaya | 6 (19.3) | 2 (9.5) | 0.335 | 2.280 (0.413-12.579) |
| | Outside Surabaya | 25 (80.6) | 19 (90.4) | | |
| | | | | | |
| Caretakers | Parents | 20 (64.5) | 9 (42.8) | 0.123 | 2.424 (0.779-7.542) |
| | Not parents | 11 (35.4) | 12 (57.1) | | |
| | | | | | |
| Caretakers education | Elementary school or not graduated | 13 (41.9) | 13 (61.9) | 0.158 | 2.250 (0.724-6.989) |
| | Junior high school or higher | 18 (58) | 8 (38) | | |
| | | | | | |
| Caretakers income | less than 1 million IDR | 14 (45.1) | 17 (54.8) | 0.010* | 5,161 (1,408-18,912) |
| | 1-5 million IDR | 17 (54.8) | 4 (45.1) | | |
| | | | | | |
| Caretakers job | Housewife or unemployed | 4 (12,9) | 6 (28.5) | 0.160 | 2.7 (0.657-11.102) |
| | Employed | 27 (87) | 15 (71.4) | | |

***Cont.*. Table 3. The Results of Bivariate Analysis between the Determinant Factors and Quality of Life of Children with HIV/AIDS**

| | | | | | |
|---|---|---|---|---|---|
| First diagnosis | Less than 2 years ago | 9 (29) | 9 (42.8) | 0.304 | 1.833 (0.574-5.855) |
| | More than 2 years ago | 22 (70.9) | 12 (57.1) | | |
| Duration between diagnosis and therapy | Less than 6 months | 29 (93.5) | 12 (57.1) | 0.002* | 0.092 |
| | More than 6 months | 2 (6.4) | 9 (42.8) | | |
| Immuno-deficiency | None to mild | 28 (90.3) | 11 (25.3) | 0.002* | 0.118 (0.027-0.511) |
| | Moderate to severe | 3 (9.6) | 10 (47.6) | | |
| Nutritional status | Good | 23 (74.1) | 8 (38) | 0.009* | 4.673 (1.1417-15.401) |
| | Bad | 8 (25.8) | 13 (61.9) | | |
| Stage when diagnosed | 1-2 | 19 (61,2) | 9 (42.8) | 0.191 | 0.474 (0.154-1.461) |
| | 3-4 | 12 (38.7) | 12 (57.1) | | |
| Adherence to therapy | Good | 27 (87) | 11 (52.3) | 0.006* | 3.136 (1.583-23.789) |
| | Bad | 4 (12.9) | 10 (47.6) | | |
| Medical care provider | Available | 30 (96.7) | 19(90.4) | 0.339 | 3.158 (0.268-37.270) |
| | Unavailable | 1 (3.2) | 2 (9.5) | | |
| Medical centers | Available | 30 (96.7) | 19(90.4) | 0.339 | 3.158 (0.268-37.270) |
| | Unavailable | 1 (3.2) | 2 (9.5) | | |
| Medical facilities and supplies | Available | 30 (96.7) | 19(90.4) | 0.339 | 3.158 (0.268-37.270) |
| | Unavailable | 1 (3.2) | 2 (9.5) | | |
| Opportunistic infection when diagnosed | Occurred | 11 (35.4) | 9 (42.8) | 0.592 | 0.733 (0.236-2.282) |
| | Not occurred | 20 (64.5) | 12 (57.10) | | |
| ARV category | First line | 30 (96.7) | 20 (95.2) | 0.777 | 0.667 |
| | Second line | 1 (3.2) | 1 (4.7) | | |

According to Chi-square test, age, monthly caregiver income, distance between diagnosis and therapy, immunological status, nutritional status and treatment adherence had a significant relationship with PedsQL. With the increase of nutritional status, a child's immunity will also increase, this can improve the health of children with HIV/AIDS both physically and mentally so that can improve their quality of life. This study uses the latest CD4/CD4% data and measurements of nutritional status were done at the time of sampling, so that we can find out the relationship of the patient's current immunological status and nutritional status with their current Quality of Life.

Those variables eventually underwent multivariate analysis using a logistic regression test. Multivariate analysis results are shown in Table 4.

**Table 4. The Results of Multivariate Analysis between the Determinant Factors and Quality of Life of Children with HIV/AIDS**

| | Variable | B | P value | OR | CI | |
|---|---|---|---|---|---|---|
| | | | | | Upper limit | Lower limit |
| Step 1a | Age | 3,048 | 0,042* | 21,076 | 1,113 | 399,275 |
| | Caretakers | 0,706 | 0,466 | 2,027 | 0,303 | 13,555 |
| | Caretakers' education | -0,628 | 0,498 | 0,534 | 0,087 | 3,276 |
| | Caretakers' income | -1,469 | 0,138 | 0,230 | 0,033 | 1,600 |
| | Caretakers' job | -0,951 | 0,439 | 0,386 | 0,035 | 4,288 |
| | Duration between diagnosis and therapy | 1,001 | 0,656 | 2,721 | 0,033 | 223,523 |
| | Immunodeficiency | -0,283 | 0,890 | 0,753 | 0,013 | 42,200 |
| | Nutritional status | 0,841 | 0,336 | 2,319 | 0,418 | 12,865 |
| | Stage when diagnosed | 0,705 | 0,406 | 2,024 | 0,383 | 10,693 |
| | Adherence to therapy | 2,032 | 0,075* | 7,632 | 0,816 | 71,403 |
| | Constant | -3,536 | 0,089 | 0,029 | | |
| Step 8a | Age | 2,933 | 0,014* | 18,780 | 1,810 | 194,844 |
| | Caretakers' income | -1,286 | 0,094 | 0,276 | 0,061 | 1,245 |
| | Adherence to therapy | 2,057 | 0,010* | 7,823 | 1,631 | 37,516 |
| | Constant | -2,741 | 0,053 | 0,065 | | |

The result of multivariate analysis using a logistic regression test showed that age and patient's adherence to therapy plays a significant role. This research found that children aged more than 5 years old had 18 times better Quality of Life compared to 2-5 years old and children with good adherence to therapy had 8 times better Quality of Life than bad adherence to therapy. This result was similar to previous study held by Burack.[5]

**Table 5. The Results of Determination and Calibration Test**

| Evaluation | Criteria | Results |
|---|---|---|
| Determination test | Nagelkerke R Squate test | 47,1% |
| Calibration test | Hosmer-Lemeshow test | p = 0.884 |

Based on determination and calibration tests, both of those factors had influenced 47,1% towards the Quality of life of children with HIV/AIDS.

The majority of the study subjects were male and had a mean age of 8.2 ± 3.64 years. This is in accordance with the statistical description of new case finding of children with HIV/AIDS in Indonesia in 2020, where boys have a higher percentage than women (56.7% compared to 43.2%) with the majority aged less than 14 years.[6]

This research found that the older the age, the higher is the quality of life. This result was similar with research held in Africa which children aged more than 5 years old had better quality of life and no psychosocial problems.[7]

In a previous study, the independent variables in this research had been observed and correlated to the quality of life. A research found that the older the children, the better the quality of life and less psychosocial problem.[6] Parents play important role in life to improve the quality of life of children with HIV/AIDS.[8] Early diagnosis and ARV therapy has proven useful for prevention, clinical benefit, increases life expectancy, and reduces the incidence of HIV-related infections in the population.[9,10] Starting ARVs at a higher CD4 or CD4% can maximize the potential for immunological recovery.[10] Immunology and opportunistic infection also played role in the quality of life in pediatric HIV/AIDS.[11] According to a study, nutritional status was also significantly correlated with quality of life. Children with HIV/AIDS who experience malnutrition have an impact on poor quality of life.[12] Patient's adherence to therapy improves patient's condition and prevents infection, so that it improves the quality of life of children with HIV/AIDS.[13] Research by Bolton-Moore showed that proper medical center, medical care workers and medical facilities can provide good outcomes for children with HIV/AIDS.[14]

In this study, age and adherence to therapy has proven to have a role in a patient's quality of life. Children aged more than 5 years old were proven to have 18 times better Quality of Life compared to 2-5 years old. This may be due to the fact that by the age increasing, the level of adherence to treatment will increase so that in the end it can also improve the Quality of Life of children with HIV / AIDS. This result was in accordance with study held by Hidayat.[6]

Based on this research, children with good adherence to therapy had 8 times better Quality of Life than bad adherence to therapy. Adherence to therapy is a complex health behavior influenced by drug regimens, patient and family factors, and patient-medical personnel relationships.[13] The better the patient's adherence to therapy, the higher the success rate in therapy. Therefore, the quality of life of children with HIV/AIDS will improve. In contrast, bad adherence to therapy will cause side effects related to ARVs and immunological and viral complication, which will lead to a decrease in quality of life.[15]

Despite the results, this research still has some weaknesses. Bias may be caused by subjectivity due to the interview. Besides, this study has not excluded all chronic diseases so it is very likely that it can affect the quality of life. Last, this research was only conducted at one time so that the subjects were unable

to observe in a certain period.

## Conclusion

Most children with HIV/AIDS who have received ARV therapy have a good quality of life. Age, monthly caregiver income, distance between diagnosis and therapy, immunological status, nutritional status and treatment adherence are significantly related to the Quality of Life of children with HIV/AIDS. Age and treatment adherence have the most significant relation with the Quality of Life of children with HIV/AIDS.

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# Study of Psychosocial Risks in the Professional Environment of Health Care Workers in Morocco

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## Abstract

Caregivers are a particular target for psychosocial risks, including stress and burnout. Burnout is a consequence of exposure to permanent and prolonged stress. It concerns professions with high mental, emotional and emotional demands and high responsibility such as nurses. The objective of this study was to describe and identify psychosocial risks, occupational stress and its components in health professionals. This epidemiological study involved 191 health professionals working in the provincial hospital centers of Kenitra and Sidi Slimane, Morocco. The questionnaire included: socio-demographic and occupational data; the Karasek scale (KJCQ) with its three components: psychological demand (DP), decision-making latitude (LD) and social support (SS). These three dimensions allow the identification of risk situations. Job strain is the combination of low decision latitude and high psychological demand. The Maslach Burnout Inventory (MBI) scale consists of three dimensions (emotional exhaustion, depersonalization and feeling of personal fulfillment at work). The positive diagnosis of burnout was chosen if the score exceeded the values defined in one of the three components of MBI (high for the first two and low for the third).

***Keywords:*** *stress, caregivers, burnout, Morocco, job strain*

## Introduction

The nursing profession requires the assessment of a patient's clinical situation, the application of medical supervision, the initiation of diagnostic tests, the determination of a plan of care and treatment and/or the execution of such plan. Nurses encounter complex situations of care on a daily basis and must intervene in this context to maintain patient stability, avoid complications and provide quality care [1]. Nurses are faced with managing situations that put the lives of individuals at risk. The urgency, unpredictability and seriousness of the interventions punctuate the practice of emergency medicine and expose the participants to intense and repeated stress. This work environment can cause chronic stress called "work stress". Occupational stress has been widely developed by health psychology [2].

Stress is defined as "a particular transaction between an individual and a situation in which the individual is assessed as being beyond his or her resources and potentially endangering his or her well-being." Thus, in the face of a disturbance, the individual assesses the problem, his or her personal and social resources to deal with it, and then develops adjustment strategies to reduce perceived stress. It is when all these transactions prove to be ineffective that work stress sets in and, if the situation persists, may lead to burnout.

The high psychological demand and high workload in Moroccan hospitals can be explained in part by the increasing shortage of medical staff and budgetary constraints. For the World Health Organization (WHO), the number of health professionals in Morocco did not reach the recommended critical threshold of 22.8 doctors, nurses and midwives per 10,000 inhabitants [3].

The increased demand for higher-quality care from an increasingly demanding population afflicted by emerging and often chronic conditions implies excessive workload and stress. 21% of Moroccans have at least one chronic disease and 75% of deaths are related to non-communicable diseases reported the Health ministry in Morocco in 2008[4]. Poor work organization, adverse socio-demographic and economic factors contribute to burnout compounded by stress, anxiety, fatigue and depression [5]. The common denominator is the existence of the discrepancy between an increased professional requirement and the reality of an often limited individual capacity. Caregivers' BO vulnerability is increased by emotional dissonance that is positively correlated with work dissatisfaction and emotional exhaustion. Emotional dissonance occurs when the work situation requires an expression that is not the one actually felt by the subject. It results from a conflict between the person and the role he holds, between the subject and the motions prescribed by the organization [6,7].

Burnout or burnout syndrome is a translation of work-induced personal suffering. Also, a multitude of research studies have focused on this sector of activity and have revealed that the bulk of the cost of psychological suffering is related to staff malaise, the most frequent consequences of which include an increase in absenteeism, a high turnover rate and a decrease in performance and productivity [8,9].

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## Method

This epidemiological survey, cross-sectional by self-questionnaire and a psychometric kit, conducted in 2018, interested 191 caregivers from two provincial hospital centers in the Rabat –Sale-Kenitra region, namely the Kenitra and Sidi Slimane CHP.

1. Participants

It includes all health care workers, nurses, who provide direct care to patients and who come under the various departments and departments of the provincial hospital centers.

2. Data collection tools

It was conducted through a self-questionnaire, the Maslash Burnout Inventory (MBI), the Karasek scale (JDCS) and the General Health Questionnaire (GHQ12).

The General Health Questionnaire (GHQ-12) is an evaluation tool developed by Goldberg and Williams (1998). It allows to quantify at the dimensional level the degree of subjective psychological suffering and, at the categorical level, to define, from threshold notes, pathological cases or not [10].

Karasek's Job Content Questionnaire in French KJCQ) ranks three dimensions of the psychosocial environment at work. Psychological demand (DP) evaluates quantity, speed, the complexity, intensity, fragmentation and predictability of the work. The decision-making latitude (LD) values the room for

maneuver, the use acquired and the development of skills. Social Support (SS) values professional and emotional support from superiors and colleagues [11]. These three dimensions allow the identification of risk situations. Job strain is the combination of low decision latitude and high psychological demand.

Maslach scale (Maslach Burnout Inventory; MBI) consists of three dimensions: Emotional Exhaustion (EE), Dehumanization or Personalization (DP) and Self-Fulfillment at Work (AP). Responses are scored on a Likert scale in seven points from 0 (never) to 6 (daily). For each dimension, the sum of the responses allows to define a low, moderate or severe level [10].

The self-survey to collect data on social and occupational variables, also the main stressors of the care environment among participants, included 4 components with 23 items

3. Statistical analysis

Analytical statistics were based on association tests such as Pearson's Chi-Two test2, correlation matrices by Bravais-Pearson and Spearman, single-factor variance analysis (ANOVA).

## Results

1. Socio-demographic and occupational stress and characteristics of caregivers

The study covered 191 respondents, 43% (n=82) male and 57% (n=109) female. The sex ratio was balanced (Female/Male) with a p<0.189 (single-sample binomial test). The breakdown of these respondents by grade shows that 65% (n=124) are state-certified nurses (Nurses) of whom 58 are male nurses and 66 are nurses, and 35% (n=67) are auxiliary nurses (Auxiliaries) of whom 23 are male and 44 are female. The khi2 independence test did not show a significant association between these two factors (sex × category) ($\chi 2$= 1,36; p<0,243).

The age distribution of nurses surveyed shows that 68% (n=130) are over 40 years of age, including 82 nurses and 48 male nurses, followed by the class of nurses between 30 and 40 years of age (17 female and 23 male), which represents 21% and finally the age category of respondents less than 30 years and representing 11% (n=21). The khi2 test did not show any significant difference between these two factors (age × sex) ($\chi 2$ = 3.37; p< 0.186).

For better organization, hospital managers adopt an hourly work system that is appropriate to patients' expectations. The khi2 test shows a strong link between sex and the schedule system ($\chi 2$=13.63; p< 0.003). In fact, 19% (n=36) follow the regular schedule system (30 female and 6 male), 60% (n=115) adopt the 12/36 system (61 male and 54 female), 6% (n=11) day work (no male) and 15% (n=29) ensure the system night shift (17 male and 12 female). A significant connection combines the hourly system and age ($\chi 2$=14,74; p<0.022). However, the nurses who provide day and night care are all over 40 years of age and 79% of the nurses who follow the regular system are over 40 years of age. However, 54% of respondents with the hourly 12/36 system are over 40 years of age, compared to 17% who are under 30 years of age. Although the khi2 test did not show a significant difference between the timing system and marital status ($\chi 2$=10.55; p<0.308), the majority of nurses who provide day and night care and the normal system are married and 87% (n=40) of single nurses provide the 12/36 system.

The joint analysis of socio-demographic and occupational variables identified three distinct groups:

- The first group is located on the positive side of dimension 1 and dimension 2 groups the nurses who provide day and night care. These nurses are generally over 40 years of age and have a seniority that exceeds 15 years of practice in the position. A lot of them are female.

- In contrast to the negative side of Axis 1, single nurses under 30 years of age with a seniority of less than 5 years and with a higher level of education are usually employed in the 12/36 hourly system.

▪ The third group, located on the negative side of Dimension 2, is generally married nurses, the majority of whom are male and follow a normal schedule. These nurses are between 30 and 40 years old, and work in the position between 5 and 15 years old.

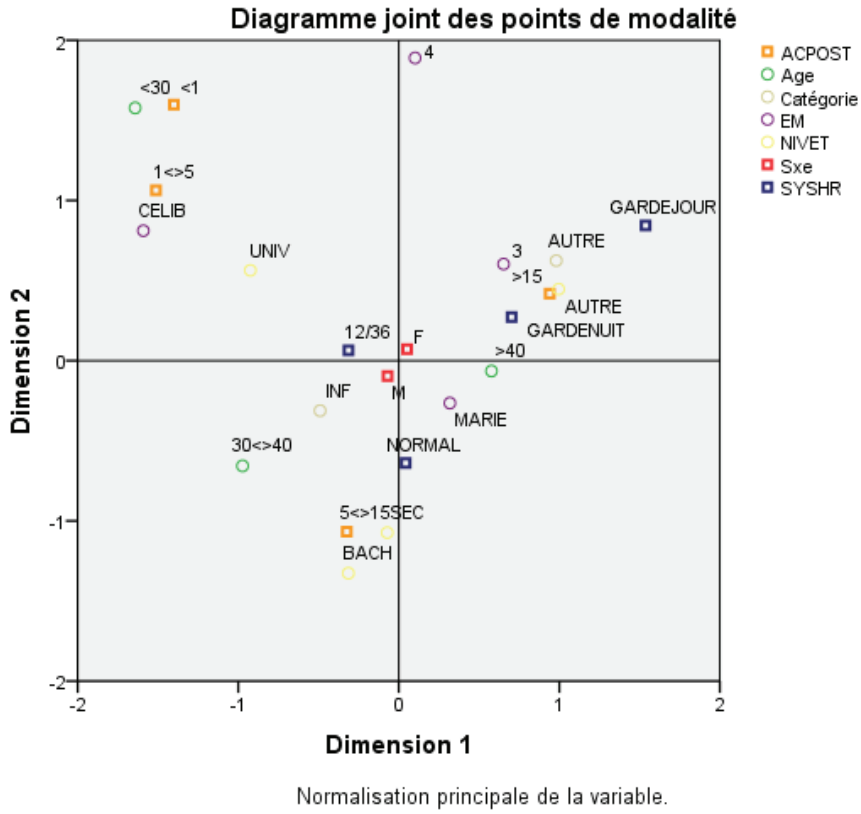


**Figure 1: Projection of the modalities of the variables studied in ACM according to two dimensions 1 and 2**

Results of responses to organizational factors items show that more than 80% of respondents responded "often" to overwork (n=153), ambiguity in role (n=157), and lack of staff (n=167), contributing to burnout. However, 40% of those surveyed responded that conflict with colleagues is a determining factor in burnout.

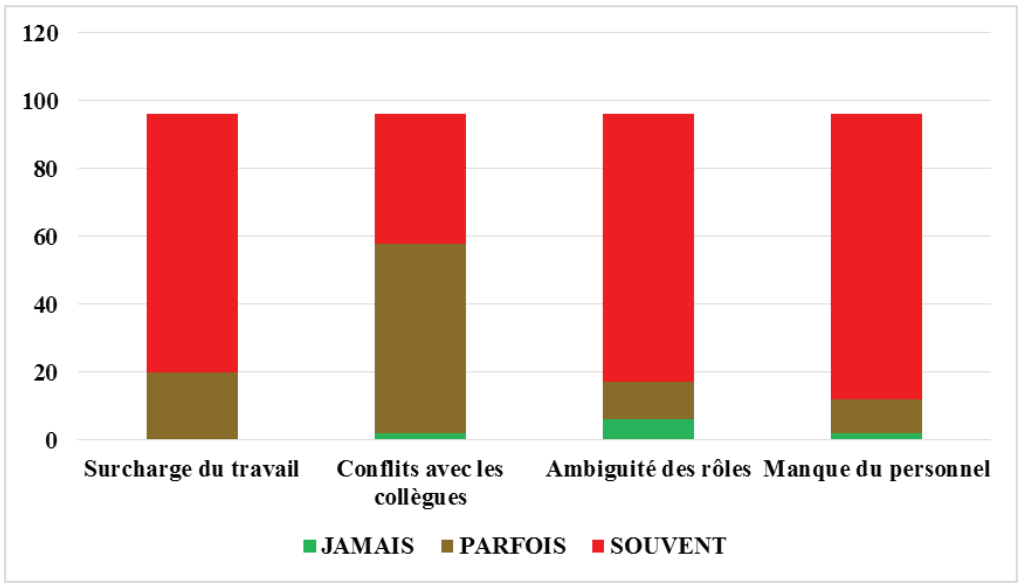


**Figure 2: Distribution of organizational factors according to their frequency**

Multiple correlation analysis shows that work overload is strongly correlated with conflicts with colleagues (r= +0.317; p<0.002) and ambiguity of roles (r = 0.241); p<0.018). However, the ambiguity of the roles is positively correlated with the question; does a lack of staff put you in a state of burnout? (r=0.860; p<0,000).

On the other hand, 92% of respondents said that the physical and/or technical environment of the care environment is a determining factor in burnout.

The breakdown of responses to the physical and/or technical environment questions shows that more than 80% confirmed that poor site design, space limitations, and humidity are environmental factors for burnout, and less than 70% said yes to inadequate lighting and heat.

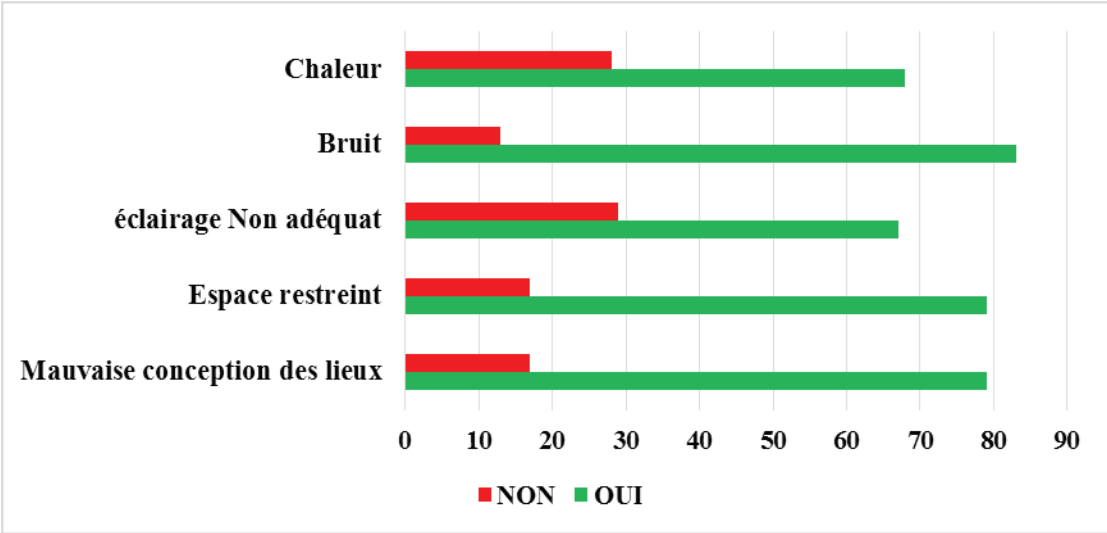


**Figure 3: Distribution of physical and/or technical factors of care according to their frequency**

2. Psychological impairment at work of caregivers:

The results of the CMA identified four distinct groups:

✓ The first group is located on the positive side of Axis 1 and is composed of female nurses generally over 40 years of age. These nurses provide night and day care with almost 15 years of service. These nurses are characterized by low depersonalization and moderate achievement.

✓ The second group on the negative side of Axis 1 is composed mainly of single nurses less than 30 years of age with a seniority of less than 5 years. These nurses, the majority of whom had a university education, developed a low level of emotional exhaustion, a high level of achievement

✓ A third group located on the negative side of Axis 2, consisting mainly of male nurses aged 30 to 40. These nurses developed a moderate level of EE and a high level of achievement and a moderate to high level of depersonalization. Nurses operate the 12/36 hourly system.

✓ The fourth group consists of high EE nurses and low achievement and high depersonalization. They are generally married, working with a normal hourly system and have a level of education less the bachelor's degree and a seniority between 5 and 15 years.

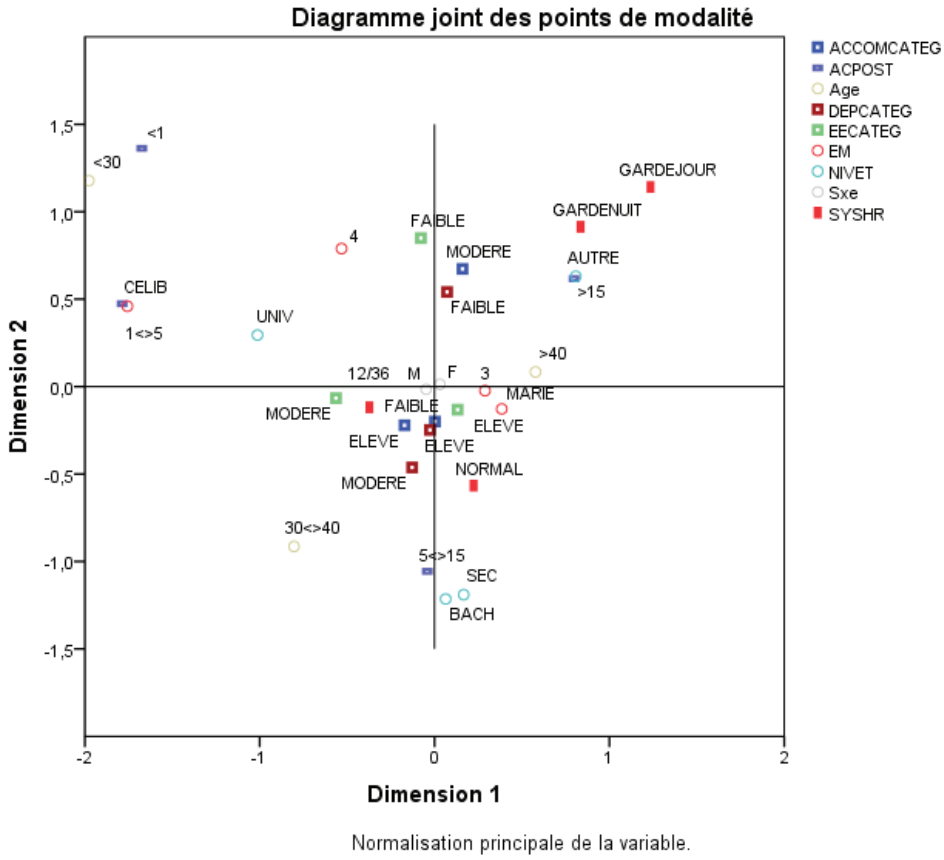


**Figure 4: Results of the multiple matching analyses of socio-demographic, socio-economic characteristics and burnout levels.**

3. Stress and burnout of caregivers:

Job strain, or tension at work, is the combination of low decision latitude and high psychological demand. If the psychological demand score is higher than the median 26 and the decision latitude score is lower than 64, the individual is in the "stressed" dial and therefore considered in the Job strain situation.

The results of the crossing between the two dimensions; table above show that 11.46% of nurses are in a state of job strain. Indeed, they have demonstrated low decision-making latitude and high psychological demand. They are therefore people in a state of stress, 32.29% of nurses are considered relaxed, they have a low psychological demand and a high autonomy to perform their work, while 41.67% of nurses considered themselves passive (nurses in this category have both autonomy and a low psychological demand), while 14.58% are assumed to be people active (high decision latitude and high psychological demand).

**Table 1: Cross study between the two dimensions LD and DP**

| | | Score DP | | Total |
|---|---|---|---|---|
| | | <26 | >26 | |
| Score LD | <64 | Passif (N=80) | Stressé (N=22) | 102 |
| | >64 | Détendu (N=62) | Actif (N=2) | 89 |
| Total | | 142 | 49 | 191 |

3. Psychological distress in the professional environment of the caregivers:

The table below shows the results of the GHQ12 khi2 independence test and some variables (gender, age, school level, marital status, time system and length of service in the position). Although the test did not show a significant association between GHQ12 and these variables, a difference in representability was reported. Among nurses who reported disorders, 58.73% (n=112) were female and 66.67% (n=127) were over 40 years of age. In terms of marital status, 80.95% (n=155) declared that they were married, while 42.86% (n=66) of them had more than 15 years of seniority in the position and 60.32% (n=93) followed the 12/36 hourly system.

**Table 2: Test khi2 between GHQ12 and certain socio-demographic and occupational variables**

| | | GHQ12 | | Total | Khi2 (p-value) |
|---|---|---|---|---|---|
| | | Normal | Distress | | |
| Sexe | M | 29 | 52 | 81 | 0,15 (p<0,659) |
| | F | 36 | 74 | 110 | |
| Age | <30 | 8 | 13 | 21 | 0,22 (p<0,88) |
| | 30<>40 | 12 | 28 | 40 | |
| | >40 | 46 | 84 | 130 | |
| Marital status | SINGLE | 17 | 24 | 41 | 1,03 (p<0,712) |
| | MARIED | 48 | 102 | 150 | |
| School level | SEC | 30 | 54 | 84 | 0,14 (p<0,98) |
| | BACH | 13 | 30 | 43 | |
| | UNIV | 22 | 42 | 64 | |
| Seniority in the position | <1 | 6 | 6 | 12 | 0,72 (p<0,86) |
| | 1<>5 | 8 | 16 | 24 | |
| | 5<>15 | 24 | 49 | 73 | |
| | >15 | 28 | 54 | 82 | |
| Hourly system | NORMAL | 12 | 26 | 38 | 1,22 (0,748) |
| | 12/36 | 42 | 75 | 117 | |
| | Day care | 2 | 10 | 12 | |
| | Night care | 10 | 14 | 24 | |
| Total | | 65 | 126 | 191 | |

## Discussion

1. Socio-demographic and occupational stress and characteristics of caregivers

The data in this study on the impact of the physical and/or technical environment of the care environment are consistent with other studies that emphasize that the environment has consequences on both the physical and the psychic. While insufficient labor forces increase the workload, this lack is a real source of exhaustion [12].

On the other hand, the material in the care service remains a fundamental factor in distributing and organizing the nursing work, the quantity and quality of the material (carts, tensiometers, tubing,...) plays a crucial role in spreading the tasks [13], the organization of the material and the resources determine how to carry out the work and therefore constitutes one of key elements that can reduce stress at work.

The role of personal factors and socio-demographic characteristics in determining the vulnerability of individuals to burnout is highlighted by several authors. Age, sex, marital status, work experience, and education or training are among the socio-demographic characteristics studied in relation to burnout [14].

2. Psychological impairment at work of caregivers:

Career burnout, particularly affecting caregivers, is a psychological and physiological condition resulting from the daily accumulation of stress factors that have worn the individual. It has its roots, in response to a multitude of stressful factors over time [15, 16]. OB is a syndrome of emotional exhaustion, depersonalization and loss of a sense of personal efficacy, which can occur in subjects working in any way with other human beings [17].

Our results showed that nurses over 40 years of age are located at the high stress quadrant and even for married nurses, something that has been cited by several studies that say that other potential explanatory variables of higher occupational stress were generally demographic factors such as age, marital status and education [18,19]

3. Stress and burnout of caregivers:

According to our study, 11% (n= 22) of nurses are in a state of job strain. Indeed, they have demonstrated low decision-making latitude and high psychological demand. So these are people under stress. This is strongly correlated with other findings that indicate that nurses have a higher degree of stress [20,21]. Working in a hospital can be a source of stress, given the workload, the lack of staff and equipment, which requires a high psychological demand and influences the well-being of the caregiver.

In our study, a significant difference in the level of stress between the sexes reflects that the demands and immediacy of stressful situations were likely to be perceived differently by the gender. Studies have already reported a higher level of stress among independent women of nationality and health unit [18, 19].

Also, a very important variable was shown in our study as a factor in occupational stress, namely the hourly system showing that night-shift nurses have accumulated a high degree of emotional exhaustion and a high degree of depersonalization. Our results are consistent with several studies that cite night work that transgresses circadian rhythms [22], which has certain consequences for the health of nurses: cardiovascular disorders [23].

4. Psychological distress in the professional environment of health-care workers

The interpretation of the GHQ12 score showed that 66% (n=126) had poor mental health (score $\geq$ 2), and its results are correlated with other studies that indicate that nurses have mental overload that influences their physical, mental and behavioral well-being. A Tunisian study reported that 62% of caregivers are mentally ill [24]. Although our research

did not show a significant association between GHQ12 and age, other studies found statistically significant associations between poor mental health and age < 30 years (p = 0.014) [24].

## Conclusion

This study shows that caregivers experience significant levels of stress, burnout and psychological distress. The mission of care is defined by specific stressors, such as noise, frequent change of tasks, and the time system.

To conclude, the strain job has been highlighted with a gender and age effect. Nurses are more stressed than men; the age of over 40 was a key factor in high work stress.

This requires the adoption of a heuristic approach based on comprehensive and integrated strategies aimed at improving the personal and professional performance of nurses and coping with daily stressors in the performance of their duties.

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**Compliance with ethical standard**

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# The Epistemological Aspects of Investigative Hypotheses

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## Abstract

This paper is about the philosophical and epistemological aspects of investigative hypotheses.

Criminal investigation is a kind of cognition, so it is important to know the basic philosophic concept of knowledge and truth. Different schools and traditions of philosophy have given different answers to these.

One of the basic elements of investigative thinking is, in addition to being reconstructive and retrospective, thinking in hypotheses. Logical, semantic, syntactic, epistemological, and ontological principles must be taken into account when formulating hypotheses. Choosing between the hypotheses should be based on preference and indifference criteria. These criteria of preference and indifference will also be logical and epistemological.

Testing, validating, and rejecting hypotheses is an essential element of investigative work. The plausibility, credibility, and accuracy of competing hypotheses are constantly changing depending on the investigative data obtained. It is a basic principle that all hypotheses should be tested and, if possible, the testing should be performed in parallel.

***Keywords****: investigation, hypothesis, truth, cognition*

## Introduction

The investigation is a kind of cognition, it is a cognitive activity.[1] One of the most important components of cognition is knowledge. The definition of knowledge is from the great Greek philosophist, Plato: knowledge is a justified true belief.[2] To avoid confusing this belief with religious belief or any other believing thing, we can say conviction instead. The condition of justification protects against accidentally hit the correct answer as knowledge.

The definition of a justified true belief has stood sound for almost two thousand four hundred years, till in the 20th century the so-called Gettier problems challenged it.[3] Edmund Gettier's examples are paradoxes where a person's beliefs meet the truth but not because of the right evidence. Thus, he introduces the fourth criterion next to the Platonic triple: justified true belief is formed on adequate evidence.

### The Concept of Truth

Cognition only fulfills its role if the acquired knowledge is true.

What is „true"? Almost every philosophic school has its answer for this. After 1945 the Soviet philosophy, the so-called dialectic materialism strongly affected the Hungarian academic world. The dialectic approach is based on the philosophist Hegel, the materialism is mostly based on Feuerbach, both of them were German philosophists. Dialectic materialism was developed in the middle of the 19th century by Marx and Engels and further developed

in the early 20th century by Lenin. The dialectic materialism claims the primacy of the material world over the world of ideas and thoughts, and also claims the constant changes of every existing thing.[4] The core concept of Lenin's epistemology is the reflection of reality on the cognitive mind.[5]

The theory of the adequacy of truth can be found from Aristotle through St. Thomas Aquinas to dialectical materialism. Truth is a correct reflection of reality on one's cognitive mind. The cognitive subject reproduces the object of cognition, as it exists outside and independently of cognition.[6] The touchstone of truth is practice, so insofar as the dialectical materialist theory of truth can be understood not only as a theory of adequacy but also as a pragmatic theory of truth.[7]

The most important feature of truth is its objectivity in terms of content. It does not depend on the cognitive subject whether the views he considers true are true; but whether these views correspond in substance to objective reality. However, the truth is not the same category as reality, these are two different concepts. Reality is objective, cannot be characterized by true or false qualities. Only a cognitive reflection of reality can have such qualities (true or false); because the reflections as products of cognition may differ from reality. The truth as a product of cognition is subjective, but in terms of its content, it is objective, because the measure of the differentiation of true and false is corresponding with objective reality. And the test of the correspondence is the practice.

Thus, truth is always objective in its content, so we do not use the term "objective truth" established in the (especially old) forensic literature, since truth other than objective in terms of content and subjective in terms of form does not exist. We also disregard the use of absolute and relative adjectives. The absolute and relative nature of truth forms a dialectical unity. In truth we always find the absolute moment, that is, the part of wholeness: it is nothing but a faithful reflection of reality. At the same time, the reality is inexhaustible, no truth can fully reflect it; in that sense, therefore, all truth is relative.

**The Investigative Thinking Way**

An important component of investigative thinking, among several others, is its retrospective and reconstructive nature. Things discovered during an investigation should always be considered as results. The investigator considers everything he or she faces as a result and tries to deduce the possible causes. Only a probabilistic conclusion can be drawn as to the reason for the result. Therefore, several explanatory hypotheses should always be set up as possible explanations.

During the investigation, we use a kind of scientific cognition method. The common steps of the scientific method are

1. data collection and analysis,

2. formulation of the question,

3. setting up the hypothesis (i.e., the possible answer to the question),

4. selection of the testing method,

5. testing, and

5. conclusion.

The conclusion may be to reject or justify the hypothesis.[8]

Hypotheses are indirect knowledge in the sense that they can be proved with the help of other knowledge accepted as true at a given level of current knowledge. In the case of hypotheses, a distinction between truth and proof is justified. Hypotheses are also true or false as statements, but due to their indirectness, their verity can only be established through the transmission of other knowledge. Consequently, the truth of hypotheses can only be determined indirectly by their degree of proof, the plausibility. Hypotheses are in a verity relationship with the facts of reality (epistemological connection) and they are in a corroborative relationship with other

knowledge (logical connection).[9]

In investigative thinking or forensic cognition, hypotheses are the more or less probable possibilities and assumptions. The exclusion or confirmation of each hypothesis is the practical goal of the investigation.

**Formulating the Hypotheses**

There are several ways to form a hypothesis from the available data.

The deduction, if we break down the existing data further, that is, we infer from the general what is special.

It is inductive to generalize the existing data, that is, to infer the general from the particular. There is no possibility of complete induction because there is always only a limited, finite number of specific data.

Abduction is when the investigator recognizes a pattern in existing data and finds an assumption that adequately explains all existing data.

In the initial phase of an investigation, almost always the abduction is used to set up the very first raw hypotheses. A corpse lying in the room, with a headshot and a handgun in his hand, provides a possibility to hypothesize an accident, a suicide, and (a deceptive set scene of) a homicide.

The formulating of proper hypotheses has logical, epistemological, and ontological requirements. Logical requirements can be divided into syntactic and semantic requirements.[9]

It is a syntactic requirement that the version shall be well-formulated: a statement, which is an allegation about the reality. A statement or allegation is a predicate in logic. Single statement if the allegation is to declare something about a single specific individual (e. g. "this hunting knife is the tool of perpetration"). An existential statement which says something about at least one individual (e. g. "the tool of perpetration is a hunting knife"); and a general statement which declares something about every individual in the universe (e. g. "hunting knives are capable of killing humans"). In the practical investigative work, the hypothesis often takes the form of a (poetic) question, but it is important to analyze the content: in fact, "couldn't she get there later?" is a hypothesis because the statement claims that she later got there; the "what if it wasn't so?" however it is not a hypothesis (by itself) because it does not contain a predicate, a logical statement.

It is also a syntactic rule that the hypothesis shall be internally consistent. It means the hypothesis shall not have a logical contradiction in it, that is, that a statement and its opposite do not occur in it. A hypothesis on the phenomena of a contradictory nature can be formulated, but there can be no logical contradiction within a hypothesis. For example, a subject of a complex crime of mutual bodily harms and public violence may be both a perpetrator and a victim, but not in the same aspect.

The third syntactic rule is the logical derivation of every conclusion.

The first semantic requirement is conceptual sharpness. The terms used in the hypothesis shall be as sharp as possible. The opposite of sharpness here is the ambiguity of the term, which can be extensive or substantive. The ambiguity of terms can be reduced by specifying their content, their criterion (realistic definition). Specifying their connections with other terms (nominal definition), the designation of their referents and subjects (interpretation), the using of analogy, and so on. A sharp term can be the „minor injury", a vaguer term could be „thoroughly beaten". A realistic definition can be any legal state of affairs (e. g. counterfeiting, bribery), a nominal definition can typically be statutory delimitation (e. g. murder or manslaughter), and so on.

An additional semantic requirement is semantic homogeneity. The terms used in the hypothesis should refer to qualitatively homogeneous, and well-defined areas of reality. Usually, this is the case in

the criminal justice system, where the past is cognized to answer questions of criminal relevance, and this homogeneous area is not mixed with the elements of other conceptual circles, such as beauty, love, providence, etc.

The last semantic requirement is that the terms that occur in the lowest level hypotheses should be interpretable empirically, but during an investigation, this is usually proper. The point is that the terms that can be linked to reality, shall not be empty abstractions. Due to the nature of the thing, forensic science uses only concepts that can be interpreted empirically.

The first of the epistemological criteria is the requirement of external consistency, i.e. the hypothesis must be consistent with at least some of the already proven statements. It cannot be entirely consistent, because then it would offer nothing new, it would only redraft the existing knowledge. But it cannot be completely contrary to the knowledge that has already been proven, because then should either be discarded without any further investigation or should be reviewed the existing knowledge. In a practical investigation, if death has been considered to be caused by crime, the hypothesis of suicide should be excluded. If, however, there is a strong suspicion of suicide, some of the already proven statements of the crime shall be reviewed. Among the syntactic requirements, the requirement of internal consistency was described, and among the theories of truth, the theory of coherence: the statement is true if fits coherently the other true statements.

The second epistemological criterion is the requirement for possible empirical revision of the hypothesis. Conclusions should be possible to derive from the hypothesis, even with the usage of other hypotheses, and the conclusions should be able to be compared with the empirical evidence, with the facts of the perceptible reality. This comparison is mainly made through so-called indicator relations. An indicator relation between version and empirical reality is, for example, cause and effect relation, part and whole relation, structure and function relation, and so on. This epistemological requirement excludes any hypothesis which can not be, theoretically and practically, proved or disproved (e.g., what the victim thought before his or her death). Respectively, this criterion allows the deduction: with knowing one side of the indicator relation, e.g. knowing the cause or part, the other half of the relation can be deduced, e.g. the cause, or the whole.

The ontological criterion requires the hypothesis to be formulated with only existing elements. Every object, act, or happening shall be a part of the known reality. Usually, during an investigation, this requirement is fit.

**Choosing Among Hypotheses**

Usually, during an investigation, there are several hypotheses, some mutually exclusive and some mutually supportive. There are some preference and indifference criteria, which can help to choose among hypotheses. These criteria can also be logical, epistemological, and ontological.[9]

Based on the logical criterion, when choosing between complex hypotheses, the one that builds on independent simple hypotheses should be preferred over the one that builds on non-independent simple hypotheses (i.e., which are derived from each other). Thus it becomes available that the pieces of evidence should not form a chain but a net: the loss of a minor piece of evidence does not necessarily destroy the whole proof.

The first epistemological criterion is greater explanatory power. Its components are scope, accuracy, and depth. The scope is the number of phenomena explained by the hypothesis. Usually, the more the hypothesis explains, the less accurate it is, and vice versa. The depth of the explanation means that it is as structural as possible, revealing internal contradictions as much as possible. During an investigation, at first glance, usually, the above relationship between scope and accuracy is not

obvious, because the scope of investigative hypotheses is usually limited: it refers to a past event. However, if the investigator looks at the issue in a broader context and is curious about the general causes of the crime, he or she inevitably comes to hypotheses of the kind that "who" does "such" crime. In these cases, the investigator will experience the above relationship between scope, and accuracy. For example, "usually only those living in deep poverty steal" is a large-scope but a less accurate hypothesis, as opposed to "an offender who saws a ground wire from a working transformer and does not suffer an accident may be an experienced person with knowledge of electrical installation."

Sophistication is also an epistemological criterion. A hypothesis is more sophisticated, the problem solving is more effective. Other epistemological criteria, like originality, heuristic force, unifying force, predictive force are less relevant when comparing investigative hypotheses.

The more flexible hypotheses should be preferred, which can adapt to new pieces of information. The flexible hypothesis can evolve further with new information. Of course, flexibility does not mean that the hypothesis is compatible with information to the contrary.

Occam's razor is also an epistemological criterion of preferences. In Latin, "entia non sunt multiplicanda sine necessitate". If one of the two hypotheses contains all of the theoretical concepts of the other hypothesis and also other theoretical concepts, then the choice of the other hypothesis is more expedient. Choose the simpler of the possible explanations offered is advisable.[10] Of course, measuring, judging, and proving which of the two hypotheses is "simpler" is not always straightforward.[11] Suicide should not be preferred over homicide just because it is a „simpler" hypothesis because it is not. But assuming a second perpetrator just because it is not possible to exclude is highly contraindicated in every case where there is no positive information about a second perpetrator. The theorem can also be formulated as assumptions should not be unnecessarily multiplied, the above Latin formula essentially means the same.

The last epistemological criterion that facilitates the choice between rival hypotheses is the possibility of a simpler empirical review. This is easy to see: During the investigation, it is advisable to start the work with the simplest versions that can be checked, so that the work can be more focused and concentrated later.

Finally, the ontological criterion: the level-appropriate hypothesis is preferred to the lower-level (reductive) and higher-level (teleological) hypotheses. During an investigation, this usually fits. A teleological hypothesis, for example, could be the one that does explain the „why" question with overall sociological and political theories, instead of the motive of the particular criminal case, which is being investigated. A reductive hypothesis, for example, could be the one that does explain the „what happened" question with very simple biological theories of death, instead of explaining the whole incident of the murderer.

**The Hypotheses and the Investigation**

Once the investigator has hypotheses, the testing should be started. The „testing" means the investigation itself: data acquisition, crime scene process, crime reconstruction, experiments, interrogations, and confrontations, etc.

It is a basic principle that all plausible hypotheses should be tested and, if possible, the testing should be performed in parallel. Parallel testing is important for time management. If one hypothesis is found to be less plausible after several weeks of investigation, the investigation of the other possible hypothesis could only begin with a delay of several weeks. If the investigation of the two hypotheses takes place more or less in parallel, such a delay should not be expected. Of course, the capacity of the investigator is limited, so it is not possible to do parallel work covering all possible hypotheses. In this case, the preference and

indifference criteria outlined above may help.

## Conclusion

Hypotheses are formed from the data available at the beginning of the investigation, which provide different explanations for the causes and circumstances of the event under investigation.

The investigation can be successful if the basic philosophical principles outlined in the article are followed in formulating the hypotheses.

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# CT Scan Finding Characteristics of Confirmed Covid-19 Patients Based on Clinical Symptom Onset Patterns

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## Abstract

Chest computed tomography (CT) scan is one of the main modalities to detect COVID-19 infection. Several CT scan findings are the hallmark to rapidly detect suspected COVID-19 infection, therefore timely treatment could be administered. This study aims to describe chest CT scan findings of confirmed COVID-19 patients based on clinical symptom onset patterns.

This study is a descriptive study with a retrospective design in confirmed COVID-19 patients. Data regarding characteristics of chest CT scan findings and the patient's history including clinical symptoms onset were collected.

There were 123 subjects in this study. The study data were categorized into modified clinical symptom onset patterns which were early, progressive, peak, absorption, and advanced phases. Most of male (57%) and female (43%) patients that were admitted to the hospital were in absorption phase of the disease with fever, cough, and dyspnea as the most prevalent symptoms. Chest CT scan findings of absorption phase included GGO (ground glass opacity) in 28 patients (76%), crazy paving pattern in 18 patients (49%), consolidation in 22 patients (59%), and fibrosis in 31 patients (84%). Chest CT scan findings of confirmed COVID-19 patients vary depending on the phase of the disease. Risk factors and secondary bacterial infection may contribute to long-term persistence of symptoms.

***Keywords****: COVID-19, chest CT scan, clinical symptom onset patterns*

## Introduction

The COVID-19 disease has symptoms similar to the common cold infection with an incubation period of about 2-14 days, therefore most cases go undetected and causes fast-growing transmission. Prompt diagnosis is essential to help prevent this disease transmission.[1] WHO and Center for Disease Control (CDC) guidelines stated that chest computed tomography (CT) scan is one of the main modalities in detecting COVID-19 infection. The typical chest CT scan findings of a patient infected with COVID-19 causing pneumonia are multiple areas of bilateral lobular and subsegmental consolidation and ground-glass opacity (GGO).[2] These findings are the key to rapidly detect a suspected COVID-19 infection, therefore timely treatment could be administered and could prevent further transmission.

Studies regarding correlation between symptom onset in COVID-19 patients and CT scan findings has been conducted by several researchers. A study by Pan

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et al.,2020, examined the time course of pulmonary changes on chest CT scans during recovery from COVID-19 pneumonia and divided them into four stages of chest CT scan findings based on the quartiles of the time course from day 0 to day 26. In stage 1 (early stage: 0-4 days) GGO was found; in stage 2 (progressive stage: 5-8 days) GGO was scattered with enhanced crazy-paving pattern findings; in stage 3 (peak stage: 9-13 days) consolidated consolidation, scattered GGO, crazy-paving pattern, and residual parenchymal bands were found; and in stage 4 (absorption stage: >14 days) the consolidation was gradually absorbed and no longer crazy-paving pattern was found.[3] This study aims to describe chest CT scan findings of confirmed COVID-19 patients based on clinical symptom onset patterns.

## Methods and Materials

### Study Design

This is a descriptive study with a retrospective design that used medical records and the examination results of non-contrast chest CT scan from patients with confirmed COVID-19 who were treated at Dr. Soetomo Surabaya General Hospital, Surabaya, Indonesia from June to October 2020.

#### *Data Collection*

Researchers identified medical record and history data of confirmed COVID-19 patients. The collected data were clinical symptoms, comorbid, and symptom onset patterns, which were calculated from the start of the patient's symptom until a non-contrast chest CT scan was performed. Evaluation of CT scan finding was conducted by 2 experimented radiologists senior (mean 10 years of experience) and one resident (mean 3 years of experience) using standardized CT reports. For cases with controversial results, two radiologists with more than 10 years experience jointly reviewed and evaluated the result.

#### *Imaging Protocols*

All patients were adults and non-pregnant who underwent non-contrast chest CT scan to detect COVID-19 pneumonia in supine position during full inspiration. A total of 69 patients were subjected to CT scans in the Emergency Room using multi-detector 128 slice CT scan (Toshiba Aquilion Vision) and 54 patients had CT scans at the Radiodiagnostic Installation using multi-detector 128 slice CT scan (Philips) and 16 slice CT scan (Hitachi). The protocol used was 120 kV voltage tube, automatic tube current (120–380) mA, 5 mm thickness, 0.5 mm slice interval, 0-5 second rotation speed, and 1-0.828 helical distance.

#### *Clinical and Imaging Interpretation*

Identification of clinical symptom data categorized by clinical syndrome according to WHO, which includes: mild disease/non-specific symptoms, mild pneumonia, severe pneumonia, ARDS, sepsis and septic shock.[4] The analysis results were reported using The Covid-19 Reporting and Data System (CO-RADS) category.[5] Severity was assessed using a visual scoring system of each lobe involved. The scores for all lung lobes are summed to show lung involvement severity.[6] This study used a modified assessment of per-lobe severity. Score 0: if there is no lesion or 0%, score 1: when the estimated number of lesions visually is 1% to 25%, score 2: when the estimated number of lesions visually is 26% to 50%, score 3: when the estimated number of lesions is visual 51% to 75%, score 4: when the estimated number of lesions visually is more than 75%. The final results were categorized into mild (total score 7 or less), moderate (total score between 8 and 17), and severe (total score 18 or more).

#### *Data Analysis*

The collected data were categorical, which were processed using SPSS 21 software and then presented in tabular form with percentages (%).

## Results

The study was conducted on 123 study subjects of COVID-19 pneumonia patients. The study data were

categorized into modified clinical symptom onset patterns. The categories included initial (0-4 days), progressive (5-8 days), peak (9-13 days), absorption (14-30 days), and advanced phase (>30 days).

***Subject Characteristics Based on Clinical Symptom Onset Patterns***

The age range of study subjects was 17-91 years, with a mean age of 52 years. Based on the characteristics of clinical symptom onset patterns, most patients were included in absorption phase with an age range of 32-70 years (mean= 55±9 years) with 37 patients. The number of subjects who were male was 70 people (57%), which was more than female subjects with 53 people (43%). In initial, peak, absorption, and advanced phases most patients were male with 18 (56%), 9 (64%), 21 (57%), and 10 (67%) patients, respectively. While in progressive phase most patients were female with 13 (52%) patients.

The 3 most common clinical symptoms were fever (66%), cough (70%), and dyspnea (58%). Fever was found mostly in initial and progressive phase, with 24 (75%) and 21 (84%) patients, respectively. Cough was mostly found in peak phase with 11 (79%) patients, while in absorption and advanced phase the number of patients who experienced cough and dyspnea were similar, with 28 (76%) and 10 (67%) patients in each phase, respectively. The study subjects in this study had variable comorbid, although some patients had no concomitant comorbid. In early, absorption, and peak phase, the most frequent comorbid was Diabetes Mellitus (DM) with 10 (31%), 11 (30%), and 3 (20%) patients, respectively. In progressive and peak phase, the most frequent comorbid was hypertension (HT), with 10 (40%) and 6 (43%) patients, respectively.

The study subjects were categorized into clinical syndromes based on WHO criteria. The results showed that most patients in early and progressive phase had mild pneumonia with 13 (41%) and 11 (44%) patients, respectively. In peak phase, there were the same number of patients for mild and severe pneumonia, which were 4 (29%) patients. Most patients in absorption and advanced phase had severe pneumonia, with 10 (27%) and 5 (33%) patients, respectively.

The subject characteristics are outlined in Table 1.

Table 1. Subject Characteristics

| Characteristics | Early phase N (%) | Progressive phase N (%) | Peak phase N (%) | Absorption phase N (%) | Advanced phase N (%) | Total N (%) |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| Male | 18 (56) | 12 (48) | 9 (64) | 21 (57) | 10 (67) | 70 (57) |
| Female | 14 (44) | 13 (52) | 5 (36) | 16 (43) | 5 (33) | 53 (43) |
| **Age (Years)** | | | | | | |
| Mean ± Standard Deviation | 43 ± 15 | 49 ± 16 | 59 ± 13 | 55 ± 9 | 59 ± 8 | 52 ± 14 |
| Age Range | 17 - 74 | 32 - 91 | 35 - 85 | 32 - 70 | 45 - 70 | 17 - 91 |
| **Symptoms** | | | | | | |
| Fever | 24 (75) | 21 (84) | 8 (57) | 19 (51) | 9 (60) | 81 (66) |
| Cough | 17 (53) | 20 (80) | 11 (79) | 28 (76) | 10 (67) | 86 (70) |
| Dyspnea | 13 (41) | 14 (56) | 6 (43) | 28 (76) | 10 (67) | 71 (58) |
| No Symptom | 1 (3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.8) |
| **Comorbid** | | | | | | |
| Chronic Kidney Disease (CKD) | 2 (6) | 3 (12) | 0 (0) | 2 (5) | 2 (13) | 9 (7) |
| DM | 10 (31) | 7 (28) | 5 (36) | 11 (30) | 3 (20) | 36 (29) |
| HT | 7 (22) | 10 (40) | 6 (43) | 7 (19) | 2 (13) | 32 (26) |
| Obesity | 1 (3) | 5 (20) | 0 (0) | 1 (3) | 1 (7) | 8 (6) |
| Malignancy | 3 (9) | 2 (8) | 0 (0) | 0 (0) | 1 (7) | 6 (5) |
| Others | 13 (41) | 4 (16) | 2 (14) | 8 (22) | 5 (33) | 32 (26) |
| No Comorbid | 9 (28) | 9 (36) | 7 (50) | 15 (40) | 6 (40) | 46 (37) |
| **Clinical Syndrome (WHO)** | | | | | | |
| None | 1 (3) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.8) |
| Mild Disease / Non-Specific Symptoms | 10 (31) | 2 (8) | 3 (21) | 4 (11) | 1 (7) | 20 (16) |
| Mild Pneumonia | 13 (41) | 11 (44) | 4 (29) | 6 (16) | 3 (20) | 37 (30) |
| Severe Pneumonia | 0 (0) | 8 (32) | 4 (29) | 10 (27) | 5 (33) | 27 (22) |
| ARDS | 3 (9) | 0 (0) | 2 (14) | 7 (19) | 4 (27) | 16 (13) |
| Sepsis | 3 (9) | 1 (4) | 0 (0) | 6 (16) | 0 (0) | 10 (8) |
| Septic Shock | 2 (6) | 3 (12) | 1 (7) | 3 (8) | 1 (7) | 10 (8) |

Characteristics of Chest CT Scan Findings by Clinical Symptom Onset Pattern

The results of this study showed that the most common characteristics in early phase were GGO in 26 (81%) patients, crazy paving pattern on 8 (25%) patients, consolidation on 14 (44%) patients, and fibrosis on 23 (72%) patients. In progressive phase there were GGO in 17 (68%) patients, crazy paving pattern in 11 (44%) patients, consolidation in 15 (60%)

patients, and fibrosis in 22 (88%) patients. In peak phase, there were GGO in 11 (79%) patients, crazy paving pattern in 4 (29%) patients, consolidation in 7 (50%) patients, and fibrosis in 10 (71%) patients. In absorption phase there were GGO in 28 (76%) patients, crazy paving pattern in 18 (49%) patients, consolidation in 22 (59%) patients, and fibrosis in 31 (84%) patients. In advanced phase there were GGO in 8 (53%) patients, crazy paving patterns and consolidation in 1 (7%) patient each, and fibrosis in 12 (80%) patients. Bronchiectasis and bronchiolectasis were commonly found in absorption and advanced phases. The most common distribution for chest CT scan findings were bilateral predominantly peripheral in all clinical symptom onset patterns. Table 2 outlines these chest CT scan findings.

Table 2. Characteristics of Chest CT Scan Findings by Clinical Symptom Onset Pattern

| Chest CT Scan Findings | Early phase N (%) | Progressive phase N (%) | Peak phase N (%) | Absorption phase N (%) | Advanced phase N (%) | Total N (%) |
|---|---|---|---|---|---|---|
| **Characteristics** | | | | | | |
| GGO | 26 (81) | 17 (68) | 11 (79) | 28 (76) | 8 (53) | 90 (73) |
| Crazy Paving Pattern | 8 (25) | 11 (44) | 4 (29) | 18 (49) | 1 (7) | 42 (34) |
| Consolidation | 14 (44) | 15 (60) | 7 (50) | 22 (59) | 1 (7) | 59 (48) |
| Halo sign | 4 (12) | 2 (8) | 2 (14) | 1 (3) | 1 (7) | 10 (8) |
| Reverse halo sign | 2 (6) | 1 (4) | 1 (7) | 2 (5) | 1 (7) | 7 (6) |
| Air bubble sign | 0 (0) | 0 (0) | 1 (7) | 3 (8) | 0 (0) | 4 (3) |
| Reticular pattern | 2 (6) | 3 (12) | 0 (0) | 1 (3) | 2 (13) | 8 (6) |
| Nodule | 1 (3) | 1 (4) | 2 (14) | 3 (8) | 3 (20) | 10 (8) |
| Fibrosis | 23 (72) | 22 (88) | 10 (71) | 31 (84) | 12 (80) | 98 (80) |
| Bronchiectasis | 2 (6) | 4 (16) | 2 (14) | 20 (54) | 8 (53) | 36 (29) |
| Bronchiolectasis | 2 (6) | 3 (12) | 3 (21) | 22 (59) | 7 (47) | 37 (30) |
| Pleural efusion | 3 (9) | 2 (8) | 1 (7) | 4 (11) | 2 (13) | 12 (10) |
| Pleural thickening | 2 (6) | 5 (20) | 2 (14) | 7 (19) | 2 (13) | 18 (15) |
| **Distribution** | | | | | | |
| Bilateral predominantly peripheral | 20 (62) | 20 (80) | 13 (93) | 35 (95) | 12 (80) | 100 (81) |
| Unilateral predominantly peripheral | 8 (25) | 3 (12) | 0 (0) | 0 (0) | 0 (0) | 11 (9) |
| Bilateral predominantly central | 1 (3) | 1 (4) | 0 (0) | 0 (0) | 0 (0) | 2 (2) |
| No predominance | 1 (3) | 1 (4) | 0 (0) | 0 (0) | 1 (7) | 3 (2) |
| No lesion | 2 (6) | 0 (0) | 1 (7) | 2 (5) | 2 (13) | 7 (6) |

**Characteristics of Chest CT Scan Findings by CO-RADS Categories**

In CO-RADS 1 group, most patients showed normal lung features and only 1 (14%) had fibrosis. In CO-RADS 2 category group, most patients had fibrosis and pleural thickening, with 7 (78%) and 4 (44%) patients, respectively. In CO-RADS 3 category group, most patients had consolidation and GGO with 4 (80%) and 3 (60%) patients, respectively. In CO-RADS 4 group, most patients had GGO and consolidation with 11 (85%) and 8 (61%) patients, respectively. In CO-RADS 5 group, most patients had GGO and fibrosis, with 35 (85%) and 33 (80%) patients, respectively. Finally, in CO-RADS 6 group, most patients had fibrosis and GGO, with 45 (94%) and 40 (83%) patients, respectively. All CO-RADS categories mostly had bilateral predominantly peripheral in their finding's distribution. Table 3 outlines these findings.

Table 3. Characteristics of Chest CT Scan Findings by CO-RADS Categories

| Chest CT Scan Findings | CO-RADS | | | | | | Total N (%) |
|---|---|---|---|---|---|---|---|
| | C1 N (%) | C2 N (%) | C3 N (%) | C4 N (%) | C5 N (%) | C6 N (%) | |
| Mean severity score ± standard deviation | 0 ± 0 | 2 ± 1 | 5 ± 3 | 5 ± 4 | 10 ± 5 | 10 ± 5 | 8 ± 5 |
| **Characteristic** | | | | | | | |
| GGO | 0 (0) | 1 (11) | 3 (60) | 11(85) | 35 (85) | 40 (83) | 90 (73) |
| Crazy Paving Pattern | 0 (0) | 1 (11) | 0 (0) | 3 (23) | 21 (51) | 17 (35) | 42 (34) |
| Consolidation | 0 (0) | 1 (11) | 3 (60) | 8 (61) | 23 (56) | 24 (50) | 59 (48) |
| Halo sign | 0 (0) | 0 (0) | 0 (0) | 2 (15) | 6 (15) | 2 (4) | 10 (8) |
| Reverse halo sign | 0 (0) | 1 (11) | 0 (0) | 1 (8) | 4 (10) | 1 (2) | 7 (6) |
| Air bubble sign | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 4 (8) | 4 (3) |
| Reticular pattern | 0 (0) | 1 (11) | 0 (0) | 2 (15) | 3 (7) | 2 (4) | 8 (6) |
| Nodule | 0 (0) | 1 (11) | 0 (0) | 1 (8) | 2 (5) | 6 (12) | 10 (8) |
| Fibrosis | 1 (14) | 7 (78) | 4 (80) | 8 (61) | 33 (80) | 45 (94) | 98 (80) |
| Bronchiectasis | 0 (0) | 2 (22) | 0 (0) | 1 (8) | 6 (15) | 27 (56) | 36 (29) |
| Bronchiolectasis | 0 (0) | 0 (0) | 1 (20) | 0 (0) | 3 (7) | 33 (69) | 37 (30) |
| Pleural efusion | 0 (0) | 3 (33) | 1 (20) | 4 (31) | 1 (2) | 3 (6) | 12 (10) |
| Pleural thickening | 1 (14) | 4 (44) | 1 (20) | 1 (8) | 2 (5) | 9 (19) | 18 (15) |
| **Distribution** | | | | | | | |
| Bilateral predominantly peripheral | 2 (25) | 1 (12) | 1 (20) | 9 (69) | 40 (98) | 47 (98) | 100 (81) |
| Unilateral predominantly peripheral | 0 (0) | 5 (62) | 3 (60) | 3 (23) | 0 (0) | 0 (0) | 11 (9) |
| Bilateral predominantly central | 0 (0) | 0 (0) | 1 (20) | 0 (0) | 1 (2) | 0 (0) | 2 (2) |
| No predominance | 1 (12) | 0 (0) | 0 (0) | 1 (8) | 0 (0) | 1 (2) | 3 (2) |
| No lesion | 7 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 7 (6) |

## Discussion

The typical findings of a patient infected with COVID-19 that causes pneumonia on a chest CT scan are multiple areas of bilateral lobular and subsegmental consolidation and ground-glass opacity (GGO).[2] These findings are the key to rapidly detect a suspected COVID-19 infection, therefore a timely treatment could be provided and prevent further infection transmission. CT scan findings in this study included GGO, crazy paving pattern, consolidation, halo sign,

inverted halo sign, bubble air sign, reticular pattern, nodule, fibrosis, bronchiectasis, bronchiolectasis, pleural effusion and pleural thickening. These findings were in accordance to several previous studies.[3,7,8] Based on clinical symptom onset patterns, the findings in progressive, peak, and absorption phases in this study were mostly GGO, crazy paving pattern, consolidation, and fibrosis, with mostly bilateral predominantly peripheral distribution. These were similar to several previous studies which reported the presence of GGO with and without consolidation in subpleural visceral with peripheral ($p<0.001$) or bilateral multifocal distribution.[3,8,9] The virus ability to reach terminal bronchi and alveoli might be the main cause of this peripheral distribution.[10] Fibrosis was found in the early phase of this study. However, fibrosis due to COVID-19 usually occurs in chronic or healing phase.[8] It could be assumed that this finding is not a COVID-19 infection process and existed before COVID-19 infection. Statistically, Indonesia is a tuberculosis endemic area with a prevalence of bacteriological confirmed tuberculosis in 2013-2014 of 759 per 100,000 population aged 15 years and over.[11]

In this study, the lung severity was in line with the CO-RADS category. Lung severity scoring progresses with clinical onset. Lung severity scoring in initial phase was mild, then increased in progressive, peak, and up to absorption phase, and then decreased in advanced phase. Study by Guan et al.,2019 compared chest CT scan findings between progressive and recovery phase. From this study, several alterations were found in progressive phase: rounded image of GGO developed into uneven GGO, increased consolidation, and sometimes pleural effusions. In recovery phase, the crazy paving pattern and air bronchogram findings decreased significantly.[12] In this study, there were prolonged clinical symptoms and CT scan findings that still showed GGO, consolidation and crazy-paving patterns in absorption (14-30 days) and advanced (> 30 days). We assumed that there are several risk factors for these events. In this study, it was found that the average age of study subject was 52 years (SD±14), with mostly male patients in all phases of clinical symptom onset pattern. We also noted the presence of comorbid factors such as DM, HT, CKD, obesity and other comorbidities. In absorption and advanced phases, it was found that most of patients still had cough and dyspnea. This is in accordance to a study by Miyazato et al.,2020 who reported prolonged or delayed symptoms of coronavirus disease. The findings of this study were dyspnea, fatigue, cough, dysosmia, and dysgeusia, which lasted more than 120 days after symptom onset.[13]

A meta-analysis study by Biswas et al.,2021 estimated risk factors such as sex, age, or comorbidity with mortality in COVID-19 patients, and proved that male patients aged ≥50 years or that had comorbidities was significantly associated with an increased mortality risk. Age ≥50 years and male sex have higher expression of angiotensin-converting enzyme 2 (ACE2) regulated by male sex hormones, making men more prone of SARS-CoV-2 infection and had worse symptoms. Another hypothesis stated that women could fight the development of SARS-CoV-2 infection due to X-linked heterozygous alleles, which is called sex dimorphisms.[14] Comorbidity is associated with decreased immune function, as in DM patients. Natural immune function is substantially reduced, which could restrict the body from producing antibodies against any infection. ACE-2 is highly expressed in lungs, intestines, kidneys, and blood vessels epithelial cells and is predominantly upregulated in DM or HT patients treated with ACE inhibitors (ACEIs) and angiotensin II type-I receptor blockers (ARBs).[15]

Secondary bacterial infection might also be the cause of prolonged symptoms. A study by Zhang et al.,2020 reported that COVID-19 patients who received invasive mechanical ventilation or were in critical condition had a higher likelihood of developing secondary infection ($p<0.0001$). The most common infections are respiratory, haematogenous and urinary tract infections. Secondary infection will

lead to a higher mortality rate.[15]

This study has some limitations. Medical intervention as well as comorbid histories of confirmed patients with concomitant infection might interfere the CT scan findings. A history of comorbid such as malignancy, CKD, and others could also raise a bias with overlapped CT findings. We have a lack of standardized timeline of evaluation in overall patients, since the CT scan has not been as the routine protocol in patient's evaluation. CT scanning is perfomed mainly to follow up the disease's progression. Some cases of this study obtained were in the state of prolonged disease onset, approximately from 30-120 days.

## Conclusion

CT scans are able to reveal distinct confirmed COVID-19 findings that are variable according to clinical symptom onset. The typical finding in early phase is GGO with a bilateral predominantly peripheral distribution. The next phases have variable findings, with GGO, crazy paving pattern, and consolidation as the most common findings with bilateral predominantly peripheral distribution. This study also showed that most patients were in absorption phase with dominant findings of fibrosis. However, GGO, consolidation, and crazy paving patterns were still found. Cough and dyspnea were still found in this phase. These results show long-term COVID-19 findings from both the symptoms and CT scan findings.

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**Ethical Clearance:** This study was approved by the ethical committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (Registration Number: 0025 / KEPK / VII / 2020).

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**Conflict of Interest:** Nil

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# Isolation and Diagnosis of the Bacteria Causing Corneal Ulceration Associated With Ocular Myiasis Infection

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## Abstract

The current study was conducted to isolate and diagnose the bacteria that company cases ocular myiasis as complications. The bacteria were collected using a cotton swab for 30 individuals who came to the Diwaniyah Teaching Hospital due to their infection with ocularmyiasis. The swabs were cultivated on many culture media, such as blood agar and MacConkey, and mannitol salt and EMB medium for diagnosis of Ecoli. The results of the culture were positive for all samples under study. Various types were isolated; including rod and coccobacilli gram-negative in addition to gram-positive cocci, fermented and non-fermented mannitol species, and the identification of the species was confirmed using the VITEK2 system, the antibiotic susceptibility test was also checked using the same test and the results were as the following Ecoli in 5 cases, Pseudomonas spp in 4 cases, Kelbssilla pneumonia in one case, S aureus in 4 cases, S.epidermides in 5 cases in addition to many other different species.

***Keywords:*** *Ocular myiasis, secondary infection, corneal ulcer, VITEK 2 compact system, Sphingomonas paucimobilis*

## Introduction

A corneal ulcer is one of the most common serious infections in the world [1]; it can be defined as a sore in the cornea [2]. The etiological agent might be bacteria, viruses, fungi, or parasite like *Acanthamoeba spp*, besides many non-infectious agents such as chemical toxic, autoimmune diseases, neurotrophic and other [3]. One of the major reasons for corneal ulceration or keratitis is waring contact lances in developed countries, while the reason might be trauma in the developing world [4]. Corneal ulcer generally occurs as secondary to prior infection as ophthalmomyiasis cases (the invasion of the eye by living fly larvae of order Diptera). It usually occurs in shepherds and farmers in rural areas, but it has been documented in urban areas as well as in patients without close contact with animals. Other risk factors that have been identified include eye infections, eye wounds, advanced age, weakening, lack of diet and treatment, and poor hygiene. Cases of ophthalmomyiasis have been recorded in many nations around the world [5]. the causative agent might be the sheep botfly or *Oestrus ovis*, which is the most common cause of human ophthalmomyiasis, besides many other species have also been described, such as the human bot fly (*Dermatobia hominis*) latrine fly (Fannia), house fly (Musca domestica), and old screw worm *chryosoma bezzina*., in addition to many other species that cause the infection accidentally when infecting the skin, the clinical feature of the infection varies from severe foreign body sensation and redness, rhinorrhoea, chemises, and swelling. Conjunctivitis may be imitated by these initial signs and symptoms of external ophthalmomyiasis[6]. Some studies assume that corneal ulceration is one of the reasons that lead to ophthalmomyias is because of the foul-smelling caused by ulceration that attracts insects [7], but the more comprehensive hypothesis is that ulceration occurred as a result of infection with the lesion because the eye was injured by the insect In addition to a change in immunity levels [8]. In general corneal

ulcer still one of the emergency defects that might lead to blindness. Since ocular myiasis is self-limited and spontaneously healing, as such as rare disease the corneal ulcer that is caused by is also neglected despite the possibility of becoming a serious problem that can lead to blindness.

The aim of the present study was isolation and characterization of bacteria responsible for corneal ulceration which occurs as a complication of ocular myiasis, besides, to study antibiotic sensitivity testing.

## Material and Methods

### Diagnosis

According to the symptoms which include itching, burning, lacrimation, rhinorrhea, foreign body sensation, and swelling, the history of the patient is very beneficial in diagnosis as the patient might recently be in close contact with an animal or not, besides, to see the larvae in the eye by an ophthalmologist.

### Bacterial sample collection

To study the secondary bacterial infection and diagnose the etiological bacterial species about 30 samples were collected from all patients who give positive for ocular myiasis infection; this was accomplished by using cotton swabs. Then samples transformed to the lab immediately and streaked on Petri dishes contain Blood, MacConkey, and Mannitol salt agar which were prepared already, the dishes incubated at 37 C for 24-48 hours. then the growth was identified using gram stain and then depended on the VITEK 2 system.

## Results and Discussion

Corneal infections almost are the second most significant cause of blindness in the world, it usually occurs as a secondary infection. Since ocular myiasis is classified as a rare disease in addition to the possibility of it being cured automatically, there were no serious studies about its complications like corneal ulcerations. The present study was carried out in Diwanyia province in Iraq. For six months on ophthalmomyiasis infected people to identify the bacteria that cause the corneal ulceration that occurs as a secondary infection.

Thirty samples from thirty individuals who visited the hospital due to infection with ophthalmomyia sis, samples have been collected with cotton swabs according to S. Ballim et al [9,10]. even the ophthalmologist didn't notice keratitis signs.

These samples have transported to the lab and soon streaked on already prepared blood, MacConkey, and mannitol salt agar culture media, the results of growing bacteria are explained in Figures 1, 2,3,4 below

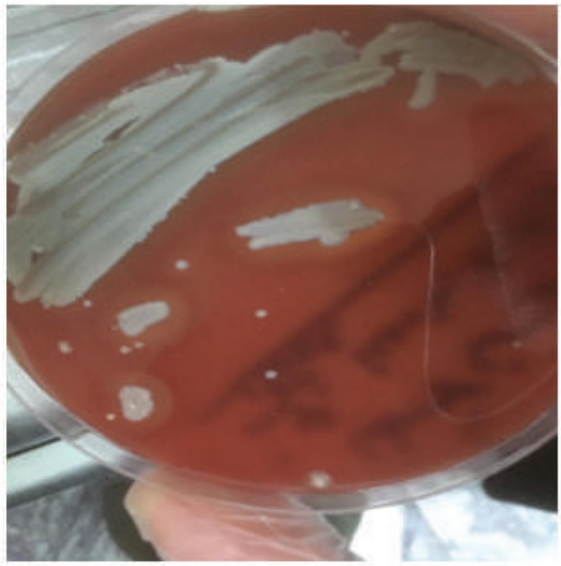

**Fig 1 growing bacteria on blood agar**

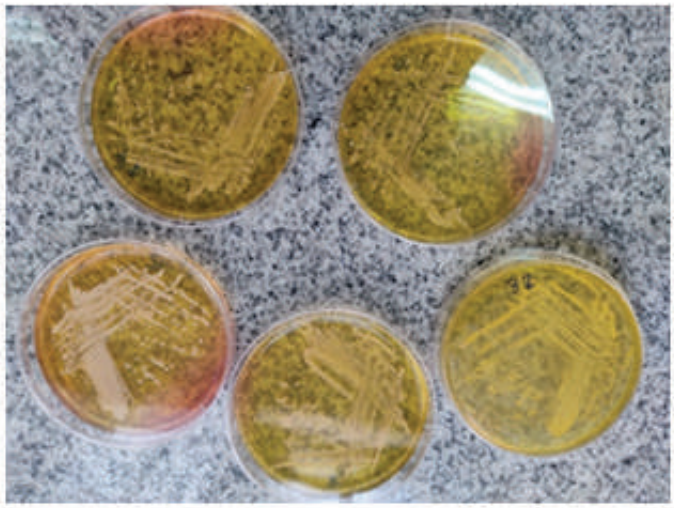

**Fig2. Growing bacteria on mannitol salt agar that ferment mannitol.**

**Fig 3 different bacteria on MacConkey agar**

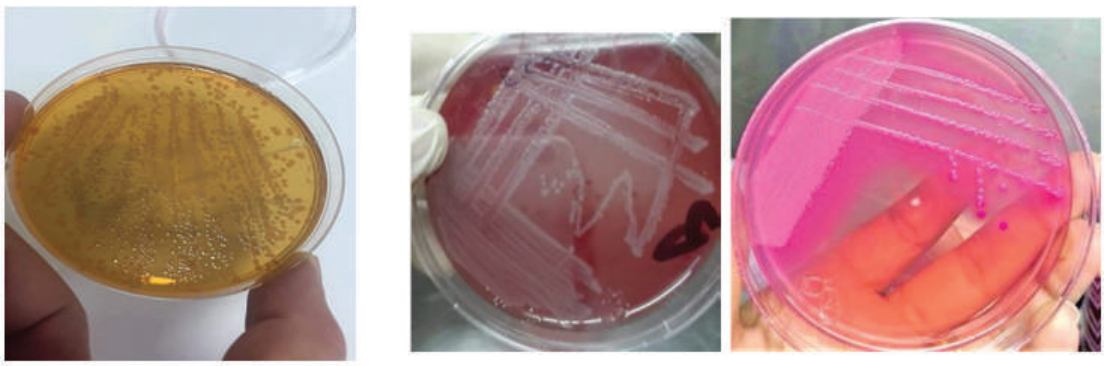

**Fig 3 different bacteria on MacConkey agar**

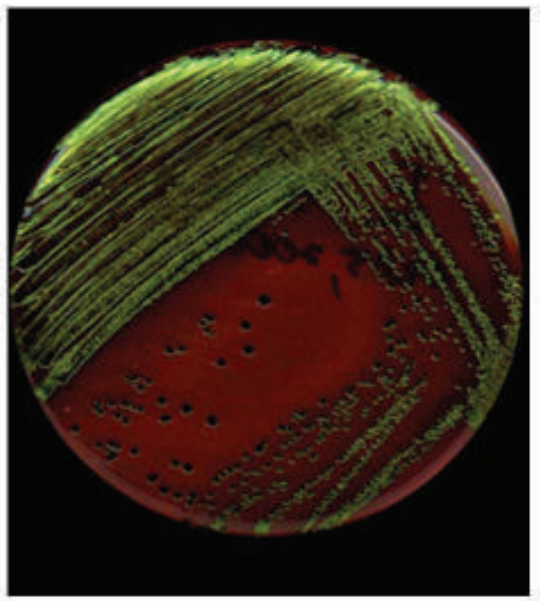

**Fig 4 *Ecoli* colonies on EMB media**

For further identification biochemical tests according to morphology on Gram stain and cultural characteristics on various media

Gram-positive cocci in clusters on gram stain, positive coagulase test, positive mannitol fermentation, and phenolphthalein phosphate test for Staphylococcus aureus Staphylococci with coagulase-negative staphylococci – coagulase-negative staphylococci negative and non-fermenting mannitol, Gram-negative bacteria, Pseudomonas aeruginosa Positive for catalase and oxidase, and green blush on agar plate *Ecoli* Gram-negative pink colonies on MacConkey and green with metallic shine on Eosin Methylene Blue (EMB).Indole and methyl red positive, *klebsiella pneumonia* gram-negative pink with slime layer on MacConkey agar indole, and methyl red negative. All these results are explained in the table1,2.

**Table 1. Biochemical tests for gram posetive bacteria**

| bacteria | Staphylococcus aureus | Staphylococcus epidermidis | S.pseudintermedius |
|---|---|---|---|
| coagulase | + | - | + |
| Catalase | + | + | + |
| Mannitol fermintation | + | - | + |

**Table 2 Biochemical tests for gram negtive bacteria**

| bacteria | E.coli | klebsiella spp | Pseudomonas spp |
|---|---|---|---|
| indole | + | - | - |
| Methyl red | + | - | - |
| oxidase | - | - | + |
| catalase | + | + | + |

Also for advanced identification and antibiotic sensitivity testing. The isolated bacteria have been sent to be diagnosed by using VITEK 2 system and the results showed many species of bacteria are represented in table 3below

**Table 3 VITEK2 compact system species identification results**

| Bacterial species | No .of cases isolated from |
|---|---|
| Klebsiella pneumonia | 1 |
| Escheichia coli | 5 |
| Staphylococcus aureus | 4 |
| Staphylococcus pseudintermedius | 3 |
| Pseudomonas earoginosa | 5 |
| Pseudomonas alcaligenes | 2 |
| Sphingomonas paucimobilis | 1 |
| Acinatobacter baumannii | 1 |
| Acrombacter deitrificans | 1 |

Original results that obtained from VITEK2 system had appendixes at the end of research. Most studies believe that infection with ophthalmomyisis is one of the rare, minor, limited infections that rarely occur recently, and if they do occur, their effect is limited to inconvenience. However, it may turn the eye into a pus cave full of smelly maggots which might encourages bacterial growing .but dependingon that most but not all of the isolated bacterial species return to the normal flora present in the eye and may have become pathogenic due to the flystrike that causes superficial scratches in the eye, on the other side immunologically larvae responsible for ocular myiasis contained have two antigens first one is the cuticle and second one represented by polypeptides that arise from salivary glands of the larvae and that the last is more immunogenic despite the direct contact between the larval cuticle and their hosts when host suffers from flystrike hypersensitivity reaction type I which is known as immediate type[11], occur lead to the proliferation of eosinophils and masts cells activation, beside IgE trigering and relaesing many mediators such as IL4,5,13 and Th2 cells response or simply just like anaphylaxis to bee venom[12]. These interferences can affect the normal environment of the eye to become pathogenic to the normal bacteria In addition to allowing the opportunistic types to cause ulcers in the cornea[13].

## Conclusion

In general, it can be said that cases of ocularmyiasis, even if they are spontaneous cure, but what accompanies them of bacterial infections may be dangerous and lead to blindness.

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**Conflict of Interest**: None

**Funding**: Self

**Ethical Clearance**: Not required

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# Study the Effect of Many Medications in Ophthalmomyiasis Treatment and the Possibility of Using these Medications Instead of Mechanical Removal

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## Abstract

This study was aimed to find the most suitable method to treat ophthalmomyiasis cases in Iraq, especially in diwanyia city. So that the study was conducted on 30 cases, where the drug xylocaine was dropped to the eye eight of them, and turpentine oil was dropped to eight others, while both anesthetics were applied to four of the patients, Then wait for 20 minutes and remove the larvae using forceps and by a specialist ophthalmologist .ten of patients were given ivermectin orally without any ivermectin to gather can immobilize the larvae thus facilitate their removal, it also found that using ivermectin is very effective in the treatment and can be used for clearing eye from larvae even after mechanical removal without any danger from side effect.

***Keywords****: ophthalmomyiasis, ocular myiasis, xylocaine, lidocaine, turpentine oil**,** ivermectin, immobilization of larvae*

## Introduction

Myiasis is the invasion by fly larvae of living or dead human (or animal) tissue. Ophthalmomyiasis contributes to the eye's larval infestation. Ophthalmomyiasis externa refers to infestation, including conjunctiva and cornea, of the external ocular structures, whereas ophthalmomyiasis interna refers to intraocular larval penetration [1]. It usually occurs in shepherds and farmers in rural areas, but it has been documented in urban areas as well as in patients without close contact with animals. Other risk factors that have been identified include eye infections, eye wounds, advanced age, weakening, lack of diet and treatment, and poor hygiene. Cases of ophthalmomyiasis have been recorded in many nations around the world[2]. The causative agent might be the sheep botfly or *Oestrus ovis*, which is the most common cause of human ophthalmomyiasis, besides many other species have also been described, such as the human bot fly (*Dermatobia hominis*) latrine fly (Fannia), house fly (*Musca domestica*), and old screw worm *chryosoma bezzina*.[3,4], in addition to many other species that cause the infection accidentally when infecting the skin, the clinical feature of the infection varies from severe foreign body sensation and redness, rhinorrhea, chemises, and swelling. Conjunctivitis may be imitated by these initial signs and symptoms of external ophthalmomyiasis. Symptoms have been reported in patients with internal ophthalmomyiasis, including photopsies, floaters, vision lines, and eye pain [5]. Management of infection with ophthalmomyiasis externa can be mainly accomplished by the mechanical removal of larvae from the eye at first and then the patient

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must be given topical analgesics and antibiotics, larvae attachment to eye tissue by their hooks make the removal difficult because the larvae escape from the edge of the eye to inside when direct slit lamp [6], using of antihelminthic are less common, in addition to only one case has treated with ivermectin successfully is recorded which" has shown benefit as a therapy and a prophylactic treatment for bot fly infestation of livestock"[2]. Some studies have mentioned the possibility of using many medications or ophthalmic anesthetics that can either paralyze larvae inside the eye or stop the motility of it. Among these compounds liquid paraffin, beeswax, heavy oil, or nail polish, it seems to be situated above the central field, Performance. The aerobic larvae are forced by such blockage air to the floor, at which point they can be caught by forceps in addition to lidocaine which can be injected into the cavity base is inhabited by the larvae, pushing the larva towards the surface. Instead, ethyl chloride they have sprays, liquid nitrogen, and insecticides. Used in conjunction or alone [7]. the topical 4% xylocaine and turpentine oil packing were used to immobilize the larvae in many orbital myiasis cases management [8]. It is worth noting that none of these compounds is used in Iraqi hospitals, despite the spread of severe cases during the spring, summer, and autumn period .in fact the epidemiological data refers to recording about 85 cases of ocular myiasis only in diwanyia city (south of Iraq) at 2020[9]. if we consider this ratio is affected by closing that occurs due to pandemic coronavirus disease, the number of cases may be more than what recorded The aim of the present study was try to find suitable and safe artificial or natural substance can be helpful with removal of larvae that cause opththalmomyiasis.

## Materials and Methods

### 1- Diagnosis

According to the symptoms which include itching, burning, lacrimation, rhinorrhea, foreign body sensation, and swelling, the history of the patient is very beneficial in diagnosis as the patient might recently be in close contact with an animal or not.

### 2- Larvae treatment

30 clinical cases were approved for the study. As the patients were divided into three groups, as follows

The first group included 8 casualties who were given 4% xylocaine and then removed the larvae using forceps, the second group includes 8 patients who were given packed turpentine oil and then removed the larvae by the same method, while 4 of the patient were given both turpentine oil and xylocaine to gather, all those 20 patients were given time about 15-20 minutes before mechanical removal process, the third group was given ivermectin orally without any surgical intervention. With it is worth noting that the patients in the third group were all residents of the village to ensure that their recovery was followed up by the village doctor and the results were recorded as for the first and second groups, they were randomly assigned. Taking into account giving the patient some antibiotics such as metronidazole, cefazolin, and amoxicillin with clavulanic acid to avoid secondary bacterial infection.

### 3. Statistical Study

Response surface methodology has been used as a statistical method depending on design expert V12 software. (A statistical method explains the relationship among several variables)[10], so in this study lidocaine and turpentine oil were the variables and their amounts and duration of administration are shown in table 1 below as suggested by the application.

**Table 1 the design actual of medication using**

| Std | Run | Factor1<br>xylocain<br>ml | Factor2<br>Turpentine oil<br>ml |
|---|---|---|---|
| 1 | 1 | 0 | 0 |
| 8 | 2 | 0.5 | 1 |
| 9 | 3 | 0.5 | 0.5 |
| 3 | 4 | 0 | 1 |
| 5 | 5 | 0 | 0.5 |
| 11 | 6 | 0.5 | 0.5 |
| 2 | 7 | 1 | 0 |
| 4 | 8 | 1 | 1 |
| 7 | 9 | 0.5 | 0 |
| 6 | 10 | 1 | 0.5 |
| 13 | 11 | 0.5 | 0.5 |
| 10 | 12 | 0.5 | 0.5 |
| 12 | 13 | 0.5 | 0.5 |

## Results and Discussion

This study was aimed to find an easy, suitable, and fast means to treat ocular myiasis cases. So that many topical ophthalmic anesthetic and anti-parasitic drugs have been used separately, it's important to know that these medications are common in most countries but it rarely used in Iraq.

Two anesthetic drops have applied on 24 patients with ocular myiasis. these anesthetics were turpentine oil, 4% xylocaine which was added to the infected eye each one separately and recorded the results after 15-20 minutes. The results are explained in table 2 below showed positive results only when used to gather.

**Table 2**

| Std | Run | Factor1 xylocain ml | Factor2 Turpentine oil ml | Response1 Motility |
|---|---|---|---|---|
| 1 | 1 | 0 | 0 | 1 |
| 8 | 2 | 0.5 | 1 | 0 |
| 9 | 3 | 0.5 | 0.5 | 0 |
| 3 | 4 | 0 | 1 | 1 |
| 5 | 5 | 0 | 0.5 | 1 |
| 11 | 6 | 0.5 | 0.5 | 0 |
| 2 | 7 | 1 | 0 | 1 |
| 4 | 8 | 1 | 1 | 0 |
| 7 | 9 | 0.5 | 0 | 1 |
| 6 | 10 | 1 | 0.5 | 0 |
| 13 | 11 | 0.5 | 0.5 | 0 |
| 10 | 12 | 0.5 | 0.5 | 0 |
| 12 | 13 | 0.5 | 0.5 | 0 |
| Maximum Variance Mean 0.7902 | | Average Variance Mean=0.28 | | Minimum Variance Mean=0.1701 |

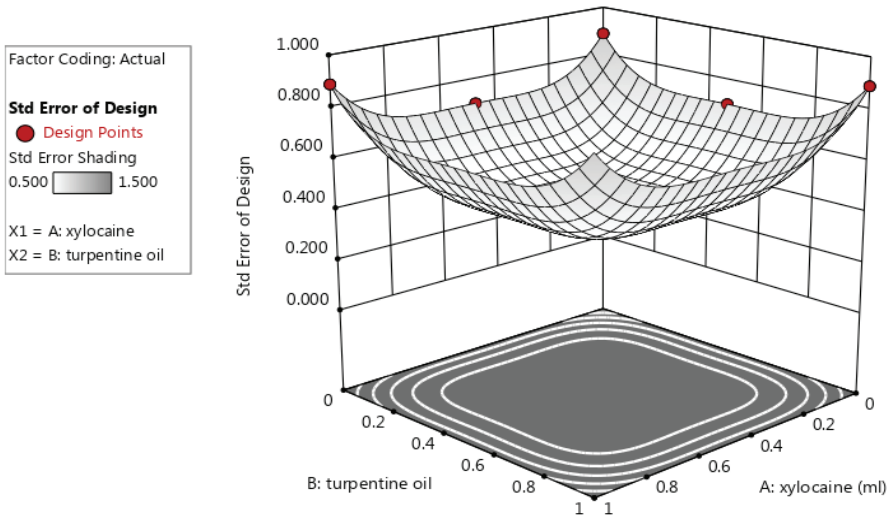


**Figure 1: 3Dexplain graph explains the activity of larvae immobilization**

Xylocaine is the brand name of lidocaine is a local anesthetic of the amino amide type, it is an activity that begins within several minutes to about half an hour [12].

Its properties are represented in table 3 below

**Table 3 lidocaine properties [13]**

| Clinical name | Lidocaine |
|---|---|
| Trade name | xylocaine |
| Chemical formula | $C_{14}H_{22}N_2O$ |
| Molar mass | 234.343gmol-1 |
| Melting point | 68 (154C) °F |
| Metabolism | Liver,[3] 90% CYP3A4-mediated |
| Duration of action | min to 20 min(IV), 0.5 h to 3 h (local) |
| Bioavailability | 35% (by mouth)<br>3% (topical) |
| Elimination half-life | 1.5 to 2 h |
| Extraction | kidney |

Since it belongs to the family of medicines called local anesthetics, its mechanism of action is represented by blocking the signal of nerve in the eyes, and thus it applied successfully to cause loss of feeling before certain procedures.it also might cause loss of feeling to larvae responsible for ocular myiasis defect by the same action. In the present study, the results of using lidocaine alone were not very effective in larvae immobilization and no paralyzing was examined also. Contrary to what was mentioned in some papers. "Agents used for paralyzing the larvae: Chloroform, Ether Lidocaine 1% and Pilocarpine [4]. But we can say using lidocaine as an eye drop in ocular myiasis cases is effective in pain relief with no side effects and helps the ophthalmologist to remove the larvae safely.

The turpentine oil is Turpentine (also known as turpentine spirit, turpentine oil, serpentine wood, terebinthine, terebinthine, and (colloquially) turpentine) is a liquid extracted from the distillation of resin obtained from living trees, predominantly pines. It is also a source of material for organic synthesis, mainly used as a specialized solvent [14]. Its properties are explained in t below able below

**Table 4 turpentine oil charecterstics[15]**

| Chemical formula | C10H16 |
|---|---|
| Molar mass | 163.238g mol-1 |
| Appearance | liquid with a high viscosity |
| Oder | Resinous |
| Melting point | -55 C(-67F:218K) |
| Solubility in water | 20mg/L(1) |
| Boiling point | 154C |

It has a variety of medical uses such as muscle, joint pain, and tooth's each, to minimize chest congestion that goes along with certain lung diseases; people often breathe in (inhale) the vapors of turpentine oil. Distilled turpentine oil is used as a flavoring in foods and beverages. It is also used for immobilizing maggots in ocular myiasis cases because it can suffocate them thus facilitate the mechanical removal process [16]. Despite the medical importance of turpentine oil, many research warnings use it due to its toxicity and irritant properties [17]. However, it can be replaced by another safe ointment that can act the same as turpentine oil.

Ivermectin is one of the important antiparasitic drugs approved by FDA that are used in many parasitic infections treatments such as filariasis, bancroftian, onchocerciasis, strongyloidiasis, and scabies. It was also used successfully for opthalmomyiasis inetrna posterior (OIP) treatment [18] the results of the present study showed that all 10 patients received a single oral dose of 10 mg (200µg\kg) of ivermectin were healed within two days.

The mechanism of action of this medication is characterized by interfering with helminths muscle and nerve functions, "it's bound to glutamate-gated chloride channels that are common to invertebrate nerve and muscle cells, Ivermectin binding pushes these channels open, increasing the flow of chloride ions and hyper-polarizing the cell membranes.[19,20]. The affected tissue is paralyzed, and the invertebrate dies as a result of hyperpolarization. Glutamate-gated chloride channels are limited to the brain and spinal cord in mammals (including humans); ivermectin cannot cross the blood-brain barrier and therefore does not reach the brain to impact mammalian channels [16]. Many recent studies have recommended using ivermectin to treat severe acute respiratory syndrome coronavirus [21]. The scientific base depends on many reports from in vitro studies indicate that ivermectin works by inhibiting alpha/beta-1 nuclear transport proteins from the host import, which are part of a main intracellular transport mechanism that hijacks viruses by suppressing the antiviral response of the host to improve infection [22]. Furthermore, docking with ivermectin can interfere with the attachment of the virus spikes to the human cell [23]. It is thought to be a host-directed agent that may be the basis for its in vitro broad-spectrum activity against yellow, HIV, Zika, and dengue fever viruses[24]. Despite this activity of ivermectin, there are no clinical trials that have reported a clinical advantage of ivermectin in patients with these viruses, despite this in vitro activity. Potential anti-inflammatory effects, which were postulated to be beneficial in people with COVID-19, were also recorded in some studies of ivermectin[25].

## Conclusion

At last, ivermectin can be safely recommended for ocular myiasis treatment either interna or external, and even when the mechanical removal is done successfully because of its important role in clearing the eye from residual larvae.

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**Funding**: Self

**Ethical Clearance**: Not required

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# Physical and Psychological Stressor Exposure during Pregnancy Impacts the Expression of Synapsin and Neuronal Cells Number of MUS Musculus Offspring

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## Abstract

***Introduction:*** Stress during gestation can lead to inappropriate fetal brain development, especially psychological stress. Psychological stress play pivotal role in offsprings' brain development.

***Objective:*** The aim of this study was to investigate the effects of maternal exposure to physical and psychological stress during pregnancy on the cerebrum in mice offspring.

***Method:*** This study was an analytical experiment study with the subject were 24 female mice *Mus musculus*. The sample were divided into treatment and control group. Treatment groups divided into three conditions, 1 group was given physical stress, 1 group with psychological stress, and 1 group was given combination of physical and psychological stress intervention. The data were analyzed with ANOVA test then followed by LSD to find the differences between all groups.

***Results:*** The ANNOVA test results showed significant differences of the expression of synapsin with p = 0.0000. The number of neuronal cells also represented significant differences with p = 0.000 on the cerebrum.

***Conclusion:*** Stress exposure during pregnancy can induce bad impacts in brain development, especially the expression of synapsin and the number of neuronal cells on the cerebrum in mice offspring.

***Keywords:*** Cerebrum, neurons, pregnancy, stress, synapsin

## Introduction

The stress that occurs during pregnancy results in an increased risk of preterm birth, a higher neonatal abnormality, and delays in motor and cognitive development. The prevalence of pregnancy stress is quite high. Psychosocial stress research during pregnancy conducted on Asian, African and white races states that 6% of pregnant women experience mild stress, 78% experience severe stress and 16% experience no stress at all.(1) In Canada, it shows that pregnant women experience low levels of psychosocial stress and 6% of high levels. Pregnant women in Spain have 30% lower chance of experiencing stress, while in Indonesia there are 64.4% of pregnant women who experience severe stress.(2)

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Previous study used exposure to light stressors proved that the number of brain neurons in the intervention group was significantly smaller than the non-intervention group.[3] The noise as stressor has been shown to reduce the number of neurons in the CNS in which is the cerebral cortex.[4] Synapsis plays a role in communication between neuron cells, so that synaptic structures play an important role in memory and learning. The greater the number of neuron cells, the more synapses. The multiple synapses cause a reduced apoptotic process. As a result, the brain processes information faster.[5] Stress during gestation can lead to inappropriate fetal brain development, especially psychological stress. Maternal who had experienced psychological stress during pregnancy have higher risks for cognitive, behavioral, and emotional problems. Exposed to psychological stress can damage structural brain development in which facilitated by programming of the hyperactivity of the Hypothalamus-Pituitary-Adrenal cortex (HPA) axis.[6]

The impact of stress on synapsin caused a decrease in synapsin-I mRNA in the amygdala by microaray analysis, the stressor showed an increase in high CRH expression and an increase in glucocorticoid receptors.[7] Also found that a disturbance at the beginning of fetal growth it will cause inhibited synapses formation which can inhibit cognitive enhancement.[8] The reduction in Syn-I will result in impaired axon differentiation, neurite growth, inhibit the formation and mechanism of synapses and allow neurological disorders in the brain.[9]

## Objectives

To compare the expression of synapsin and neuronal cells number of *Mus musculus* offspring exposed to physical stress, psychological stress, the combination of psychological and physical stress, and without stress exposure.

## Materials and Methods

This study used experimental laboratory research method with posted-only control group design. Animal subject was obtained from Faculty of Veterinary, Universitas Airlangga in which 24 pregnant female mice Mus musculus. Sample size was calculated based on replication formula of Frederer. Samples for each group has 6 pregnant mice. Pregnant Mus musculus were divided into 4 groups, 1 control group in which was not treated and 3 treatment groups. Treatment group 1 (G1) were given exposure to physical stress by making the pregnant mice swimming every morning for 5 minutes. Treatment group 2 (G2) were given exposure to psychological stress by giving noisy sound every morning with an intensity of 90 dB in a soundproof box for one hour. Treatment group 3 (G3) were given exposure to psychological stress then followed by physical stress every morning. There were no pregnant mice experiences preterm birth, abortion, or death. The interventions start from 6th day until 15th day of pregnancy. On the 16th day pregnant mice were sacrificed and the fetuses were taken by section caesarea. Then three newborn were selected based on the heaviest, moderate, and light weight. Selected newborn mice were sacrificed by decapitation. The brain of the selected offspring were taken and immunohistochemistry preparation. The expression of synapsin-I in the cerebrum were examined through immunohistochemistry with Syn-I antibody and calculated with Immuno Reactive Score (IRS) which is viewed under microscope with 5x visual field with 400 magnification. The number of neuronal cells in the cerebrum were calculated after Hematoxylin Eosin staining under the microscope with 5x visual field with 400x magnification. The data were analyzed with ANOVA test then followed by LSD to find the differences between all groups.

## Results

The research sample consisted of 24 female mice based on inclusion criteria and randomized into four groups. Mean and standard deviation of the expression of synapsin in the cerebrum can be seen in Table 1.

**Table 1. Data of the expression of synapsin in the cerebrum.**

| | Mean ± SD | | | |
|---|---|---|---|---|
| | G1 | G2 | G3 | Control |
| Synapsin | 7,80 ± 1,56 | 6,20 ± 0,82 | 4,03 ± 0,46 | 9,70 ± 1,71 |

Table 1 showed that treatment group 3 has the lowest synapsin expression in the cerebrum of *Mus musculus* offspring. Treatment group (G2) which had been exposed to psychological stress has lower expression of synapsin compare to physical stress exposure group (G1). The differences of synapsin expression between groups were analyzed by one-way ANOVA test then followed with Least Significant Difference (LSD) test. ANNOVA test resulted p= 0.000 which there are significant differences in the expression of synapsin in the *Mus musculus*' offspring cerebrum.

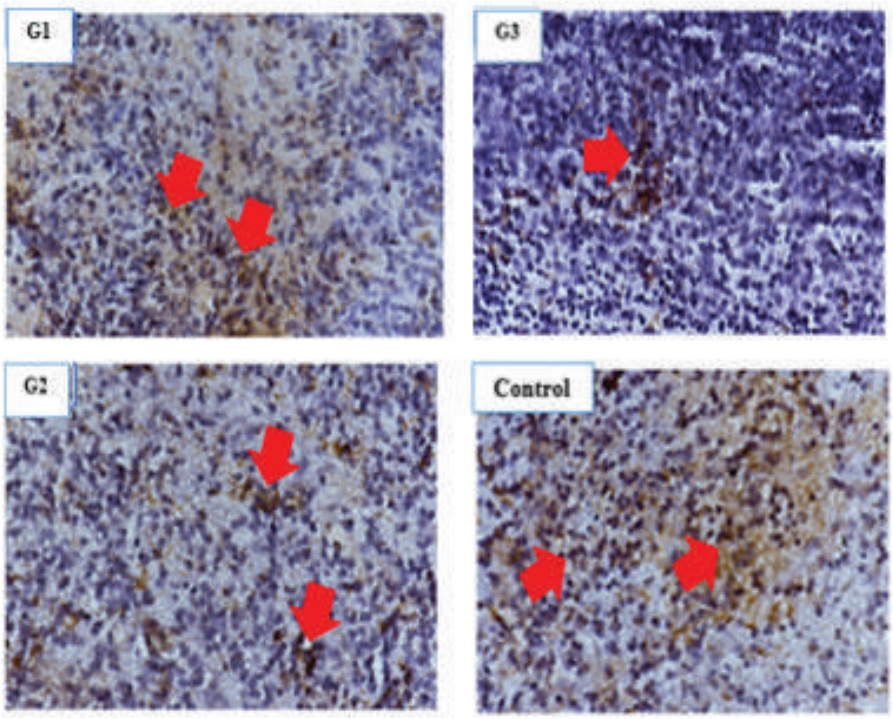


**Figure 1. Comparison of synapsin expression in the cerebrum of Mus musculus offspring (G1, G2, G3, Control). The red arrow indicates the presence of synapsin expression in the cerebrum which is indicated by the presence of a chromogenic brown color. Viewed after immunohistochemistry with 400x magnification.**

The number of neuronal cells in the offspring's cerebrum were calculated after Hematoxylin Eosin staining procedure. Mean and standard deviation of the number of neuronal cells in the cerebrum can be seen in Table 2.

**Table 2. Mean of neuronal cells number in the cerebrum.**

| | ***Mean* ± SD** | | | |
|---|---|---|---|---|
| | **G1** | **G2** | **G3** | **Control** |
| Neuronal cells number | 9,67 ± 1,19 | 7,87 ± 1,89 | 6,00 ± 1,46 | 11,63 ± 0,87 |

The results showed the mean difference between G1, G2, G3, and Control group, as shown in Table 2. The decrease in neuronal cells number occurred in the stress exposure groups. Statistical analysis used the One Way Anova test with p-value = 0.000, which means significant differences between groups. Post Hoc LSD test (Least Significant Difference) shows significant differences between G1, G2, G3, and Control group.

## Discussion

Stress during pregnancy affects the well-being of the mother and the fetus. Stress caused disadvantages such as low birth weight, premature birth, and delayed child development after birth. It can cause brain mass atrophy and reduce its weight.[10]

This research found that on the cerebrum of newborn *Mus musculus* showed the mean of synapsin expression in the physical stress exposure group (G1) proved to be lower than the control group. Physical activity for 1 hour can significantly increase oxidative stress.[11] Stress and mental conditions have a critical impact on physical health because the mind and body are one unit and psychological stress can adversely affect the health of the body.[12]

Stress changes brain function by modifying the structure and function of neurons and astrocytes. Astrocytes play an important role in synaptic transmission by expanding the subtle processes around the synapses. These astrocytes express GluA1, a subunit of glutamate receptors that is known to prolong astrocyte processing. Astrocytic structural changes are associated with decreased levels of the GluA1 protein.[13]

This study also showed that in the cerebrum of the newborn *Mus musculus*, the mean synapsin expression in the psychological stress exposure group (G2) proved to be lower than the control group. The effects of stress not only result in changes in the number of synapses, but stress can also result in significant remodeling of the ultra-structural morphology of the individual excitatory synapses. In particular, exposure to stress reduces the length of the synaptic active zone in the dentate gyrus and reduces the thickness of the postsynaptic density in the CA1 area.[14]

The stress-induced morphological changes of the GABAergic tissue are complemented by in vitro-electrophysiological findings, documenting malfunctioning GABAergic neurotransmission in the pressurized hippocampus.[15] In addition, vivo-electrophysiological studies focused on tissue function documented long-term potentiation-induced impairment and decreased basal synaptic transmission at the hippocampal CA3-CA1 synapses, and this was accompanied by decreased dendritic spine density in the CA1 and CA3 pyramidal neurons.[16]

Stress patterns can be divided into two main categories, namely physical stress and psychological stress.[17] Various studies have shown that these two stress patterns can lead to behavioral disorders, brain atrophy and cognitive dysfunction, abnormal neurotransmitters and cytokines, irregular hormone levels, and increased inflammatory factors in experimental animals.[18] Synapse loss has been reported in PFC depression patients. Synaptic dysfunction has been suggested as a key factor contributing to emotional distress. The mechanistic theory prevailing in perinatal psychiatry to explain mood-related effects on offspring is through changes in the mother's Hypothalamic-Pituitary-Adrenal (HPA) axis during pregnancy. Research result found that basal levels of plasmatic corticosterone were higher in animals under prenatal stress compared to controls. Corticosterone is a fat-soluble glucocorticoid that penetrates the placenta and interacts strongly with various fetal cells and tissues.[19]

This research found that mean of neuronal cells in the cerebrum was shown to be lower than in the G1 than control group. Structural changes include disorders of atrophy and neurogenesis. In addition, chronic stress can increase plasma cortisol and cause a decrease in the number of dendritic branches and the number of neuron cells, as well as structural changes in synaptic terminals and decreased neurogenesis in the hippocampal tissue.[20] Research on exposure to psychological stress in the form of noise causes the release of stress hormones, including glucocorticoids.[21] When suffering from stress, the central nervous system (CNS) is affected, the individual can experience anxiety and depression, which can have detrimental effects. Brain-Derived Neurotropic Factor (BDNF), a well-known neurotropic factor, is widely expressed among various brain areas, playing an important role in neuron maintenance and survival and neurogenesis.[22] Chronically elevated GC impairs neurotrophic factor expression.[23] This causes changes in neuron structure such as decreased synaptic activity, changes in morphology and neuron proliferation capacity.[24] The effects of stress were more pronounced in the psychological group than in the physical group at the end of the treatment week even though exposure to psychological stress was responded more slowly than exposure to physical stress.[25]

Physical and psychological health during pregnancy affects fetal nerve development.[26] The combined exposure group of physical stress and psychological stress (G3) showed a lower mean number of neuron cells than the group given one exposure (G1, G2). Stress involves two-way communication between the brain and other systems through nervous and endocrine mechanisms. Stress exposure and stress hormones can produce maladaptive effects in this brain region throughout the course of life. Psychological stress give worse impacts than physical stress with the result that the mean number of neuron cells is lower in the psychological stress exposure group than in the physical stress exposure group.

## Conclusion

The expression of synapsin and neuronal cells number in Mus musculus offspring cerebrum exposed to physical and psychological stress proved to be lower than pregnant mice with no stressor exposure.

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# Distribution of Virulence genes in *Streptococcus pneumoniae* Isolated from Different Baghdad Hospitals

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## Abstract

*Streptococcus pneumoniae* causes serious infections (pneumonia, meningitis and bacteremia) with significant morbidity and mortality rates. The goal of this study is to isolate and identify *S. pneumoniae* from clinical samples in Baghdad City hospitals in order to explore the presence of three virulence genes (*lytA*, *ply* and *psaA*) in clinical isolates. A total of 120 separate clinical samples were obtained from inpatients and outpatients with upper and lower respiratory tract infections at different hospitals in Baghdad. Twenty-five isolates of *S. pneumoniae* were identified by using cultural, morphological and biochemical characteristics as well as the diagnosis by VITEK2 system. Clinical samples were including sputum 19 isolates (76%) and urine 6 isolates (24%). The results of collected specimens showed that male's percentage is highest than female's patients, which was out of 25 isolates are 15 (60%) males and 10 (40%) females, The results also showed that it is much more common in elderly people than in younger people. The genomic DNA of bacterial isolates was applied in a polymerase chain reaction (PCR) to amplify certain genes. The PCR results were: Six isolates (24%) show positive results for the presence of *lytA* gene. Five isolates (20%) showed positive results for the presence of *ply* gene. Twenty-three isolates (92%) show positive results for the presence of *psaA* gene. As concluded from the present study, that the results exhibited that the presence of the virulence genes can be considered as a serious issue.

***Keywords:*** *virulence genes, Streptococcus pneumoniae, Baghdad hospitals*

## Introduction

*Streptococcus pneumoniae* bacteria carries a plethora of factors that helps in disease pathogenicity and immune evasion. The most common habitat is the upper respiratory tract, which not only assist in colonization but also provide an opportunity to cause an invasive disease. It is gram–positive facultative anaerobic pathogen, alpha- hemolytic bacteria [(1)]. Estimation of recent data show that this bacteria is responsible for 14.5 million annual infections worldwide and more than 800,000 deaths in children less than five years of age [(2)]. The genome of *S. pneumoniae* is quite plastic that means they can readily acquire DNA from environment and can recombine with the incoming DNA thus acquiring virulence and new genes for adaptation so it has a horizontal gene transfer that leads to a wide variation in pneumococcal genome and explain the presence of a large number of virulence genes attributing to disease pathogenicity [(3)]. The virulence factors of pneumococcal include its` capsule, cell wall components, pili and recently identified pneumococcal proteins including pneumolysin (*ply*), autolysin lytic amidase (*lytA)*, pneumococcal surface adhesion A (*psaA*), choline-

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binding proteins (CBPs), Neuraminidase, biofilm, IgA protease and Lipoteichoic acid [4]. Many risk factors for streptococcal infection such as age, race, immunodeficiency, other illness, previous antibiotic therapy and day–care attendance may play an important role in streptococcal diseases [5]. The epidemiological data of *pneumococcus* in Iraq is little at present time, therefore recent study aims to analyze distribution of virulence factors of isolates identified from Iraqi patients in Baghdad hospitals, to provide data support for development pneumococcal infection prevention strategies and vaccination.

## Materials and Methods

### Collection of samples

A total of 120 clinical samples were obtained, include: sputum samples that were collected from patients by asterial disposable cotton swaps. In addition, clean midstream urine samples collected from patients. The specimens sent to the laboratory, and examined.

### Isolation and Identification of *Streptococcus pneumoniae*

The collected samples were inoculated on blood agar that already prepared and incubated at 37ºC for 24-48 hours with ~5% CO2 (in a candle-jar). Isolates were examined for their shape, size, color, pigments, and hemolytic activity [6]. The identification of *Streptococcus pneumoniae* done by microscopic examination (Gram stain) [7]. In addition to biochemical tests performed according to [8]. Including Catalase test [9], Optochin test [10] and diagnosis by VITEK2 device.

Bacterial isolates preserved using long-term preservation (20 ml of glycerol to 80 ml of Brain Heart infusion broth) [11].

### Molecular detection of virulence genes

DNA extraction of *S. pneumoniae* isolates done by a genomic DNA extraction kit supplemented by the manufacturing company (Promega, USA).

### Polymerase Chain Reaction (PCR) amplification analysis

In the current study, four primers were selected, especially for pneumococcal which responsible for virulence factors genes, including the *LytA* (encodes the autolysin), *ply* (encodes the pneumolysin) and *PsaA* gene (encodes the adhesion). Each 25 PCR tube reaction contains 1.5 µl of each forward and reverse primer, 3 µl of DNA template, 14 µl of deionized water, and 5 µl of PCR PreMix (BIONEER, Korea), as well as a negative control that contains all components except DNA. Vortex PCR tubes, and then placed in the thermocycler PCR device according to table 1.

**Table 1: Optimum of PCR reaction for *lytA*, *ply* and *psaA* genes detection.**

| Target gene | PCR conditions | Temperature (ºC) | Time | No. of cycles |
|---|---|---|---|---|
| lytA ply | Initial denaturation | 95 | 3 min. | 1 |
| | denaturation | 95 | 30 sec. | 30 |
| | Annealing | 58 | 30 sec. | |
| | extension | 72 | 30 sec. | |
| | Final extension | 72 | 7 min. | 1 |
| Target gene | PCR conditions | Temperature (ºC) | Time | No. of cycles |
| psaA | Initial denaturation | 95 | 3 min. | 1 |
| | denaturation | 95 | 30 sec. | 30 |
| | Annealing | 55 | 30 sec. | |
| | extension | 72 | 30 sec. | |
| | Final extension | 72 | 7 min. | 1 |

## Results and Discussion

### Isolation and identification of *Streptococcus pneumoniae*

A total of 120 separate clinical samples (sputum and sterile urine samples) were obtained from inpatients and outpatients at different hospitals in Baghdad. About 30 samples showed negative growth of bacteria while 90 samples showed positive growth of bacteria. 50 isolates where non-*Streptococcus spp.* and 40 isolates where belonging to the *Streptococcus spp.* but only 25 isolates confirmed to be *Streptococcus pneumoniae*, , this expectation was observed after studying the shape of the colonies, size, color and surface texture after it was developed in the traditional differentiate. Selective and modified media cultures and in addition to the biochemical tests and VITEK2. Twenty-five isolates were recovered on subculture and confirmed as *S. pneumoniae* from different clinical sources including: sputum 19 isolates (76%) and urine 6 isolates (24%). Out of 25 isolates are 15 (60%) males and 10 (40%) females, the results of collected specimens showed that male's percentage is highest than female's patients. Distribution according to age groups demonstrated in figure 1.

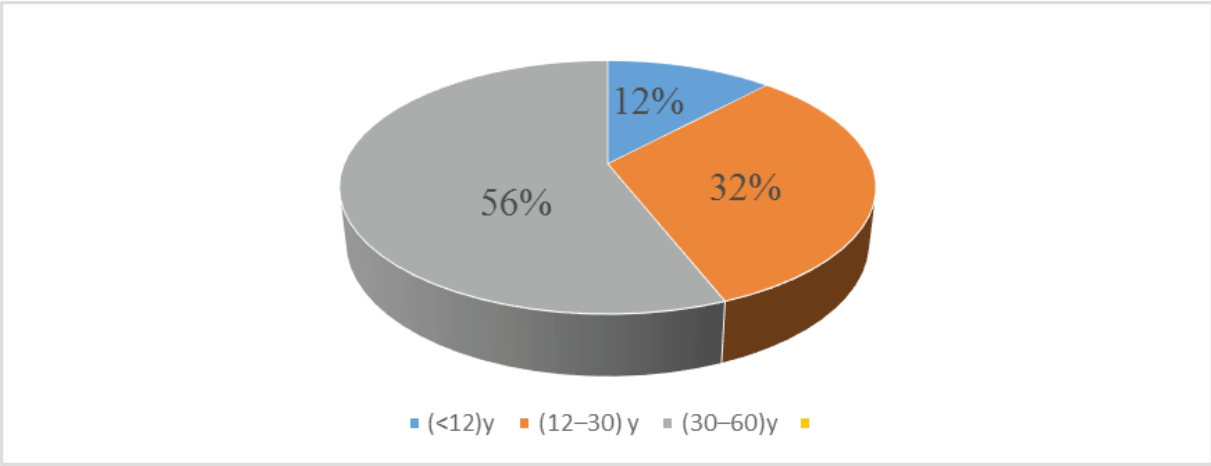


**Figure 1: Patients distribution according to the age groups**

This result approximately agree with Motaweq and Naher, (12) that the age group 51-60 years had the highest prevalence, with (19.7%), followed by age groups over 60 years (14%), 31-40 years (11%) and 1-10 years with (10.8%).

More *S. pneumoniae* were isolated from males 15 (60%) than females 10 (40%) This may be due to the fact that smoking is more common in males [12]. Despite variations in percent of isolates, Al-Taaie [13] found that *pneumococci* had a total incidence among those aged over 49 years.

Pneumococcal pneumonia is much more common in elderly people than in younger and middle-aged people, according to Pejcic *et al*. [14]. *Pneumococcus* is the most common organism found in hospitalized elderly patients suffering from CAP (community acquired pneumonia), according to Gupta *et al*. [15].

The high prevalence of pneumococcal isolate in the 51-60 age group may be attributed to immune system impairment, and the majority of elderly people are infected with chronic diseases [16]. Females had a higher number of cases than males in the age group 31-40. Most females in this age group are likely to be taking a variety of medications and hormones for pregnancy-related issues, putting them at risk of infection due to lowered immunity [17]. In contrast to our results, increased infection rates with pneumonia

in the 1-10 age group could be due to low immunity and increased exposure to contaminated materials. Pneumonia is also the leading cause of death from infectious disease around the world. It killed almost 1.6 million children under the age of five in 2008 [18].

**Molecular detection of *S. pneumoniae* genes by PCR**

**Detection of Autolysin *(lytA)* gene**

The extracted DNA was amplified for *lytA* gene by PCR. Amplified product size was 319bp as shown in figure 2. Six isolates (24%) show positive results for the presence of *lytA* gene.

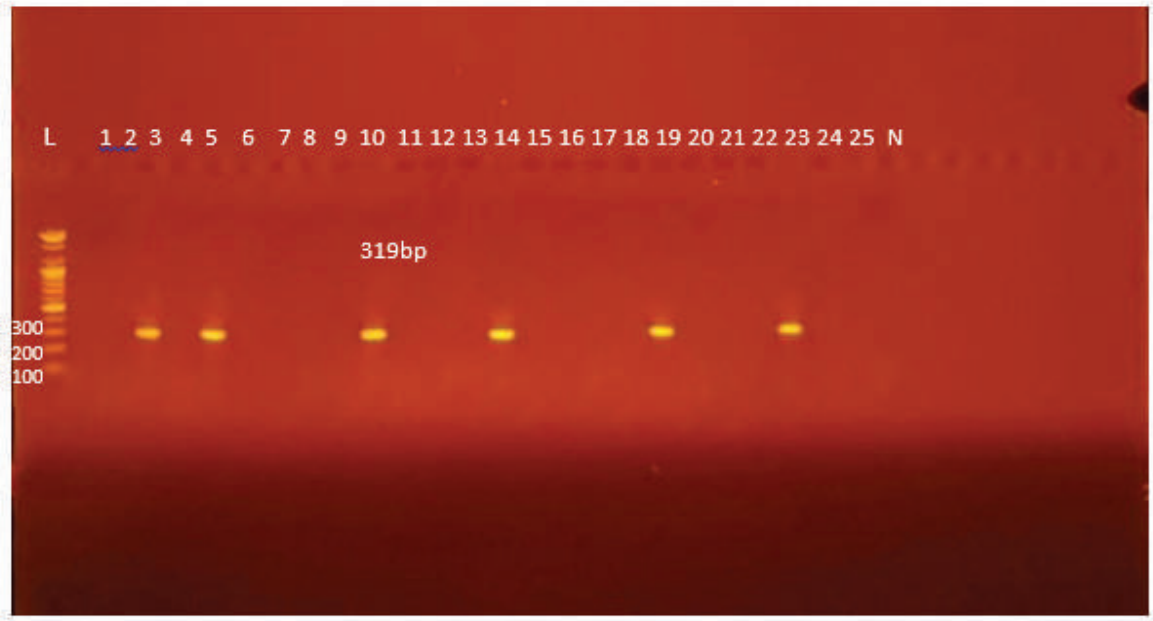


**Figure 2: Gel electrophoresis for PCR product of *lytA* gene for *S. pneumoniae* using 1.5% agarose gel at 60volt for 15 minutes and then at 90volt for another 15 minutes. Lane L: 100bp DNA ladder, Lane (1-25): results for *lytA* gene; Lane N: Negative control**

This result is identical with the result obtained by Asadi *et al*. [19] which found that *lytA* gene detected in (23%) from Middle ear effusion samples. Moreover, this result in disagreement with Abdul-Lateef *et al*. (20) results which found that *lytA* gene is present only in 4 isolates out of 8 (50%) and Irajian *et al*. [21] study, which found that *lytA* was present in (100%) isolates when using multiplex PCR with other virulence genes. Also, disagree with the results obtained by Kurola and Paula [22], that found the prevalence of *lytA* is (51%) of isolates by PCR or with Motaweq *et al*. [23], which showed that the *lytA* gene was (89.2%).

**Detection of Pneumolysin *(ply)* gene**

The extracted DNA was amplified for *ply* gene by PCR. Amplified product size was 347bp as shown in figure 3. Five isolates (20%) show positive results for the presence of *ply* gene.

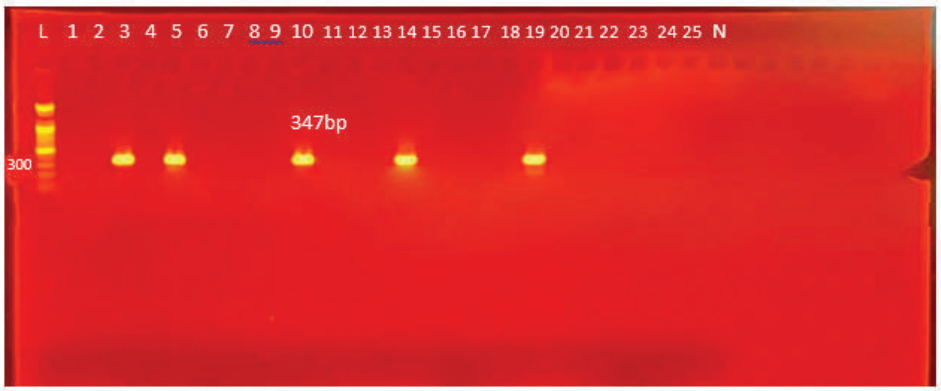


**Figure 3: Gel electrophoresis for PCR product of *ply* gene for *S. pneumoniae* using 1.5% agarose gel at 60volt for 15 minutes and then at 90volt for another 15 minutes. Lane L: 100bp DNA ladder, Lane (1-25): results for *ply* gene; Lane N: Negative control**

This result in disagreement with Abdul-Lateef *et al*. [20] results which found that *ply* is present only in 4 isolates out of 8 (50%). Also, disagree with the results obtained by Sourav *et al*. [24] that found the isolates gave (71%) PCR positive result for pneumolysin (17 out of 24 isolates). Moreover, this result totally disagrees with the result obtained by Kurola and Paula [22], which found that (85%) *ply* gene, during the testing of *S. pneumoniae* isolates. These variations may be due to a variation in the marker's sequence. However, all *S. pneumoniae* isolates found to generate pneumolysin extracellularly in blood agar, implying that pneumolysin encoded by several genetic loci, and that other bacterial exotoxins may have the potential to guide cell lysis and thus aid in microbial spread through tissues by causing significant damage to the extracellular matrix or eukaryotic cells' plasma membrane [25].

**Detection of Pneumococcal surface adhesion A *(psaA)* gene**

The extracted DNA was amplified for *psaA* gene by PCR. Amplified product size was 512bp as shown in figure 4. Out of 25 only 23 isolates (92%) show positive results for the presence of *psaA* gene.

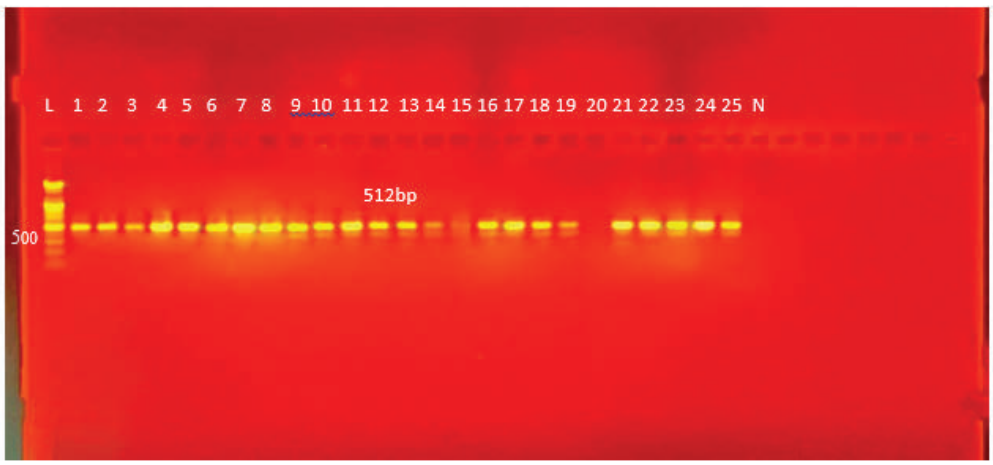


**Figure 4: Gel electrophoresis for PCR product of *psaA* gene for *S. pneumoniae* using 1.5% agarose gel at 60volt for 15 minutes and then at 90volt for another 15 minutes. Lane L: 100bp DNA ladder, Lane (1-25): results for *psaA* gene; Lane N: Negative control**

This result is in agreement with the result obtained by Morrison *et al*. [26], which found *psaA* gene detected in all isolates of *S. pneumoniae* from clinical samples. However, this result is in disagreement to the result obtained by Abdul-Lateef *et al*. [20] that *psaA* is present in 2 isolates out of 8 (25%) and Alhajem *et al*. [27] result that showed that the *psaA* gene was present in only 9 out of 11(50%) isolates.

## Conclusion

*S. pneumoniae* is a very sensitive bacterium that is difficult to handle due to its quick degradation in culture conditions. The distribution of the three virulence genes of *S. pneumoniae* isolates in our study showed that, the number of different virulence factors in different clinical isolates, S3, S5, S10, S14, S19 have the three types of virulence factors. While, other isolates such as S15, S20 do not have any of them. The other isolates are variable. Which played a role in colonizing the host and thereby generating illness.

**Conflict of Interest**: None

**Funding**: self

**Ethical Clearance**: Not required

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# Prevalence of Biofilm Genotype Pattern( *algD −/pslD −/pelF –*) with Multidrug-Resistant in Clinical Local *Pseudomonas Aeruginosa* Isolates

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## Abstract

The study was designed to explore the distribution and association of the biofilm genotype pattern( *algD −/pslD −/pelF –*) with multidrug-resistant in clinical local *Pseudomonas aeruginosa* isolates. A total of one hundred isolates of *Pseudomonas aeruginosa* were included in this study, which has been collected from different specimens, from July to September 2020. The isolates included were 34 from burns, 19 from wounds, 23 from ear infections, 22 from urinary tract infections (UTI), and 2 from cystic fibrosis (CF). Identification of the isolates was carried out using microscopical, cultural characterization on MacConkey agar, Cetrimide agar, then Pseudomonas agar. Biochemical tests were performed, and further identification was carried out by the VITEK_2_compact system. Genotypic identification has been completed *by16SrRNA*. To assess the frequency of multidrug-resistant of *Pseudomonas aeruginosa* (MDR), the antibiotic susceptibility test was done. It was carried out by using different groups of antibiotics (10 antibiotics) using the Kirby–Bauer disk diffusion method. The results showed that the resistance were Ceftazidime(62%),Gentamicin(26%,(Piperacillin-tazobactam(25%), Ticarcillin(24%), Meropenem(20%), Cefepime(18%),Amikacin(17%) Levofloxacin(16%),Colistin(15%) Imipenem(10%). Biofilm production was assessed using a microplate examination method. The results showed that 93% of isolates were positive for biofilm production, while (7%) were non-biofilm producers. There were differences in the rates of biofilm-production distributed into 21 (21%) were strong biofilm producer (OD was more than 2.156), 25 (25%) intermediate biofilm producer, and 47 (47%) were weak biofilm producer (OD was less than 1.078), and the non -biofilm producer was 7(7%).

Three virulence factors genes ( *algD*, *pslD*, and *pelf* ) were chosen, which responsible for the phenotypic pattern of biofilm formation and identified as genotypic *algD −/pslD −/pelF –* pattern. The differences in genotypic pattern prevalence among the MDR-positive isolates of different origins were statistically significant. Chi-square analysis showed a highly significant association between strong biofilm capacity and genotype pattern ($p<0.0001$), also the analysis showed a highly significant association between moderate biofilm capacity and genotype pattern ($p<0.002$). Chi-square analysis showed a highly significant association between weak biofilm capacity and genotype pattern ( $p<0.001$).

In the current study the percentage of resistance among *P. aeruginosa* local isolates for multiple antibiotics (MDR) was relatively low, maybe due to the combination strategies based on appropriate anti-pseudo-antibiotic agents that may be used to improve treatment from the related infections, according to these results, *P. aeruginosa* local isolates that produced biofilm were mostly (70%) indicated as non-MDR.

***Key words:*** *Biofilm formation, MDR,* **Pseudomonas aeruginosa,** *genotype pattern*

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## Introduction

*Pseudomonas aeruginosa*, the primary human pathogen in the *Pseudomonas* genus, it is an important opportunistic bacterium and a leading cause of nosocomial hospital-acquired infections. *P. aeruginosa* is a regular cause of nosocomial pneumonia, urinary tract infections(UTI), it may also colonize in healthy humans without causing the disease [[1]. *Pseudomonas. aeruginosa* is a motile, aerobic Gram-negative rod bacterium located in a range of biotic or abiotic habitats, such as soil, water, animals, plants, and insects [2]. *Pseudomonas* species are aerobic, non-spore-forming, straight, or slightly curved gram-negative rods of 0.5–1.0μm by 1.5–5.0μm. They're motile with one or more polar flagella, very strict oxygen aerobic respiratory metabolism, but in certain cases, nitrate has been used as an alternative that allows anaerobic growth[3]. It can produce a variety of pigments, such as pyocyanin (blue-green) and fluorescein (yellow-green), and also the capacity of some strains to produce other pigments, like yellow pyoverdin, dark red pyorubin, and pyomelanin (dark black) [4]. It has great genomic content (~6.5 Mbp) for variations in metabolism and adaptation for several environmental roles and mismatch repair systems [5].

Multidrug-resistant *P. aeruginosa* (MDR-PA) is one of the great concerns as it does not only cause fatal and serious infections, it increases the length of stay in the hospital, leading to increased treatment costs[6]. The main types of antibiotic resistance developed are natural (Intensive) resistance, acquired resistance, cross-resistance, and multidrug resistance as well as pan-resistance[7]. Once biofilms are recognized as the source of disease, management becomes very problematic. Typically, instant controller by extra of high -dose for antibiotic required for long term administration. Biofilm is formed of surface-adjusted aggregates of bacteria integrated with self-made extracellular polymeric substances(EPS), which decrease the probability that the bacteria will penetrate the immune cells as well as antibiotics within the biofilm and serves as a useful defense against the host immune system and antibacterial compounds, resulting in continuous colonization leading to treatment failure[8].

The biofilm components of *Pseudomonas aeruginosa* consist of three distinct exopolysaccharides, which include alginate, Psl, and Pel [10]. Alginate is a polymer made up of -D-mannuronic acid and -L-guluronic acid that contributes significantly to the structural support and protection of biofilms. Psl is a polysaccharide made up of repeated pentasaccharides, made up of D-mannose, D-glucose, and rhamnose Psl is necessary for biofilm development and for the biofilm structure to be protected. Pel is the third polysaccharide found in *Pseudomonas aeruginosa* biofilm and is high in glucose[60]. Different virulence factors such as lipopolysaccharide, flagellum, type IV pili, type III secretion system, proteases, alginate, exotoxin A, quorum sensing(QS), biofilm formation, type VI secretion systems, and oxidant generation in the airspace, may be cited as pathogenicity, and they affect in various ways on the immune response [11]. Quorum Sensing is a communication method that bacteria use to regulate the density of the population by producing as well as sensing small diffuse signal molecules. This form of intercellular bacterial signaling coordinates gene regulation and controls several cooperative behaviors, which include biofilm formation, virulence traits, metabolic demands, and host-microbe interactions [12]. This study aims were: isolation and characterization of *Pseudomonas aeruginosa* from different clinical specimens, also screening of the multidrug-resistant, and biofilm formation isolates, evaluate the phenotypic and genotypic characteristics of biofilm production rates, and the association between resistance patterns and their biofilm capacity.

## Materials and Methods

One hundred samples from Anbar Governorate were randomly included in the study. These were obtained from patients admitted to the Urology and Dermatology Departments in Al-Ramadi Teaching

Hospital and Burn department. All the samples were collected during the period from July to December 2020. The study isolates were obtained from urinary tract infections (UTI), wound infections, including burns, otitis media, and other types of wounds, out of these isolates, 100(100%) isolates were identified as *P. aeruginosa*., 93(93%) isolates were produced biofilm.

All isolates were identified as *P. aeruginosa* according to morphological, cultural, biochemical characteristics, VITEK-2 and *16S rRNA* proposed by [13, 14].

**Antimicrobial Susceptibility Test(AST):**

Depending on the definition of the Clinical and Laboratory Standards Institute (CLSI-2019) using the agar diffusion method. All these isolates were tested for as well as (*Pseudo* ATCC:15442) was used as a standard strain**.**

**Quantification of biofilm production**

**Microtiter plate assay:**

Microtiter plate assay was achieved according to [15]. The results were compared according to the following equations (Table 1).

**Table (1 ): The results were calculated based on the following equation:**

| Mean OD630 | Biofilm Intense |
|---|---|
| OD≤ ODC* | Non-producer |
| ODC<OD≤ 2ODC | Weak |
| ODC<OD≤ 4ODC | intermediate |
| OD>4ODC | Strong |

***Cut off value (ODC)=Mean OD of negative Control +3 (Standard Deviation of control).**

**Methods of PCR for detection of specific genes**

**1 Primers Solutions:**

The primers were established based on the National Center for Biotechnology Information NCBI and provided by the Promega Company as a lyophilized product of various concentration of picomol (Table 2).. Solution final concentration of (10 pmol/µl) was prepared separately by dissolving 10µl of stock solution for each primer and added to 90µl free nuclease distilled water un-ionic($ddH_2O$), mixed well and kept in (-20$^oC$). They were mixed by vortex to homogenize before use.

**Table (2): Sequence of PCR primer and molecular size of PCR products.**

| Gene | | Sequence of forward and reverse (primer 3/-5/) | TM(C0) | Product (bp) | Reference |
|---|---|---|---|---|---|
| 16srRNA | F | AGAGTTTGATCCTGGCTCAG | 58 | 1500 | [16] |
| | R | CTACGGCTACCTTGTTACGA | | | |
| algD | F | CTACATCGAGACCGTCTGCC | 58 | 593 | [17] |
| | R | CATCAACGAACCGAGCATC | | | |

***Cont...*** **Table (2): Sequence of PCR primer and molecular size of PCR products.**

| | | | | | |
|---|---|---|---|---|---|
| PslD | F | TGTACACCGTGCTCAACGAC | 56 | 369 | [17] |
| | R | CTTCCGGCCCGATCTTCATC | | | |
| PelF | F | GAGGTCAGCTACATCCGTCG | 58 | 789 | [17] |
| | R | TCATGCAATCTCCGTGGCTT | | | |

**F=Forward sequence, R=Reverse sequence.**

**PCR program for *16SrRNA, algD* and *pelF and pslD* genes detection:**

PCR was used for the detection of *Pseudomonas aeruginosa*. PCR tubes containing The mixture were transferred to the preheated thermocycler and began the program as shown in the tables below (Table 3,4 and 5).

**Table (3): PCR amplification program for*16SrRNA* detection.**

| Step | Temp.(C0) | Time | NO. of cycle |
|---|---|---|---|
| Initial denaturation | 95 | 5min | 1 |
| denaturation | 95 | 1 min | 35 |
| Annealing | 58 | 40 second | 30 |
| extension | 72 | 45 second | |
| Final extension | 72 | 5 min | 1 |
| Hold Temperature | 4 | 3min | - |

**2.12 Statistical Analysis:**

Data analysis was performed using the available statistical package SPSS-22. Data was reflected in simple frequency and percentage measurements. The significance of the difference in different percentages (quality data) was evaluated using the Chi-square test ($X^-$). Statistical significance was considered whenever the P-value for the relevance check was equal to or less than the P-value for the relevance check ( 0.05).

## Results and Discussion

**Isolation and identification of *Pseudomonas aeruginosa*:**

The current study has been carried out on 100 clinical samples from including catheter-acquired urine and wound infections and also burn, cystic fibrosis, and otitis media patients from July 2020 to September 2020. Microbial isolates were collected from patients who are admitted to the Urology and Dermatology Wards of the Al-Ramadi Teaching Hospital (Ramadi, Iraq) and the laboratory of Al-Ramadi Teaching Hospital. The isolates were identified by various types of media and chemical characteristics of *Pseudomonas aeruginosa.*

**Prevalence of *Pseudomonas. aeruginosa isolates***

After all confirmation tests for the identification

of *Pseudomonas aeruginosa*, a total of one hundred isolates were obtained . The results showed that the highest ratio of *P.aeruginosa* isolates was from burns with percentages of 34(34 %). This may be due to the pathogen has several potentially virulent factors which help it to colonize and infect mammalian tissues, like protease, pyocyanine, and hemolysin, which promote adherence to host cells, destroy host tissue, and disrupt the defense system[18,19]. As a result of the damage to the skin barrier in burn patients and repeated scrub of the burn site with high resistance to topical povidone-iodine as well as the cross-contamination with *Pseudomonas* strains are often more likely to occur[20][21]. Abdullah et al,2019 [22] indicated that the percentage of *P.aeruginosa* from the wound was 14%, and this slightly close to our results.

However, the above results indicated that *P.aeruginosa* is one of the most species that causes wound and burn infections because this pathogen is opportunistic and can cause infection to any deficiency in the body's defense system.[23]. The percentage of other infections were (23%) in the ear, (22 %) in the urine of total clinical isolates. Our study also showed that the ratio of bacteria isolated from the urine was 22 %. Whereas, this result varied from [24], which found that the isolation rate from ear infections was 11.6%. This may be due to the difference in isolation sites, the number of isolates, type of samples, and the distribution of isolates that may vary depending on the location of the infection, and some other reasons, like the type of sterilization and disinfectants used in hospitals besides the methods used in sterilization. Since this pathogen is resistant to many sterilizers and antimicrobial agents(Fgure 1) [25].

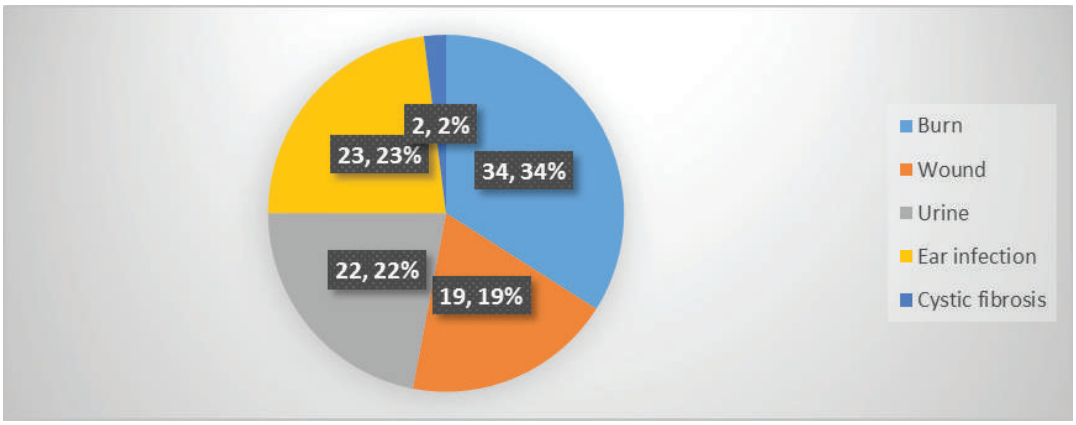


**Figure (1): The source of the specimens and their number and percentage (%).**

**Kirby Bauer Disk Distribution Susceptibility Results:**

One hundred *Pseudomonas aeruginosa* isolates from the burn, wound, urine, ear, and CF were tested for antibiotic sensitivity by the Kirby Bauer Disk Diffusion method as recommended by (CLSI, 2019). The antigram of the isolates studied showed variable resistance toward most of the antibiotics under study as shown in figure (2). Susceptibility was tested for 10 antimicrobials agents including Ceftazidime(CAZ),Imipenem(IPM),Piperacillin-tazobactam(PRL),Levofloxacin(LEV), Meropenem (MEM), Gentamicin(GN), Colistin(CO), Amikacin (AK), Ticarcillin(TC), Cefepime(FEP/CPM). The isolates were resistant to all antibiotics used in our study. An increase in bacterial resistance to many antibiotics considered to be a major therapeutic challenge. The results showed that the highest resistant percentage was 62% to Ceftazidime and the lowest percentage was 10% to Imipenem antibiotic.

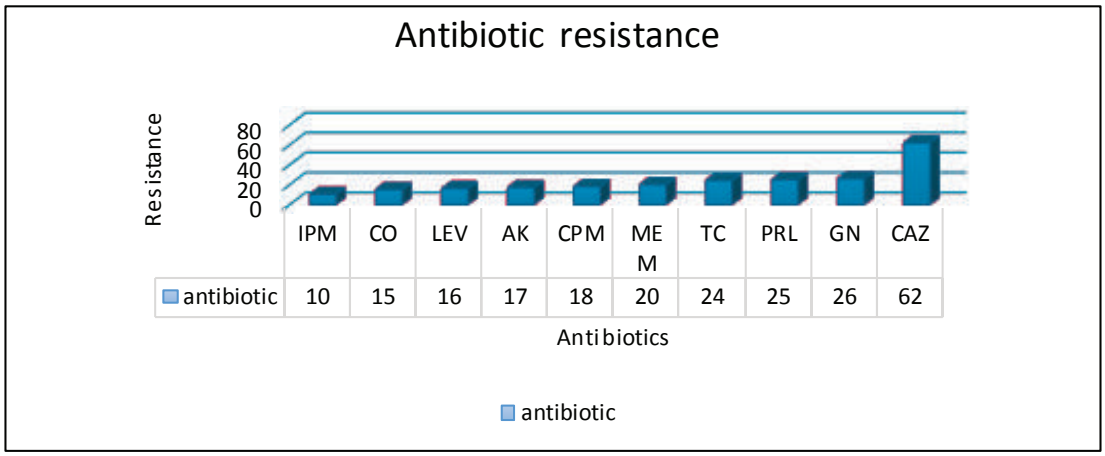


**Figure (2): Antibiotic Resistance ratios among *P. aeruginosa* isolates. Ceftazidime(CAZ),Imipenem(IPM), Piperacillin-tazobactam (PRL), Levofloxacin(LEV), Meropenem(MEM), Gentamicin(GN), Colictin(CO), Amikacine(AK), Ticarcilin(TC), Cefepime (FEP/CPM).**

The present study showed a different percentage of *P.aeruginosa* resistance to ceftazidime in comparison to some previous studies, and this percentage of *P.aeruginosa* resistance to ceftazidime was lower than the result achieved in the study by [26], and higher than[27],[28].

**Biofilm estimation by microtiter plate assay**

The results of the quantifiable biofilm formation assay showed that the various *P. aeruginosa* biofilm producers were classified as strong, moderate, and weak. In the qualitative biofilm formation assay, a spectrophotometric technique was used under a set of experimental situations. Our results showed 100 isolates were *P. aeruginosa*, 93% were biofilm producers, distributed into 21% strong biofilm producer (OD was more than 2.156), 25% intermediate biofilm producer, and 47% were weak biofilm producer (OD was less than 1.078), and the non -biofilm producer was 7%.

Isolates from the wound were 19% which distributed into 9.52 %, 12 %, and 29.78 %, strong, intermediate, and weak biofilm producers respectively. 19% isolates were collected from UTI out of them, 19% isolates were biofilm producers. 23.40% isolates were weak biofilm producers, 20% were intermediate, 14.28% isolates were strong biofilm producers. Non -biofilm producer was 42.85%. Out of 100isolates of *P. aeruginosa*, 23% were collected from ear infections, 19% isolates, 23 isolates were biofilm producers. Distributed into 27.65% isolates were weak biofilm producers, 20% were intermediate, 14.28% isolates were strong biofilm producers, and the non –biofilm producers were 2%. Two percent were collected from CF, and they were biofilm producers. Out of these 2 isolates, 4.25% isolates were weak biofilm producers. However, the TCPM is considered as the standard phenotypic test for the assessment of biofilm formation and it was the most specific test in the current study. It was also an easy test in the laboratory to detected biofilm formation in quantitative ways. Furthermore, the verification of the TCPM results is accomplished by using an ELISA reader which considerably decreases the subjective errors shown with other phenotypic tests.

In the present study, the TCPM strong biofilm formation in 21% of isolates. These results were higher than those by Panda et al, 2016 [29] who reported 11.00%. In a study performed by Jabalameli et al, 2012 [30], biofilm production has been observed

in more than 96% of the isolates which 22.9% were weak biofilm formers, 26% were moderate, and 47% were strong. The results of this study showed tht the biofilm producers have been observed in more than 93% of the isolates , which werer 25% moderate, while this study did not agree with our results 21% of strong biofilm and weak biofilm 47%.

**Molecular Identification of the local isolates**

Using housekeeping genes in the molecular analysis (e.i *16S rRNA*) led to the advancement toward rapid techniques diagnostic for the identification *of P. aeruginosa* isolates [31]. The PCR technique is a highly sensitive and fast tool utilized for bacterial detection is that highly conserved and unable to change over time, and provides a specific sequence to each type [32].

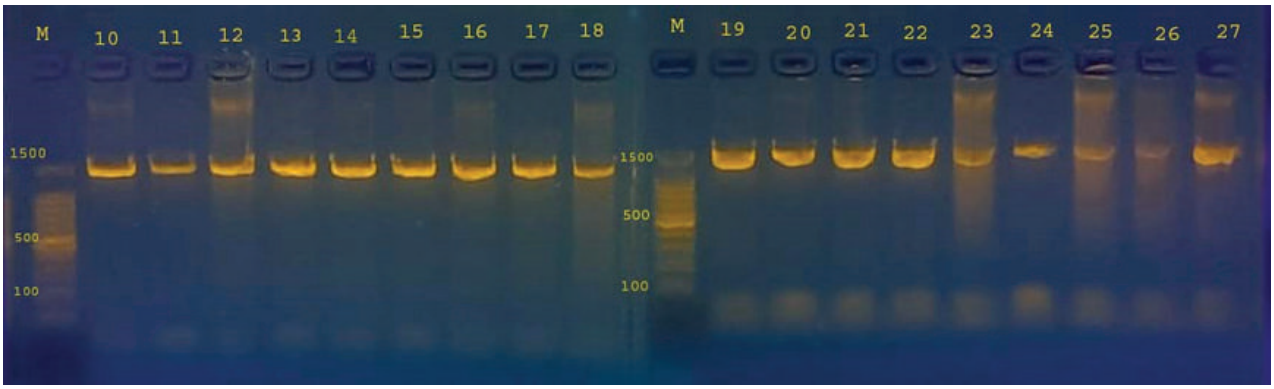


**Figure (3): Agarose gel electrophoresis (1.5%agarose, 7 V/cm$^2$for 90min) of *16srRNA* gene (1500bp). Lane M 1500bp DNA ladder, Lanes10-27 represent bands of *P.aeruginosa* isolates, stained with ethidium bromide and visualized on a UV trans illuminator.**

In this study, all isolates are diagnosed genetically by using a PCR technique depending on the *16S rRNA* gene. Figure 6 showsed agarose gel electrophoresis ( 90 min with 1.5% agarose ) for *16S rRNA* with PCR products (amplified size 556 bp). All of the isolates 51/51(100%) gave positive results during this genotyping test, where its bands appear at the same level in the agarose gel, this result matched with several studies such as [33][34]. Since the genetic diagnosis results of the *16S ribosomal RNA* were conforming to the morphological and biochemical test results; all bacterial isolates that were subjected to diagnosis were confirmed as *P. aeruginosa* isolates (Figure 3).

**Relationship between biofilm characteristic and genotype patterns among *P. aeruginosa* clinical isolates**

Biofilm phenotypes accounted for 93 % (n = 93) out of 100 isolates , being distributed in the following groups: 21% (n = 21) produced strong biofilm; 25% (n = 25) produced moderate biofilm; 47% (n = 47) produced weak biofilm, whilst 7% of isolates (n = 7) were identified as non-biofilm producer (Table 6). A high existence of biofilm-encoding genes were found, 73.89% (n = 17) of the isolates presented all three *algD*, *pslD*, and *pelF* genes, at the same time (considered as *algD* +/*pslD* +/*pelF* + genotypic pattern), 17.38% (n=4) presented only two gene *algD*, *pslD* (considered as *algD* +/*pslD* +/*pelF* - genotypic pattern), 4.34%(n=1) showed only one gene *algD* +( considered as *algD* +/*pslD* -/*pelF* - ), while 4.34% (n =1) had none of the three genes and identified as *algD* −/*pslD* −/*pelF* − pattern. The present study revealed a high prevalence of *algD*, *pslD*, and *pelF* genes, being presented simultaneously in a considerable percentage (73.89%) of *P. aeruginosa* isolates, the result is similar to those found by [35]. Other genes

related to biofilm formation, such as *pslA* and *pelA* were noticed by Ghadaksaz et al [36].with a rate of 83.7% and 45.2%, respectively, and pournajaf et al[37], with a rate of 89.5% and 57.3%, respectively within *P. aeruginosa* clinical isolates. However, minute data is available about the frequency rate of *pslD* and *pelF* genes in different areas of the world.

Chi-square analysis showed a highly significant association between strong biofilm capacity and genotype pattern ($X^2$= 25, d.f=1, p<0.0001), also the analysis showed a highly significant association between moderate biofilm capacity and genotype pattern ($X^2$= 12.42, d.f=2, p<0.002), and Chi-square analysis showed a highly significant association between weak biofilm capacity and genotype pattern ($x^2$= 13.77, d.f=2, p<0.001)(Table 6).

**Table (6): Relationship between biofilm characteristic and genotype patterns among *P. aeruginosa* clinical isolates.**

| Genotypic biofilm pattern, No. (%) | Phenotypic biofilm pattern, No. (%) | | |
|---|---|---|---|
| | Strong | Moderate | Weak |
| algD +/pslD +/pelF + | 2(8.69) | 5(21.73) | 10(43.47) |
| algD +pslD +/pelF − | 0(0) | 2(8.69) | 2(8.69) |
| algD +/pslD -/pelF - | 0(0) | 1(4.34) | 0(0) |
| algD -/pslD -/pelF - | 0(0) | 0(0) | 1(4.34) |
| P-value | 0.0001 | 0.002 | 0.001 |
| Total | 2(8.695) | 8( 34.782) | 13(56.521) |

In agreement with other studies byBanar, Ghadaksaz, and Kamali [17][36] [10][, which have shown a significant association between the biofilm-forming ability and the existence of related genes (p-value < 0.0001). The ability of biofilm production despite the absence of biofilm genes studied indicates other genetic determining factors of biofilm contribute to matrix development in *P. aeruginosa*[38,39,40]*]*. By similarity, the absence of biofilm production with the existence of genes may be due to chromosomal mutations in diverse regulatory and controlling systems, influence the production of efficient biofilm-related proteins. Other researchers reported that 31.03% of *P. aeruginosa* isolates contained the *pslA* gene and none of them were phenotypically positive for biofilm production in Congo red agar and microtiter plate assays[41]. Lima et al and Hou et al [42]reported that the mutations in *lasI/lasR* and *rhlI/rhlR* systems lead to phenotypical changes in quorum sensing proteins as the reason why these isolates are unable to produce biofilm[41][42] While, in another study, Abidi et al [43] reported that biofilm production was significantly higher in MDR isolates. In a study by Bogiel et al [44] on the *algD* gene's existence, 99 (92.5%) CRPA isolates were positive, these results are consistent with the results of the research carried out by Ellappan et al [45] showing that 92.9%. According to the findings of the current study, we can conclude:

the highest percentage of *P. aeruginosa* antibiotic resistance was 62% towards Ceftazidime(CAZ) and the lowest was 10% against Imipenem(IPM), most isolates of *P. aeruginosa* were the highest production of biofilm (93%). while few isolates were non-producer (7%), and there is a variation of genotypic patterns of biofilm production capacity *algD, pelFand pslD* in MDR local *P. aeruginosa* clinical isolates, the results showed that most biofilm producers were mainly considered as non-MDR.

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**Ethical Standard**: The study was formally approved the research plan by the ethical committee board at the Anbar health directorate.

**Informed Consent** was taken from all the participant patients before being enrolled in the study. **All data and materials are available**

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# The Effect of Long Storage of Whole Blood Components on the Level of 2,3 Diphosphoglycerate and Lactic Acid in the Blood Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia

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## Abstract

The purpose of this study was to analyze changes in levels of 2,3 DPG and lactic acid on WB storage time. This research is an observational analytical with time series design was conducted at the Clinical Pathology Installation and Blood Bank Dr. Soetomo General Hospital Surabaya in the period July - September 2020. Levels of 2,3 DPG and lactic acid were measured in 16 bags of Whole Blood components on the day 1, day 5, day 10, day 20 and day 30. Statistical analysis was performed using the Friedman test. The results were statistically significant if $p < 0.05$. The Friedman statistical test showed that there were significant differences in levels of 2.3 DPG ($p < 0.001$) and levels of lactic acid ($p < 0.001$) during storage. The results showed that the level of 2,3 DPG in WB which was stored decreased according to the duration of storage. The yield of lactic acid on stored WB increased with the duration of storage. Therefore, saving WB is recommended to be given within <6 days to reduce the risk of acidosis. Further research is needed for other parameters that can affect the storage process.

***Keywords:*** *2,3 diphosphoglycerate, lactic acid, whole blood*

## Introduction

Blood transfusion is an important part of modern health care. Blood transfusion is a series of processes for transferring donor blood to the recipient's blood circulation as a treatment effort. The main purpose of giving blood to a patient is to rapidly improve tissue oxygenation. Whole Blood component transfusion is used for resuscitation of patients with massive bleeding, hemorrhagic shock, and patients who need blood quickly.[1]

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The ideal WB transfusion is actually using fresh blood, which is blood that has just been taken from a donor until 6 hours after collection. The advantage of using fresh blood is that the clotting factors are still complete, including labile factors (V and VIII), the function of erythrocytes and platelets is still relatively good. Fresh blood is difficult to obtain because it takes more than 4 hours to check blood groups, cross reactions and transportation and the risk of disease transmission is relatively high.[2]

Fresh blood (fresh whole blood) is difficult to get, therefore WB transfusion can use stored blood. Storage of WB is recommended at refrigerator temperature (2-6°C) with storage time depending on the anticoagulant used. The advantage of using stored blood is that it is easily available at any time,

but the disadvantage is that clotting factors, especially factors V and VIII, have been used up and the ability of erythrocyte oxygen transport decreases. This is caused by a progressive decrease in levels of 2,3 Diphosphoglycerate (2,3 DPG).[3]

The decrease in the level of 2,3 DPG causes the release of oxygen in the tissue is low, resulting in tissue hypoxia. Blood with a low 2,3 DPG does not increase tissue oxygenation even though the hemoglobin level is raised. Blood with a low 2,3 DPG is not appropriate for patients requiring rapid oxygenation or resuscitation.[3] The level of 2,3 DPG is important to pay attention to in blood transfusions, because blood stored quickly can lose 2,3 DPG so that its ability to deliver oxygen will decrease.[4]

Whole blood (WB) during storage at the blood bank will experience a series of chemical changes that affect the viability and function of transporting oxygen from the lungs to the tissues. This change is known as a storage lesion. It is estimated that 1-5% of erythrocytes will be damaged during the time of donor collection and every day the erythrocyte viability will continue to decrease as a result of decreased levels of Adenosine Triphosphate (ATP). If the ATP level decreases, there is a loss of membrane lipids, the membrane becomes stiff and its shape changes from disc to spherical (without a central palor and small size), then this can cause potassium to leave and sodium to enter the cell. This lysis of red blood cell (RBC) causes a decrease in the RBC count and an increase in hemoglobin and iron.[5]

Erythrocytes do not have a nucleus and mitochondria so that energy is generated for oxidative metabolism through the breakdown of glucose. The breakdown of glucose into lactate or pyruvate is generally referred to as glycolysis. The process of glycolysis causes glucose to be consumed, resulting in a decrease in glucose levels. Glycolysis that occurs also results in the production of lactic acid by producing a low pH. Lactate, as an end product of red blood cell metabolism, will increase during storage. Transfusions of stored blood with increased lactate concentration by a mean concentration of 8 mmol/L during the first week of storage will increase metabolic acidosis.[6]

This research was conducted on the blood component of WB and measured the levels of 2,3 DPG and lactic acid at the storage period of the 1st, 5th, 10th, 20th, and 30th day. The selection of 2,3 DPG and lactic acid was based because they were the most influencing parameters for oxygenation to the tissues. The choice of measurement time for the day 1, day 5, day 10, day 20, and day 30 of measurements is based on the literature which states that the WB components are stored for 35 days at most to avoid the increased risk of contamination by bacteria.

## Method

This research is an observational analytical study with a time series design which was conducted at the Clinical Pathology Installation and Blood Bank Dr. Soetomo General Hospital, Surabaya, Indonesia. The samples were 16 units of WB components stored at refrigerator temperature (2-6°C) on storage day 1, day 5, day 10, day 20 and day 30. Samples were taken by consecutive sampling technique from July-November 2020.

WB which is stored on the 1st day at 2-6°C temperature is accommodated in a plain tube, as much as 3 ml by clamping the hose in the WB bag first then cutting the ends and accommodating in a tube as much as 3 ml. The hose connecting the WB bag is closed again using a heat sealer. A plain tube containing 3 ml of WB was centrifuged at 3000 rpm for 15 minutes. Plasma was removed and put into an aliquot. The aliquots were then stored in a refrigerator at -200C until 16 samples were collected. Measurement of 2,3 DPG was carried out by the competitive ELISA method based on the insert kit from Bioassay[7] and measurement of lactic acid was carried out by the enzymatic method based on the insert kit from Randox.[8] The remaining WB blood components are again stored at 2-6°C. The same

method used for the first day sampling was carried out for the fifth day, tenth day, twentieth and thirtieth day of sampling. Data analysis was performed using the SPSS statistical program. The statistical test used is the Friedman test. The result of the test is statistically significant if the p value is <0.05.

## Results and Discussion

The results of the normal distribution test using the Shapiro-Wilk test showed that the chlorhexidine gluconate group data was normally distributed (p >0.05), while the n-propanol and hydrogen peroxide groups were not normally distributed (p <0.05) (Table 1).

**Table 1. Characteristics of the Research Sample.**

| Group Blood | Whole Blood (Bag) | Amount (%) |
|---|---|---|
| A + | 6 | 37.5 |
| B + | 5 | 31.25 |
| O + | 5 | 31.25 |
| AB + | 0 | 0 |
| Total | 16 | 100 |

The sample of this study used 16 bags of Whole Blood (WB) components and the most WB bags were obtained with group A+ as many as 6 bags (37.5%) and no AB+ blood group was obtained as shown in Table 1.

Table 2 shows the results of the examination of 2.3 DPG on days 1, day 5, day 10, day 20 and day 30 of WB storage in the blood bank. There was a difference in the mean level of 2.3 DPG which was statistically significant at storage on day 1, day 5, day 10, day 20 and day 30 (p <0.001), namely the highest on day 1 (32.2 ± 11.2) compared to day 5 (30.0 ± 8.9), day 10 (28.4 ± 7.6), day 20 (27.1 ± 6.9) and day 30 (26.2 ± 7.2).

**Table 2. Comparison of 2.3 DPG Levels (n = 16).**

| Parameter | Range | Median | Mean | SD | P value |
|---|---|---|---|---|---|
| 2,3 DPG (µmol/ml)<br>Day 1<br>Day 5 | <br>23.5 - 58.7<br>22.4 - 56.8 | <br>28.5<br>27.0 | <br>32.2<br>30.0 | <br>11.2<br>8.9 | |
| Day 10 | 21.6 - 52.9 | 26.6 | 28.4 | 7.6 | <0.001 |
| Day 20<br>Day 30 | 21.3 - 51.7<br>19.5 - 51.4 | 26.2<br>25.2 | 27.1<br>26.2 | 6.9<br>7,2 | |

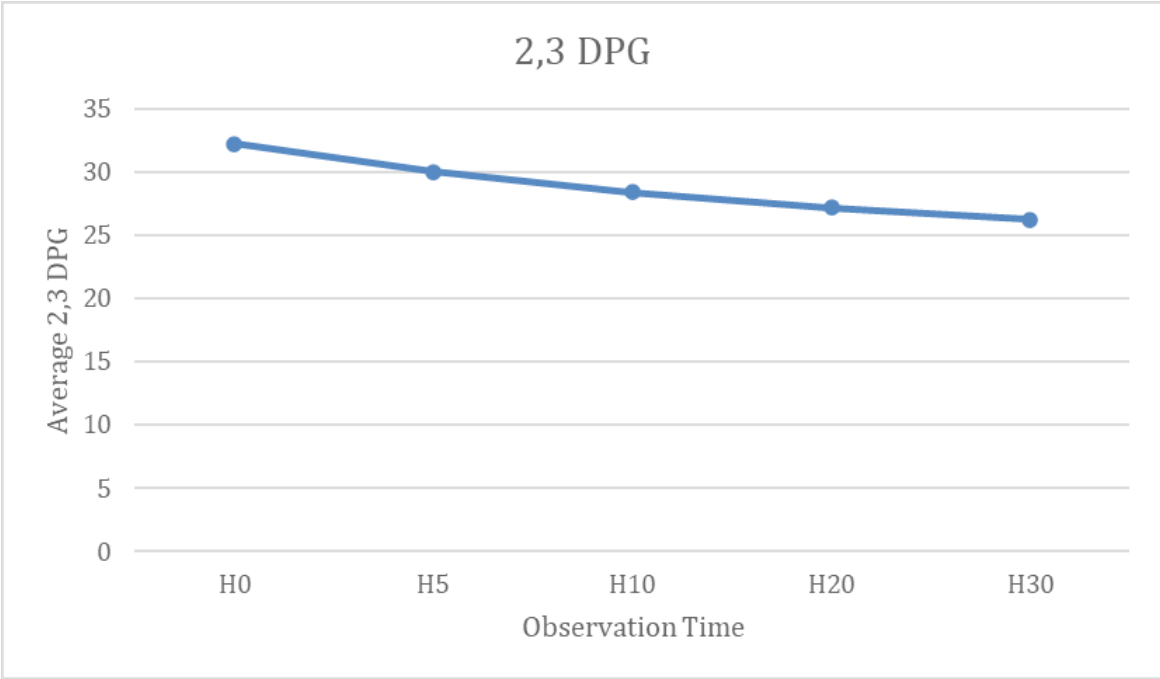


**Figure 1. Line diagram of the average 2.3 DPG levels between observation times.**

The results also showed that the levels of 2.3 DPG on day 5 were significantly lower than on day 1 ($p < 0.001$) and levels of 2.3 DPG on day 30 were significantly lower than on day 1, day 5, day 10 and day 20 ($p < 0.001$, respectively) (Figure 1).

Decreased levels of 2,3 DPG during the storage period, WB can be caused by the glycolysis process of red blood cells against WB components which are said to be the main cause of Red Blood Cell (RBC) Storage Lesions. During the storage period in the refrigerator, the temperature of 2-6°C red blood cell metabolism continues and glycolysis will also continue.[9,10]

Blood stored in the preservative Citrate Phosphate Dextrose Adenine (CPDA) and stored at a temperature between 2-6°C will undergo biochemical structural and functional changes that affect the viability and function of red blood cells due to differences in the atmosphere compared to invivo. These changes are called storage lesions, namely decreased concentrations of Adenosine Triphosphate (ATP) and 2,3 Diphosphoglycerate (2,3 DPG), decreased blood pH, increased potassium and lactate concentrations, changes in erythrocyte shape, loss of erythrocyte vitality, and hemolysis. 2,3 DPG is important for Hemoglobin (Hb) affinity for oxygen and for oxygen delivery to tissues. 2,3 DPG binds to deoxyhemoglobin and stabilizes it and facilitates the transport of oxygen from the lungs to the tissues by oxyhemoglobin. A decrease in 2,3 DPG leads to an increase in oxygen affinity for hemoglobin and therefore less oxygen delivery to the tissues.[11]

A reduction in 2,3 DPG levels in blood transfusion therapy should be considered because blood stored quickly can lose 2,3 DPG. However, the in vivo regeneration of 2,3 DPG after transfusion of depleted blood is a rapid process. The rate of in vivo regeneration depends on the quality and quantity of blood transfused as well as the state of the recipient.[12] In a study conducted by Juel reported that after 42 days of storage at $4^0$C, the red blood cell concentration of 2,3 DPG decreased only by 92% from its original level.[14]

Table 3 shows the results of lactic acid tests on days 1, day 5, day 10, day 20 and day 30 of WB storage in the blood bank. There was a statistically significant difference in the mean levels of lactic acid

at storage on days 1, day 5, day 10, day 20 and day 30 ($p < 0.001$), namely the lowest on day 1 ($4.7 \pm 0.6$) compared to day 5 ($8.4 \pm 1.2$), day 10 ($12.7 \pm 2.0$), day 20 ($19.1 \pm 2.2$) and day 30 ($22.2 \pm 1.8$).

**Table 3. Comparison of Lactic Acid Levels (n=16).**

| Parameter | Range | Median | Mean | SD | P value |
|---|---|---|---|---|---|
| Lactic acid (mmol/ml) | | | | | |
| Day 1 | 3.7-5.9 | 4,7 | 4,7 | 0.6 | <0.001 |
| Day 5 | 6.8-11.2 | 8,4 | 8.4 | 1.2 | |
| Day 10 | 9.9-16.6 | 12.4 | 12.7 | 2.0 | |
| Day 20 | 16.9-23.1 | 18.4 | 19.1 | 2.2 | |
| Day 30 | 19.3-25.2 | 22.0 | 22.2 | 1.8 | |

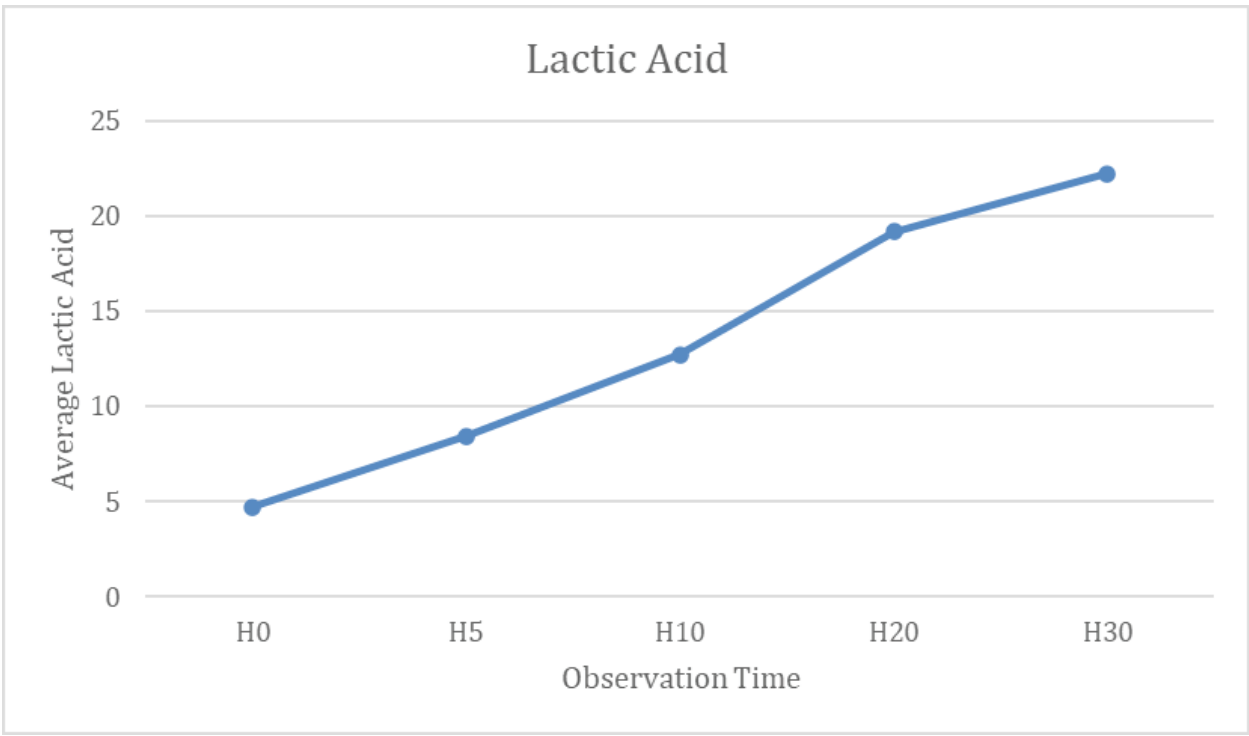


**Figure 2. Line diagram of mean lactic acid levels between observations time.**

The results also showed that the levels of lactic acid on day 5 were significantly higher than on day 1 ($p < 0.001$) and the levels of lactic acid on day 30 were significantly higher than on day 1, day 5, day 10 and day 20 ($p < 0.001$, respectively) (Figure 2).

The increase in lactic acid levels occurs during storage in the blood bank at a temperature of 2-6$^0$C due to glycolysis for ATP production and is associated with changes in pH. Blood stored for 35 days showed a higher lactic acid content than fresh blood.[15]

Glycolysis (breakdown of glucose) in normal red blood cell metabolism is the only source of energy for red blood cells. Red blood cells do not have mitochondria and depend entirely on glycolysis for energy needs. Red blood cells produce energy through the anaerobic glycolysis pathway, one glucose

molecule through anaerobic glycolysis produces ATP and pyruvic acid molecules, then pyruvic acid will be converted into lactate as the end product of red blood cell metabolism. Lactate as the end product of red blood cell metabolism will increase during storage.[16]

Increased levels of lactic acid in stored Whole Blood (WB) can affect the condition of the recipient's body or worsen the condition of the recipient's body experiencing acid base balance disorders.[15] Transfusion of stored blood with an increased concentration of lactic acid and an average concentration of lactic acid 8.0 mmol/l during the first week of storage, will increase metabolic acidosis.[13] The normal level of lactic acid in the blood is 0.6-1.5 mmol/l or 0-20 mg/dl and the level of lactic acid that can be accepted by the body is around >2,0-5 mmol/l or >18.02-45.05 mg/dl.[17,18]

## Conclusion

In summary, acid 2,3 DPG in stored WB decreased and lactic acid in stored WB increased with the duration of storage. It is recommended that WB transfusion be given to save WB blood <6 days so that the risk of acidosis is lower. Also recommended further research is needed for other parameters that can affect the storage process.

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# The Effect of Practical Exercises for the Technique of Ballistic Training to Develop Some Functional Capabilities of the Goalkeepers of the National Youth Football Team)

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## Abstract

The importance of the research lies by recognizing the importance of using the Ballistic training method, which is one of the important methods in explosive strength training and the characteristic force with speed, and this method works by overcoming other traditional strength training such as weight lifting, and overcoming the shortage in speed as a result of these exercises in a position, a set of special exercises for this method to train goalkeepers for the youth national football team, and the researcher decided to tackle the problem according to the following question:

The question? ... Do special exercises prepared by the researcher using the method of Ballistic training contribute to raising the level of development of some functional abilities of the individuals of a research specimen...?

The answer to this question is through the results that the researcher will obtain for all field procedures that he will prepare and implement on the research specimen, and the research aims to develop a set of applied exercises for the method of Ballistic training to develop some functional abilities of the individuals of the research sample, as well as to identify the rate of development Also, as for the imposition of the research, there were statistically significant differences between the results of the pre and post tests for the values of some functional abilities and in favor of the results of the post tests of the individuals of the research sample, and the researcher used the experimental method in a comparative method for one group, and the research specimen consisted of four Goalkeepers for the Iraqi national youth football team, and then the sample was tested with pre-tests of functional capabilities, after which the special applied exercises according to the Ballistic method were applied to the members of the research specimen, as the duration of their application lasted eight weeks with three training units per week, after which Post-tests were conducted on them, and the statistical application Statistical Package for the Social Science (SPSS) was used to obtain and discuss the results of tests of the goalkeepers. The special applied exercises according to the Ballistic method have a positive effect on the functional capabilities of the goalkeepers. The recommendations, the use of the Ballistic training method in the goalkeeper's position was emphasized because of its clear importance in the development and upgrading of the career capabilities of the goalkeepers in football.

***Keywords:*** *Ballistic training, effect of practical exercises, functional capability of goalkeepers, develop of functional capability of Athletics.*

## Introduction

The use of modern training methods and various sports sciences with the science of sports training leads to achieving the goals of training, as the science of physiology and sports training and their importance in achieving through the interconnectedness of the work of the nervous-muscular system and the cardiovascular system and how to develop them using the method of Ballistic training Which leads to raising the level of the player's performance, and football is the first popular game in the world and attracts the largest number of society because it is an interesting game.

"Since the football law allowed the goalkeeper in (1871) to use his hand in the penalty area, he became the distinguished player in the team. However, the ball law did not exclude him from any advantage over any other player in the team, as he can deal with the ball like any other player either. Inside or outside the penalty area in part of the stadium "(1), and due to the goalkeeper's use of his hands to block the ball, he plays a distinct role in the team's performance in all physical and functional aspects. That is why we see that the goalkeeper is distinguished from the rest of the players with some special specifications commensurate with the nature of his physical and functional performance from the rest of the team, and given his right to hold the ball in his hand inside the penalty area, his physical exercises for the hands are more than the players who are not allowed by the law of the game to hold the ball except in the side throw, and as for the skillful side, the goalkeeper uses his distinct physical abilities in Playing skills and planning performance, the nature of his role in staying inside the penalty area necessitates him to contribute a certain amount in the planning side, and the importance of research comes through recognizing the importance of using the ballistic training method, which is one of the important methods in training exercises. Explosive force and force characterized by speed and this method is also called the ballistic resistance method influencing the development of a set of special exercises for this method to train goalkeepers for the youth national football team and to identify the effect of these exercises in developing some of their functional abilities.

**Ballistic Training:** define as exam training to measure the ability of the muscles to perform movements at the maximum possible speed at weak and medium resistances ranging from (30-50%)" (2) and it is also expressed by "explosive movements against resistance at the highest possible speed" (3) that is, used "to overcome the resulting lack of velocity. One of the traditional weight training and includes ballistic training exercises (lifting weights with lightweight and at high speeds, medical balls, weight jacket, weight kit) (4).

**Anaerobic capabilities (functional capabilities):**"Anaerobic ability of football players is the basis for the physiological numbering process, and it means the ability of the muscles to perform muscle contractions at the maximum within a period of time from ten seconds to a minute or two, so that dependence is on the lactic system. In energy production, the anaerobic capacity also represents the ability of muscles to work under oxygen debt and bear performance under conditions of muscle fatigue resulting from the accumulation of lactate in the blood" (5), and the physical capabilities are divided into two types: (6)

1- Maximum anaerobic capacity: It is the ability to produce the maximum energy or work possible in the anaerobic phosphate system, and it includes all physical activities that are performed with the greatest speed or force and in the lowest possible time ranging between (5-10) seconds.

2-Anaerobic capacity: it is also called anaerobic endurance, which is the ability to maintain or repeat maximum muscle contractions depending on the anaerobic energy output of the lactic acid system, and it includes all physical activities that perform the maximum possible muscle contractions, whether fixed or moving while facing fatigue for up to a minute or

two.

## Objectives

1. Developing a group of practical exercises for the technique of ballistic training to develop some functional capabilities of goalkeepers for the national youth soccer team.

2. Identify the effect and rate of training development applied to the technique of ballistic training to develop some functional capabilities of goalkeepers, the national youth football team.

## Materials and Methods

**Methodology:**

The researcher used the experimental method by the comparison method for the one group by the method of pre-testing and post-testing due to its suitability to the nature of the problem that the researcher wants to address, as it is one of the most appropriate scientific approaches to solve the research problem.

*Research sample:* The research samples were selected by the deliberate method from the goalkeepers of Iraq national team for the youth category preparing to participate in the 2021 Asian Nations Youth Football Championship, of four goalkeepers and they represent (66.6%) of the original community of six goalkeepers who were summoned from the youth clubs participating in the league for the (2020-2021) soccer season in Iraq for these ages, and the researcher made procedures for their homogeneity in terms of height. And age, weight and training age.

To find the homogeneity of the members of the research sample, the researcher conducted the torsion coefficient in the variables of age, height, and weight Note Table No. (1)

**Table No.1: Arithmetic means, standard deviations and the median, and functions of the coefficient of torsion in length, weight, and age of the main experimental research sample.**

| # | Variables | unit | Mean | Standard deviation | Median | sequences |
|---|---|---|---|---|---|---|
| 1 | Biological age | 1 year | 19.25 | 0.957 | 19.5 | -0.855 |
| 2 | Weight | Kg | 73.5 | 6.455 | 75.00 | -0.892 |
| 3 | Height | Cm | 184.25 | 1.708 | 184.5 | -0.753 |
| 4 | Training time | 1 year | 5.25 | 0.975 | 5.50 | -0.855 |

Where it appeared that the values of the torsion modulus were distributed between (-1) and (+1), that is, there is homogeneity in the variables of age, weight, height, and training age within the curve of the normal distribution.

*Heavy Weight tools:* (Weighted bra, leg and arm lifts, elastic ropes, medicine balls, Vertie max machine)

(3-5) tests for the special functional abilities of soccer goalkeepers:

1- Short anaerobic capacity test (phosphogenic capacity) [7].

- Aim of the test: to measure short functional ability.

 Test description: The time taken to travel 30 meters is measured and the anaerobic capacity is measured.

$$\text{Anaerobic capacity} = \frac{\text{Body mass X 9.8 M. / S. X 2 X 30 M.}}{\text{T}}$$

 Recording: The values for the anaerobic capacity of each individual are extracted, and the unit of measure is Newton. M. / S.

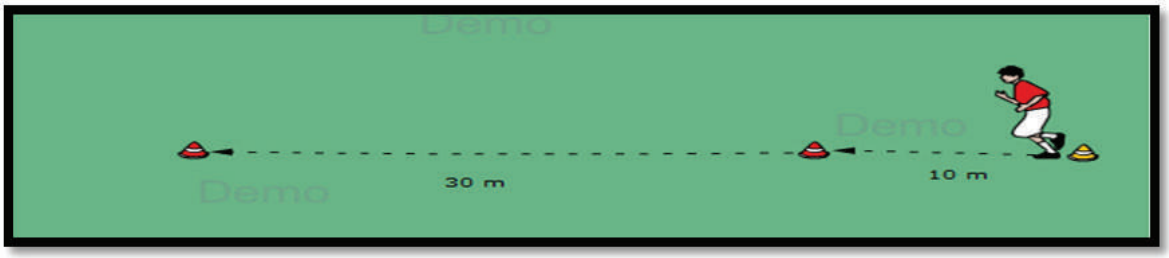


**Figure No. (1) shows the anaerobic capacity (phosphogens) test.**

2-Test of the average lactic anaerobic capacity (anaerobic capacity) [8].

The objective of the test: to measure the lactic anaerobic capacity for 60 seconds.

- Hardware and tools: 40 cm (15.75 inches) high box, electronic stopwatch, weighing scale, calculator.
- Method of performance: the tester stands facing the side of the box, and one foot is placed on the box (the leg preferred by the laboratory) while the other leg is free on the ground, and when the timing starts, the player begins to raise the free leg and place it next to the man above the box and repeat this performance in rhythm Two counters one-two (one up - two down) and the laboratory must perform the largest number of steps within 60 seconds, as shown in Figure (2).
- Conditions: The step is not counted if the tester bends the torso forward or bends the free leg.
- Recording method: the laboratory calculates the number of steps that it performs within 60 seconds, which is the time of performance, and the lactic anaerobic capacity is calculated by the following equation:

The unit is kg. Meters / minute.

To calculate the lactic anaerobic capacity in units of real power in watts, the result is divided by 6.12 Kg. M./ min. as (one wattage equals 6.12 kg. M. / min.)

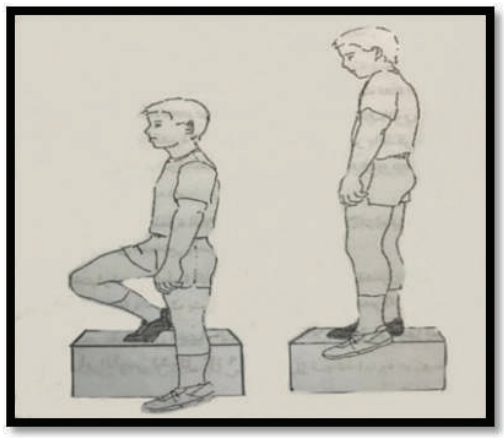

**Figure (2) shows the anaerobic (lactic) step test.**

*The exploratory experience:* An exploratory experiment on the functional abilities understudy on a sample consisting of four goalkeepers from the same community of origin and did not share the main experiment on (11/14/2020) and on the stadium (Captain Ali Kazem in the Ministry of Youth and Sports) and the purpose of that is he is:

1 Knowing the time required to carry out tests.

2- The validity of the means and tools used.

3- How ready the players are to carry out the tests.

4- The work team knows how to carry out tests, use tools, and diagnose errors.

5- Diagnosing and overcoming errors that appear in the exploratory experiment.

*Research pre-tests:* With the assistant work team to apply the functional pre-tests on (Thursday) 11/26/2020 and at 9 am in the stadium (Captain Ali Kazem, located in the Ministry of Youth and Sports), and the results of the tests were recorded in the previously prepared form.

*The main experiment of research:* The researcher developed a set of exercises according to the mechanism of the ballistic style exercises that includes special exercises for the goalkeeper as he deals with multiple variables in holding and blocking balls, whether in terms of moving by movement frequency and taking steps or through vertical and horizontal jumping to reach the ball and prevent it from entering the goal and estimating the distance to exert strength and move to catch or block the ball in addition to estimating the time required for moving to catch or block the ball. The modest being a former goalkeeper and a current coach, and the implementation was carried out under the direct supervision of the researcher and the in charge of the goalkeeper coach for the youth national football team. Corresponding to (20/1/2021).

*The mechanism for implementing the action road map for these exercises is as follows: -*

1- The number and ages of the research sample were determined, who are the goalkeepers of the youth national football team.

2- The appropriate time period for the preparation stages of this sample has been determined, and that it does not conflict with the curriculum for preparing this sample, which is the period of the second special preparation phase in the sports season for the members of this sample.

3- The time period has been set for two consecutive months and for a period of (8 weeks) to draw the training circuits therein and determine the number of these departments to suit the procedures of the training method used. Capacity under study.

4- The number of daily training units has been determined to suit the goal of applying these exercises to develop the capabilities under study and to suit the goal of the ballistic style exercises used for the biological effect of these units, and the number of units will be 24 daily training units.

5- The number of daily training units in one week has been determined, which are (3 units per week) for a period of eight weeks, as indicated by (Abu Al-Ela, 1996), that "the changes resulting from training mostly occur. During the first period of applying the exercises within 6-8 weeks "[9].

6- The time of the independent variable under study has been determined according to the application of plastic-style exercises, and the time will be (in minutes) for the total exercises implemented in one training unit, which is the time taken from the main part of the daily training unit, and the total time for applying this time will be throughout the period of applying these exercises In the preparation stage for soccer goalkeepers, it is (30 to 50) minutes multiplication by 24 daily training units the result would be (940,54 min.) the total time for training and applying the exercises set.

7- The number of exercises carried out in one training unit has been determined according to the application of the plastic training method, (6 to 8) exercises in each training unit, and after the end of the course, it is repeated according to the intensity and size of the training unit, and the rest period is between one exercise and another (2-3) Minutes and rests between each group (25-90) seconds.

8-The mechanism for implementing the ballistic training method has been determined by defining the rules for ripples of the load for exercises by implementing the repetitions and determining the mechanism for carrying out the training load according to the rule of daily ripple and the weekly wave to reinforce this mechanism in the rules of training this method in terms of size, intensity and comfort to target the development of capabilities understudy and avoid the phenomenon of overload.

9- The relative weights were determined in the ballistic training exercises according to the scientific foundations of the weight-loading rule in the Ballistic training method, taking into account the individual differences between the weights of the goalkeepers.

10- The principle of rolling and the gradual load was used in planning the training load during the competition time at a ratio of (1:2) during the period of the experiment with its three components (intensity, volume, comfort). the researcher relied on the principle of gradual training in the training volume up and down in the fourth week to be hospitalization and increase in the fifth, sixth and seventh week, and reduce it in the eighth week for the same purpose.

11- The principle of reducing the training volume and using the principle of the work-to-rest ratio (1:2) was used during the average menstrual cycle, as the training load was escalated in the first, second, and third week and decreased in the fourth week, and in the second month the same principle was also used.

12- The same exercises were used during the twenty-four training modules for learning and adapting to each exercise, and then the intensity escalated in the last week. The exercises also differed and difficulty was used in their performance in the second month.

*Post-tests for research:* were applied according to the approved mechanism for functional pre-tests, as it was conducted on (Monday), on January 25, 2021, 9 AM morning, in the stadium (Captain Ali Kazem, located in the Ministry of Youth and Sports), and the results of the tests were recorded in the form. Pre-prepared.

## Results

The statistical application (SPSS) to extract the values of the research variables.

(Arithmetic mean, standard deviation, median, coefficient of torsion, evolution ratio, mean variances, standard error, (t) test of corresponding samples).

The results of the functional tests under investigation were processed on the research sample by statistical means, and the results are shown to us below.

**Table No. (2): It shows the values of the arithmetic mean and the standard deviations of the pre and post-test of the investigated functional abilities.**

| # | Variables | Unit | Pre-test | | Post- Test | |
|---|---|---|---|---|---|---|
| | | | Mean | Standards deviation | Mean | Standards deviation |
| 1 | Short anaerobic capacity (phosphogenic capacity) | N*m/s | 1072.11810 | 465.2736732 | 11380.88985 | 670.4314487 |
| 2 | Medium lactic anaerobic capacity (anaerobic capacity) | Wat | 39713.10455 | 3887.708239 | 49229.55883 | 5313.098935 |

**Table No. (3): It shows the mean values of the differences, the standard error, the calculated T-value, and its significance for the physiological functional capabilities.**

| # | Variables | Unit | Mean differences | Standards deviation | T value | SIG | Field | Development percentage |
|---|---|---|---|---|---|---|---|---|
| 1 | Short anaerobic capacity (phosphogenic capacity | N*M/s | 656.77175 | 162.5566086 | 4.04 | 0.027 | moral | 6.12% |
| 2 | Medium lactic anaerobic capacity (anaerobic capacity) | Wat | 9516.45427 | 1647.669325 | 5.776 | 0.010 | moral | 23.96% |

Note: Each value in the significance field is less than (0.05), the differences are significant and with a degree of freedom (3)

## Discussion

When reviewing the results of the functional abilities tests shown in Table (3), as well as what the results of the T-test showed for the corresponding samples about the presence of a moral difference in favor of the post-test, and this is what the rate of development showed, and the researcher attributes the reason for these moral differences and to all the special functional abilities of the goalkeepers The surveyed respondents indicated the effectiveness of the effect of (special applied exercises according to the Ballistic method on goalkeepers) and used in the training curriculum on the members of the research sample, which was planned according to the scientific foundations, as well as the codification of the proper training load (intensity, size and comfort) commensurate with the characteristics and capabilities Individuals of the research sample, where the exercises were graded in difficulty and repetitions, the length of their implementation, which took eight weeks, and this gave the work to be scientifically studied and according to the individual and physiological capabilities that achieve functional adaptation to improve the physical and health level of the player and to achieve the requirements of the physiological preparation of goalkeepers In football, the preparation should be directed towards training functional abilities, as indicates that "when training functional capabilities, the direction of training must be determined. To train in terms of quantity, quality and style according to the basic system of energy extension "[10], and because the football game requires changing positions during matches, which gives the characteristic of diversifying the energy systems of the body between the air system and the anoxic system, and this is what he mentioned. The anaerobic ability of soccer players is the basis for the physiological numbering process, and it means the ability of the muscles to perform muscle contractions at the maximum during a period of time from ten seconds to a minute or two, so that the dependence on the lactic system in the production of energy, and the anaerobic ability also represents the capacity of the muscles To work under oxygen debt and endure performance under conditions of muscular fatigue resulting from the accumulation of lactate in the blood. "[11] As for soccer goalkeepers, he states. "The energy system used for a soccer goalkeeper consists of two anaerobic systems: (95%) phosphate (ATP-CP) and

(5%) lactic acid [12]. And to confirm the importance of these functional abilities for the soccer goalkeeper, the results of the research showed the development rates in} short anaerobic capacity (phosphogenic capacity), and medium lactic anaerobic capacity (anaerobic capacity), as regulating exercises prepared according to non-oxygen energy systems in line with a training method Ballistic and with the functional state that the research sample was subjected to helped to develop these functional abilities, as the exercises used in the method of training ballistic up to thirty seconds and thus the work of the anaerobic energy system is not oxygenated (phosphogenic and lactic) and this is what (Jamal Sabri Faraj, 2012) that "the most prominent characteristic of this method is the frequency of the explosive action upon performance, and the number of repetitions ranges (6-8 repetitions), which lasts from (20-30 seconds), and that the amount of resistance (30%) upwards is according to the training objective. Effectiveness requirements "[13].

## Conclusions

1- We conclude that the application of a set of special exercises according to ((the method of ballistic training)) and the mechanism of their construction had a direct impact on the development of some functional capabilities of football goalkeepers.

2- We conclude by how to deal with the components of the training load ((size - intensity - density)) and through field application of them in the total training units that were implemented and applied to the individuals of the research sample, which contributed to raising the level of development of functional capabilities.

3- We conclude that the mechanism of calculating the relative weights adopted for all parts of the body in the application of exercises in a ballistic method using the weights and caters for these weights for these parts for each guard from the members of the research sample according to their equations, contributed clearly and positively to the development and raising of their functional abilities.

4- We conclude that the development of some functional capabilities under consideration, namely ((phosphogenic capacity)) and ((anaerobic capacity)), which is a true reflection of the work and because of the mechanism of model rationing of the ripples of the components of the training load under consideration.

### Recommendations

1. The attention of all coaches working in the field of football specialization in the training curricula of the goalkeeper position because of its clear importance, and this center is considered one of the important centers for any football team.

2. That the appropriate and typical period be determined within the numbers of stages and how to define intermediate training courses to achieve the development of the functional capabilities under consideration.

3. Diversifying exercises and using models of different and new exercises to search for suspense and get away from boredom to apply single exercises in all training units.

4. The use of modern special and approved devices and tools according to the method used that contribute to the implementation of the exercises for the ballistic training method under consideration.

**Ethical Clearance:** University of Baghdad, College of Physical Education & Sport Sciences, and Iraq football Association.

**Source of Funding:** Self.

**Conflict of Interest:** Iraq Football Association.

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# Analysis of Physico-Chemical and Bacteriological Parameters of Liquid Effluents from the Provincial Hospital Center in Sidi Kacem Morocco

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## Abstract

**Background:** The effluents generated by the hospital activities and which are rejected into the municipal sewerage system without any prior treatment, present a chemical, biological and physical risk for public and environmental health. They cause a degradation of the aquatic ecosystem, and favor the propagation of antibiotic resistant germs. **Objective:** The aim of this study is to carry out physico-chemical and bacteriological analysis of the effluents of the provincial hospital of Sidi Kacem in Rabat-Salé-Kénitra region, Morocco, in order to evaluate the degree of wastewater pollution and to reduce its environmental impact.

**Methods:** 24 samples of wastewater were collected from the Hospital's main sewer according to Moroccan Norm NM 03.7.059 and transported to the Provincial Laboratory of Epidemiology and Environmental Health (LPEHM) for analysis. These analyses were conducted according to the requirements and methods recommended by Rodier 1996 and 2009.

**Results:** The results of the bacteriological analysis showed a very high bacterial load of fecal coliforms ($36{,}7.10^4$ CFU/100 ml), total coliforms ($40.10^4$ CFU/100 ml), and fecal streptococci ($21.10^4$ CFU/100 ml). The physicochemical parameters revealed a significant load of organic and mineral matter expressed in terms of nitrates (41,59 mg/l), nitrites (0,53 mg/l), sulphates (222,75 mg/l), ammonia (1,95 mg/l), electrical conductivity (3202 µs/cm), hydrogen potential PH (7.82), all of which are well above the upper limit stipulated by the regulations. The COD/BOD5 ratio (7.66) higher than 3 means that these effluents are hardly biodegradable.

**Conclusion:** These results show that hospital effluent is loaded with mineral, organic and bacteriological pollutants and requires a monitoring system and pre-treatment before they are discharged into the municipal sewers.

***Key words:*** *Hospital effluent, bacteriological analysis, physicochemical, environmental impact.*

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## Introduction

Water is much more than a simple human need. It is an essential and irreplaceable element for the continuity of life. Since the end of the last century, problems related to water scarcity and pollution have

arisen worldwide[1].

In Morocco, as in most African and Mediterranean countries, the scarcity and/or high cost of water and fertilizers make the use of raw wastewater for irrigation an alternative of choice for farmers. This is a very old practice throughout the world[2]. Every year, more than 7000 ha are irrigated directly with raw wastewater discharged from cities, or about 70 million $m^3$/year of wastewater reused in agriculture without any prior treatment[3].

This practice has a negative impact on the environment and human health. These impacts are further exacerbated when domestic wastewater receives special effluents such as hospital effluents. These have the reputation of being very dangerous because of the micro-organisms they could disseminate; the danger is rather linked to the antibiotic resistance of some bacteria. On the other hand, in quantitative terms, a 1000-bed hospital would be polluting as a city of 10000 habitants [4].

The hospitals have an important demand for drinking water, whereas in a domestic environment the consumption is 150 to 200 L per habitant per day, in hospitals the average value is 400 to 1200 L. This important consumption of water gives rise to large volumes of waste water loaded with pathogenic microorganisms [4].[5]

The hospital effluents are polluted waters produced by different medical units. They are represented by domestic, chemical, pharmaceutical and radioactive discharges, and also discharge from laboratory and care rooms. These different sources give rise to hybrid liquid discharges, both domestic and industrial, which are very specific to medical care and research activities[6].

Many biological and chemical contaminants in significant quantities are transported by hospital wastewater such as viruses, bacteria, endocrine disrupting compounds, radioelements, residual pharmaceuticals, and active residues of hygiene products and other molecules [7],[8]. Some of these substances persist even after advanced treatment of wastewater by sewage treatment plants, and can contribute largely to the spread of antimicrobial resistant bacteria and genes[6].

The municipal wastewater treatment stations are not designed to treat medicinal and biological waste. In this context, the problem of hospital effluent discharge is becoming increasingly important[9].

The liquid hospital discharges threaten the environment much more than urban effluents [10]. These discharges are nowadays in the center of the preoccupations of all the health actors and those of the environmental protection, because they constitute a potential source of the transmission of infections and epidemic diseases[11].

To confront the problem of hospital wastewater, the objective of this study is to analyses the physicochemical and bacteriological parameters of the liquid effluents in the provincial hospital of Sidi Kacem in order to make more conscious of the importance to treating them before they are discharged into the public sewage system, in the aim of reducing their environmental impact.

## Materials and Methods

### *Presentation of the study site*

Sidi Kacem is a medium-sized city in the northwest of Morocco in the Rabat-Salé-Kénitra region, its population numbers are 90000 (according to 2015 census). far from Meknes with (45 km), Tanger (210 km), Fez (85 km) and Rabat (120 km), located next to the Rdoom river coming from Meknes and crossing the great fertile plain of Gharb, the province of Sidi Kacem covers an area of 4060 $km^2$ (5,7% of the national area) [12].

The provincial hospital of Sidi Kacem is a multidisciplinary health establishment. It was built in 1986, on a total area of 7 hectares, with a capacity of 210 beds, organised in 7 departments and several

services, its water consumption is estimated at 260 $m^3$ / day with a rate of 1.90 $m^3$ / day / bed. The hospital's sewage network contains several collectors, draining the discharges of various departments.

***Type of study***

This is a prospective study carried out in the provincial hospital of Sidi Kacem with the objective of carrying out physicochemical and bacteriological analyses of the liquid effluents of the different hospital services

***Sampling methods and frequency***

This study was carried out in a period of 6 months from March the first to August thirty-one with a frequency of 4 samples per month, one sample per week every Monday at 12 o'clock, with a total of 24 samples. the samples were collected from the principal sewer, in appropriate bottles of 500 ml according to the Moroccan Norm NM 03.7.059[13] and transported an icebox maintained in a temperature of 4°C to the provincial laboratory of epidemiology and environmental hygiene (LPEHM) for analysis.

**Sample analysis:**

***Physicochemical analysis***

The temperature measurement was carried out at the time of sampling, while the other analyses were carried out at the provincial laboratory of epidemiology and environmental hygiene (LPEHM) according to the recommendations of Rodier 1996 [14] and 2009 [15]. The physicochemical analyses concerned the measurement of hydrogen potential (pH), temperature, electrical conductivity at 20°C, nitrate ($NO_3^-$), nitrite ($NO_2^-$), sulphate ($SO_4^{2-}$), ammonia ($NH_4^+$), suspended solids (SS), chemical oxygen demand (COD), biochemical oxygen demand (BOD5). A calibration for all parameters and a control of all factors affecting the quality of the analyses, were carried out according to the requirements and procedures described by the NM ISO 17025 adopted by the LPEHM.

## Bacteriological Analysis

These analyses were carried out according to the requirements and methods recommended by Rodier 1996[14] and 2009[15], and conjoint decree no. 2942-13 of 07 October 2013 [16]. The culture media used were prepared according to the norm and respecting rigorous procedures of quality. Before using the culture media, a control plate with a suitable temperature for each type of media was checked for 24 hours. a Regular and daily quality control of reagents, distilled water, materials and equipment, autoclaving operation as well as the ambient conditions of the environment (temperature and humidity measurement) was performed.[17]

Statistique Tools

All the information was entered and processed using the Excel program.

***Results and discussion***

***Physicochemical parameters:***

The results of the physicochemical analysis for each parameter are presented in the figures: 1, 2, 3, and 4. The values shown represent the average of four samples taken during the same month.

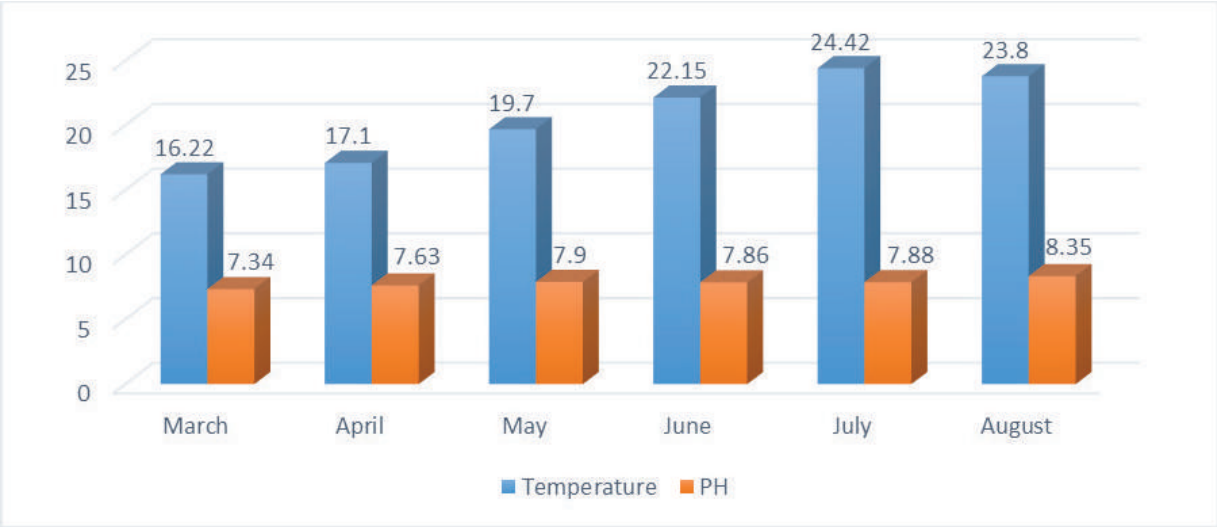


**Figure 1: Variation the average of temperature and pH values of hospital effluents.**

***Temperature***: In our study, the average temperature values of the wastewater from the different samples taken show a variation ranging from 16,22°C to 23,8°C, with a total average value of 20,56°C. This observed temperature variation is closely linked to the climatic change and the seasonal periods, and is suitable for the development of micro-organisms and the phenomenon of self-purification of wastewater. These results is conform to the WHO standards (1989) which fix the maximum temperature at 30°C. However, these results are comparable to those reported by : Touzani and al with a temperature of 20.79 °c[18] and Tahiri and al at El Ghassani Hospital in Fez with a value of 17.11 °C [19].

***Hydrogen Potential (pH):*** The analysis of the wastewater samples shows an average of pH values between 7.50 and 8.35. This interval is slightly alkaline, and can have harmful consequences for the environment, the suitable interval of pH for the aquatic environment is between 6 and 7.2. These results are in conformity with the Moroccan norms ranging between 6.5 and 8.5. However, the pH values reported are higher than those found by Touzani and al in Taza hospital[18] and similar to those found by Berrada and al [17]

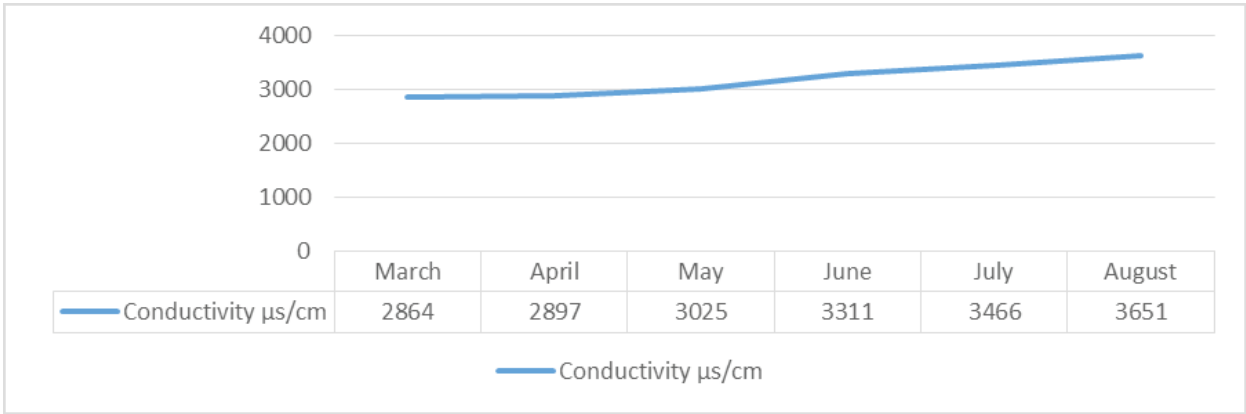


**Figure 2: variation in the conductivity during the study period.**

***Electrical conductivity (EC):*** The average values of electrical conductivity recorded in the wastewater during the six month study period showed values between 2864 µs/cm and 3651 µs/cm, these results exceed the upper limit stipulated by the regulation which is 2700 µs/cm[20], and similar to those found by Berrada and al with the values ranging from 3340 µs/cm to 3560 µs/cm [17], and El Mountassir and al with the values ranging from 5202 to 8940 µs/cm[21] . While Bouchaib and al [22] and El ogri and al [23] found a result below the norm, with 1500 µs/cm and 1195.60 µs/cm respectively.

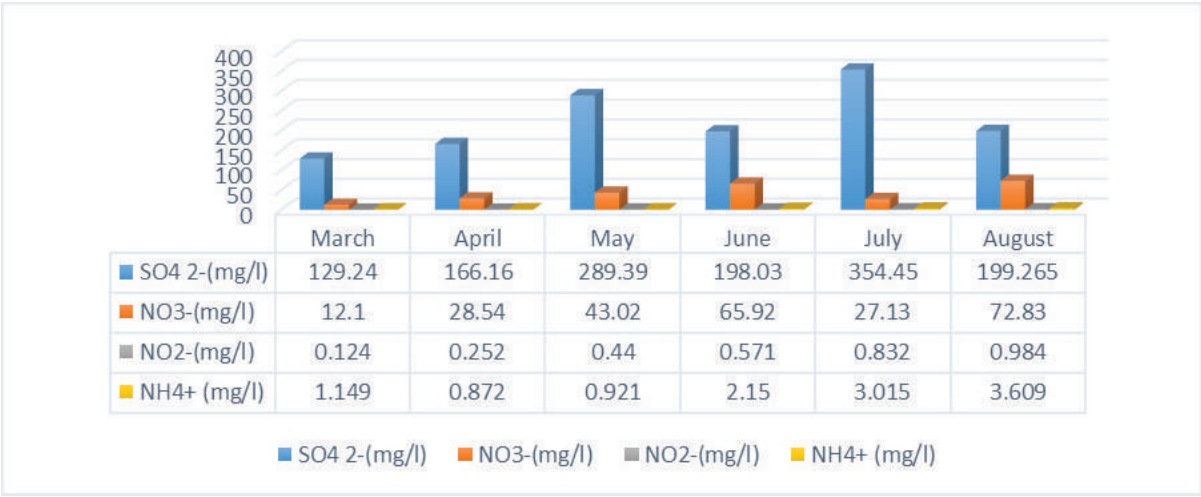


**Figure 3: Concentration of nitrogen compounds and sulphate in wastewater.**

***Sulphates ($SO_4^{2-}$):*** The average concentration of sulphates in the samples analyzed in our study ranged from 129.24 mg/l to 354.45 mg/l, with a total average value for six months of around 222.75 mg/l, these results do not exceed the limit value set by the regulations (500 mg/l)[16].

***Nitrates ($NO_3^-$):*** In our study, the nitrate concentration in the samples analyzed showed values ranging from 12.1 to 72.83 mg/l with a total average value of 41.59 mg/l. These results exceed the limit value set by Moroccan norms (30 mg/l) [16]. ***Nitrites ($NO_2^-$):*** The concentration of nitrites in the analyzed samples showed values ranging from 0.124 to 0.984 mg/l, exceeding the maximum value set by the national regulation which is 0.5 mg/l[16]. Similar results were found by El Mountassir and al [24] on the other hand Touzani and al found a result in conformity with the regulation[18].

***Ammonia ($NH_4^+$):*** The presence of a large quantity of ammonia $NH_4^+$ in water indicates an incomplete degradation process of organic matter, which is a contamination indicator of the water by human discharges, probably due to the transformation rate of urea into ammonia[25]. The results of the analyzed samples are between 0.872 and 3.609 mg/l, these concentrations are very high compared to the WHO standard of 0.5 mg/l, and are similar to that of Berrada and al with concentrations ranging from 0.935 to 4.03 mg/l[17]

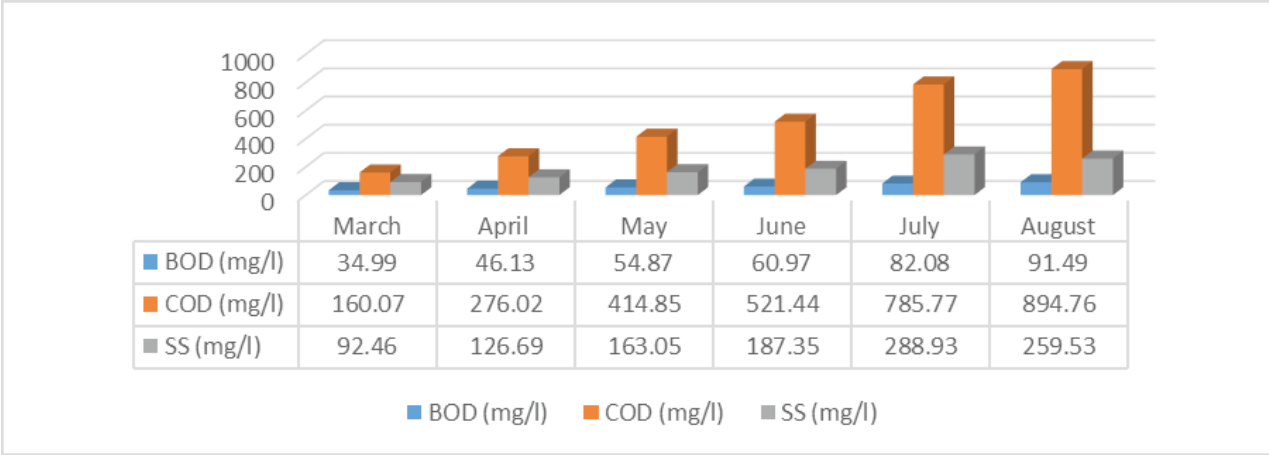


***Figure 4: Variation in BOD5, COD and SS concentrations of hospital effluent.***

***Suspended solids (SS):*** Represent all mineral and organic particles contained in the wastewater. The knowledge of the concentration of colloidal elements in wastewater is necessary to evaluate the impact of pollution on the aquatic environment[26]. The average values found in our study are between 92.46 and 259.53 mg/l, these values largely exceed the WHO standard which is 20 mg/l, and the Moroccan norm of 30 mg/l, these high concentrations can have a very harmful impact on the environment through the soil clogging and the reduction of the light penetration in the water [15]. These results can be explained by the increased hospital activity during the study period. Our results are similar to those found by Touzani and al with an average of 165.99 mg/l[18], Nourdine and al at Mohamed V hospital in Meknes with 424.25 mg/l [27].

***Chemical Oxygen Demand (COD):*** COD measures the quantity of oxygen necessary to oxidize the organic and inorganic oxidizable matter contained in an effluent[15]. The average values of COD recorded during the six months of the study show an evolution between 160.07 and 894.76 mg/l with a total average value of 508.81 mg/l, which largely exceeds the Moroccan norm fixed at 120 mg/l [16], and the WHO norm which is 90 mg/l, These recorded values are similar with those found by : El Mountassir and al with an average of 1593.5 mg/l[21], and El ogri and al with an average of 3901 mg/l[23].

***Biochemical oxygen demand (BOD5):*** BOD5 measures the quantity of biodegradable organic matter in the water. This biodegradable organic matter is evaluated by the consumption of oxygen by the microorganisms involved in natural purification mechanisms[1]. The results found show an evolution of the average values between 34.99 and 91.49 mg/l with a total average value of 61.75 mg/l in the six months of study, which is higher than the WHO standard of 30 mg/l, and comparable to other results found by : Touzani and al with an average of 488.11 mg/l[26], and Nourdine and al with a value of 737.5 mg/l[27].

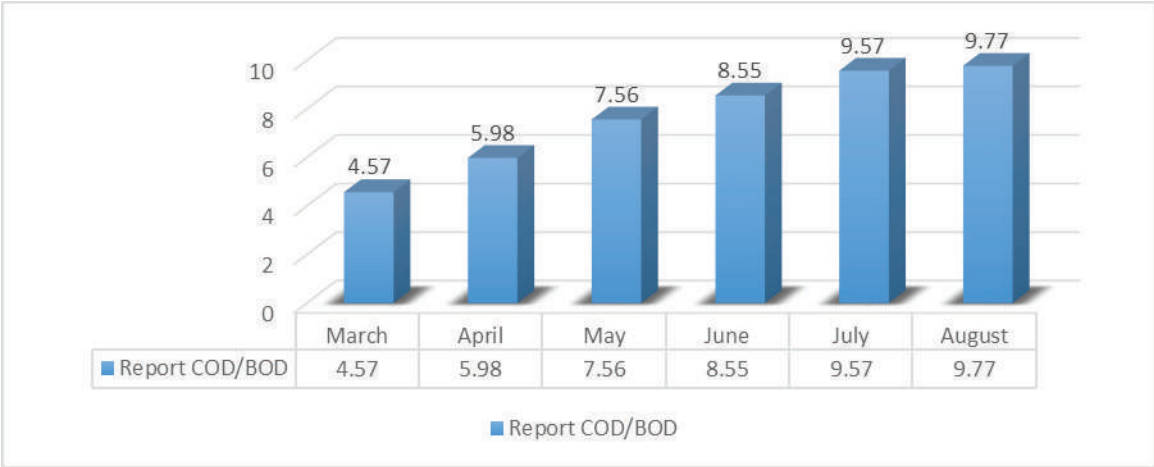


**Figure 5: Variation of the biodegradability report during the study period.**

***COD/BOD5:*** The COD/BOD5 report makes it possible to estimate the biodegradability of the organic matter, to determine the degree of pollution and to optimize the physicochemical parameters of effluent, in order to propose an adapted treatment [28]. In our study the result of the average values of this report is between 4.57 and 9.77, with a total average value of six months at about 7.66, this value higher than 3 means that these effluents are hardly biodegradable. Other studies have similar results: Sarhane and al with an interval between 6.5 and 8.21[29], and El Mountassir and al with values ranging from 5.7 to 10.85 [21], on the other hand Tahiri and al[19] and Touzani and al [18] found results lower than 3: 2.83 and 2.10 respectively.

**Bacteriological parameters:**

The germs sought, identified and counted in the effluents are firstly fecal coliforms, total coliforms, fecal streptococci, staphylococci and clostridium. The results obtained are presented in figures 6 and 7.

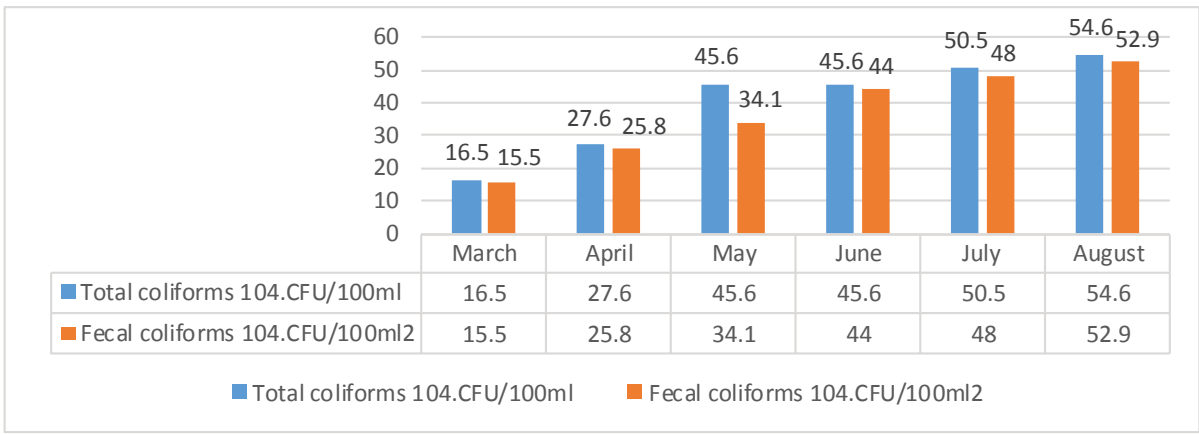


***Figure 6: Concentration of total and fecal coliforms in hospital effluent.***

***Fecal coliforms:*** The fecal coliforms could provide information on the ecotoxicity rate of the studied effluent. The concentration of fecal coliforms can be an indicator of the degree of water pollution and also an indirect indicator of the presence or absence of antibiotics or disinfectants.[21] In our study the average concentration of fecal coliforms found in the provincial hospital of Sidi Kacem is 36,7.

$10^4$ CFU/100 ml, largely exceeds the limit values recommended by the WHO of 1000 CFU/100 ml, is also comparable with different studies, for example: El Mountassir and al with values between 39,1.$10^4$ and 45.$10^4$ CFU/100 ml [21], Nourdine and al with an average of 7,44.$10^7$ CFU/100 ml[27], on the other hand Evens Emmanuel found a value of 2.$10^3$ CFU/100 ml[5].

***Total coliforms:*** The total coliforms are enterobacteria that include bacterial species that live in the intestine, but also in the environment (soil, vegetation and water). This bacterial group is used as an indicator of the microbial quality of water because it contains bacteria of fecal origin, such as Escherichia coli. [1] In our study, the samples analyzed showed the presence of total coliforms with a proportion ranging from 16,5.$10^4$ to 54,6.$10^4$ CFU/100 ml, with a total average value of 40.$10^4$ CFU/100 ml, this value largely exceeds the limit values recommended by the WHO of 1000 CFU/100 ml, and similar with other results: El Ogri and al with a value of 3,9.$10^7$ CFU/100 ml [23], Touzani and al with an average of 2,95.$10^4$ CFU/100 ml[18], and Tahiri and al with 2.$10^4$ CFU/100 ml[19].

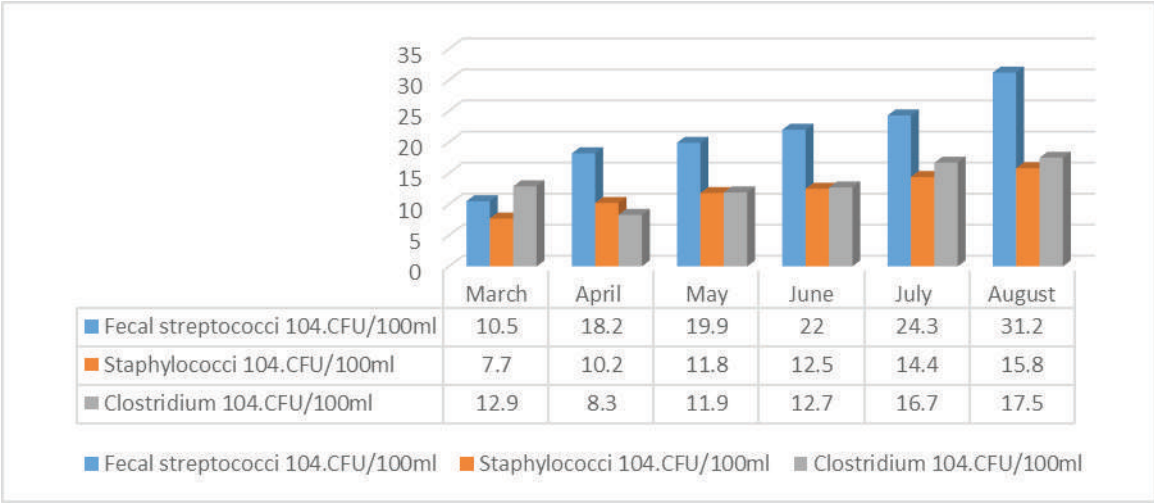


***Figure 7: Concentration of Fecal streptococci, staphylococci and clostridium in the effluent.***

***Fecal streptococci:*** In our study, the analysis of the samples showed an evolution of the values of fecal streptococci ranging from 10,5.$10^4$ to 31,2.$10^4$ CFU / 100 ml, this progressive increase in the concentrations of streptococci is justified by the nature of the care provided, the infectious diseases received and the increase in the flow of patients during the period of the study. The average total value was 21,$10^4$ CFU/100 ml. These results are similar to those found by: Nourdine and al with an average of 2,28. $10^6$ CFU/100 ml[27]. On the other hand, Tahiri and al found results between 1,1.$10^3$ CFU/100 ml and 2,1.$10^4$ CFU/100 ml[19].

***Staphylococci:*** The analysis of the samples revealed a concentration of staphylococci between 7,7.$10^4$ and 15,8.$10^4$ CFU / 100 ml, these results showed a progressive increase, with a total average value of 12.$10^4$ CFU / 100 ml, and comparable with those found by : Nourdine and al with an average of 3,6.$10^7$ CFU/100ml[27], and Boillots and al which is in the order of 6,08.$10^2$ CFU/100 ml[30]. This could be explained by the low consumption of water, cleaning products, disinfectants and detergents by the hospital[27].

***Clostridium:*** The analysis of the samples showed the presence of clostridium with proportions ranging from 8,3.$10^4$ to 17,5.$10^4$ CFU / 100 ml. These values show an evolution of the concentration during the study period. The total average value of clostridium

is in the order of 13,33. $10^4$ CFU / 100 ml. Similar results were found by Nourdine and al with a variation ranging from 10. $10^4$ to 60. $10^4$ CFU / 100 ml[27]. The clostridium is known for its high resistance to disinfection and they can survive in water much longer than coliforms. This resistance could explain their concentration in hospital effluents[21].

## Conclusion

In this study, it was found that hospital effluents are heavily loaded with physical, chemical and biological pollutants. We therefore hope, in the light of the results obtained by this study, to have generated a real awareness in the competent and responsible authorities to create a system for treating hospital effluents such as in developed countries. because the impact of these effluents on human and environmental health is very harmful and can lead to serious consequences.

**Conflicts of Interest:** All authors have no conflict interest to declare.

**Source of Funding:** The source of the research cost from self.

**Ethical Clearance:** Nil

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# Microbiological Study of Surfaces in the Hospital Environment Case of the Provincial Hospital of Sidi Kacem, Morocco

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## Abstract

**Background**: The hospital environment is largely colonized by pathogenic microorganisms. this contamination varies qualitatively and quantitatively according to the services and the capacity of the microorganisms to survive in inert environments, the elimination of these microorganisms requires a well formed staff and well detailed protocols which meet the international requirements. The microbiological monitoring of the hospital surfaces is a key factor to prevent nosocomial infections, it allows us to elaborate a diagnosis of the microbial ecology in order to lead preventive and corrective actions. **Objective**: The aim of our study is to identify the present bacteria on the surfaces of the provincial hospital of Sidi Kacem in Rabat-Salé-Kénitra region, Morocco, in order to reduce the rate of nosocomial infections.

**Methods**: 167 samples were taken from different sites in the hospital wards, using the swabbing technique, according to ISO/DIS 14698-1, the identification of the isolated germs has been realized by the classical biochemical gallery and the API (Biomerieux, France).

**Results**: A total of ten (10) bacterial species were isolated with a predominance of Bacillus sp (27%) and coagulase-negative staphylococci (29%), Staphylococcus aureus (18%), and Klebsiella pneumoniae (13%) followed. Thirteen (13) samples were a negative culture with a positivity rate of 92%. The distribution of isolated strains by department shows a predominance of bacterial strains in the intensive care unit (19%) and the emergency department (17%).**Conclusion**: These results show the importance of developing a risk management approach based on cleaning and disinfection protocols and procedures, as well as training and awareness programmers.

***Key words:*** *Hospital environment, Microbiological monitoring, Nosocomial infections.*

## Introduction

The nosocomial infections are a global health problem for their frequency, severity, and socio-economic cost. [(1)] They cause a significant amount of mortality and morbidity.[(2)] Their global prevalence is between 1 and 20%, and the global incidence is from 5 to 10%, with strong disparities between countries in terms of frequency and type.[(3)]

A study led by (WHO) world health organization in 2009 on the prevalence of nosocomial infections in four of the six regions WHO (South-East Asia, Europe,

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the Eastern Mediterranean, and the Western Pacific) has revealed that 8.7% of hospitalized patients have contracted a nosocomial infection.[2] The nosocomial infections in the developed countries affect 5 to 10% of hospitalized patients. In some developing countries, the rate is 25% of hospitalized patients.[4]

In the USA the nosocomial infections prevalence is 4.5%, in Canada 10.5%, France 6.7%, Belgium 6.2%, Brazil 19.1%, and 14% in Tunisia.[5]

In MOROCCO, the number of studies led in this field remains weak. Thus the national investigation on the prevalence of nosocomial infections in 1994 on 6584 hospitalized patients in 17 hospitals (2 university, 10 regional and 5 provincial) has shown an 8.1% global prevalence. This prevalence is 10.3% in the University Hospital Centre (CHU) Ibn Sina of Rabat (January 2010) and 12.4% in the CHU Hassan II of Fez (2009).[6]

The absence of risk management, preventive measures, and precautions against nosocomial infections in the Moroccan public hospitals might lead to severe health consequences, for patients the consequences can be emotional, physical, and social. For health staff: credibility loss, demotivation. For the organization: the health care cost augment due to overconsumption of resources (prolongation of hospitalization) and the alteration of its brand image.[6]

The hospital environment constitutes an ecological niche of microorganisms that can have a clinical significance, this contamination varies qualitatively and quantitatively from one establishment to another, and within the same establishment depending on the services, the patients, the care practiced, and the survival capacity of the microorganisms in the environment.[7],[8]

The microbiological monitoring of the hospital environment represents one of the major axes in the strategy to fight against nosocomial infections. It makes it possible to identify the pathogens responsible for infections.[9]

The direct involvement of the hospital environment in the occurrence of these infections is discussed and remains difficult to evaluate. Some consider its role as negligible, whereas others claim that it can be a relay in the cross-transmission and that the microbiological contamination of surfaces would favor the emergence of nosocomial infections in the most fragile patients.[10]

The aim of our study is to research the microorganisms and more specifically the pathogenic bacteria found on surfaces and medical devices in hospitals in order to evaluate the degree of biocontamination and to reduce the rate of nosocomial infections.[8]

## Materials and Methods

### *Frame and type of study*

This study is both qualitative and quantitative, about the microbiological monitoring of the hospital environment. in the provincial hospital center of Sidi Kacem with a total bed capacity of 210 beds. this study was carried out during a period of six months, from January the first, to June thirty 2020.

### Sampling points and technique

167 samples were carried out in different rooms in the hospital services. we have used the swabbing technique according to the ISO/DIS 14698-1 norm (Clean rooms, mastered environments and Biocontamination mastery), in the search for specific germs on flat surfaces and in areas which are difficult to access and not flat.

A sterile swab moistened in a sterile isotonic liquid was rubbed against precise flat areas in 25cm$^2$ of surface (floor, wall, trolley, door, night table, etc.) and non-flat areas (wrist, oxygen mask, tap, stethoscopes, etc.). The chosen points were the ones closest to the patients, health staff and visitors. The samples collected were quickly transported an icebox

maintained in a temperature of 4°C to the Provincial Laboratory of Epidemiology and Environmental Hygiene for analysis, in coordination with the provincial delegation of Sidi Kacem.

### *Sample analysis*

Every sample is immersed in a liquid nutrient broth (LBN) and incubated at 37°C for 18 to 24 hours. This broth is used to inoculate plates containing the blood agar, which are incubated at 37°C for 48 hours. the Colony count, aspect, size, and color of colonies were noted, and purification of every type of colony was performed by depletion of the PCA (Plate Count Agar) medium. this identification of the isolated germs has been realized by the classical biochemical gallery and the API (Biomerieux, France). The culture media, distilled water and reactants, materials, and equipment of sterilization, and other conditions, were regularly controlled according to the requirements of the ISO 17025 norm.

## Results

During our six (6) months study, 167 samples from different surface points in the seven (7) hospital services, were analyzed in the Provincial Laboratory of Epidemiology and Environmental Hygiene (LPEHM). Thirteen (13) samples were negative. A total of ten (10) bacterial species were isolated from different surfaces. The distribution of bacterial strains on the surface of the hospital services was represented in the table 1. We have noted the presence of the following bacterial flora: coagulase-negative Staphylococci (29%) and Bacillus (27%) were predominant, followed by Staphylococci aureus (18%) and Klebsiella pneumoniae (13%). Finally, Pseudomonas aeruginosa with 3%, Enterobacter cloacae (3%), E. coli (3%), acinetobacter sp (2%), Serratia rubidaea (1%), and Proteus vulgaris with 1%.

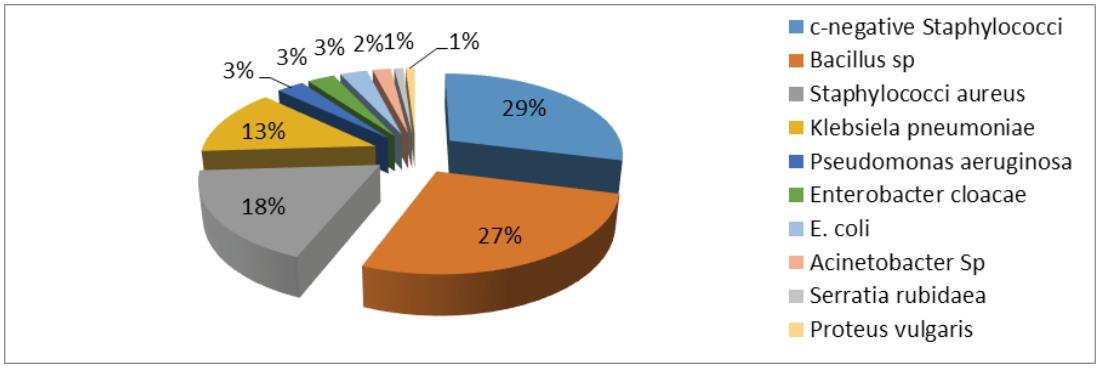


**Figure 1: Nature and percentage of isolated germs**

Figure 2 represents the distribution of the different bacterial strains isolated according to the gram coloration. We have noticed a predominance of gram- with a percentage of 70%, while gram+ represents only 30%.

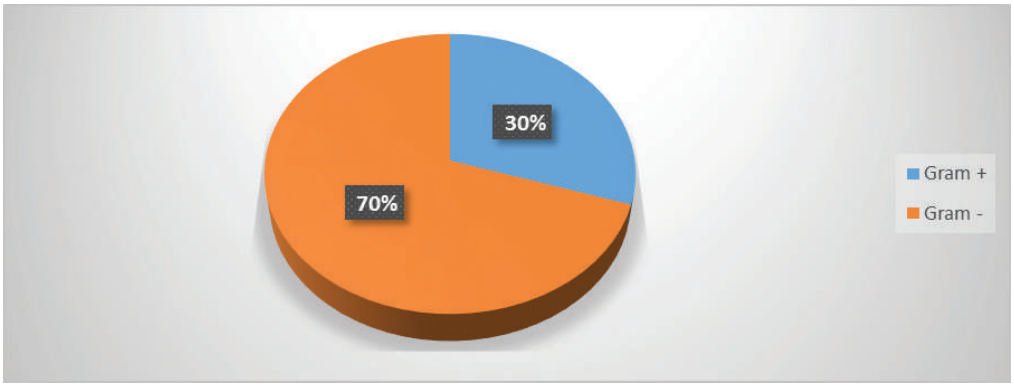


**Figure 2: Repartition of isolated germs according to the gram coloration.**

Figure 3 represents the distribution of the different isolated bacterial strains in the seven analyzed hospital departments. We have noticed a predominance of the bacterial strains in the intensive care unit (19%), and in the emergency department with 17%. 15% in each of the medicine and surgery departments, 14% in the maternity department, 12% in the pediatric department and 8% in the operating room.

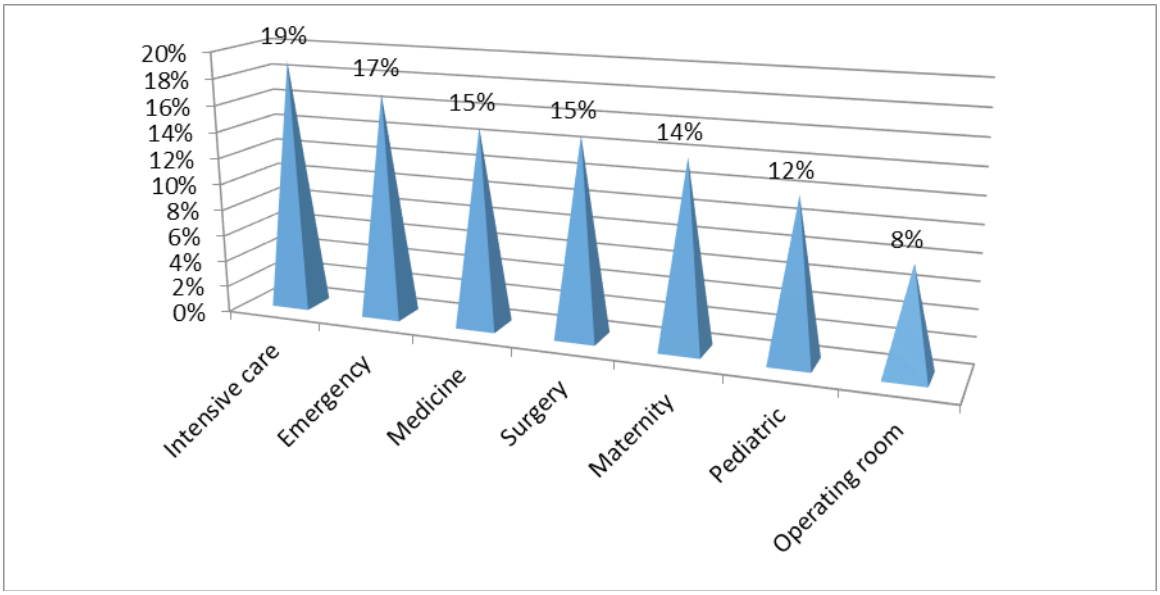


**Figure 3: Repartition of germs by department**

The microbiological study of the different surfaces sites of the seven hospital departments, has shown the presence of a varied percentage: from 23.52% to 40% of Bacillus sp, between 17.64% and 40% of coagulase-negative staphylococci, and 10.71% to 20% of Staphylococcus aureus, these three bacterial strains are present in all the departments studied. The Klebsiella pneumoniae is isolated in all departments except the Maternity and the Operating Room with a variation in percentage going from 9% to 29.41%. The Enterobacter cloacae has been found in the emergency departments with a percentage of 3.84%, 4.76% in the medicine and 13.63% in the surgery. Serratia rubidae is isolated only in the emergency department with 7.69%. The Pseudomonas aeruginosa and Proteus vulgaris were found only in the intensive care unit with 17.85% for the first and 7.14% for the second. E. coli and acinetobacter sp were isolated both in surgery with a percentage of 4.54% and 9.09% respectively, with the presence also of E. coli in the operating room with 17.64%, and 9.52% for the acinetobacter sp in the medicine department.

**Table 1: Percentage by department of isolated bacterial strains**

| Services / isolated germs | emergency | medicine | Pediatric | Surgery | Maternity | Intensive care | Operating room |
|---|---|---|---|---|---|---|---|
| S. aureus. | 19,23% | 14,28% | 17,64% | 13,63% | 20% | 10,71% | 17,64% |
| Proteus vulgaris | - | - | - | - | - | 7,14% | - |
| Serratia rubidaea | 7,69% | - | - | - | - | - | - |
| E.coli. | - | - | - | 4,54% | - | - | 17,64% |

***Cont...*** **Table 1: Percentage by department of isolated bacterial strains**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pseudomonas aeruginosa | - | - | - | - | - | 17,85% | - |
| Bacillus sp | 27% | 23,80% | 23,52% | 27,27% | 40% | 25% | 23,52% |
| Enterobacter cloacae | 3,84% | 4,76% | - | 13,63% | - | - | - |
| Acinetobacter sp | - | 9,52% | - | 9,09% | - | - | - |
| Klebsiella pneumoniae | 19,23% | 19,07% | 29,41% | 9,09% | - | 21,42% | - |
| coagulase negative staphylococci | 23% | 28,60% | 23,52% | 22,72% | 40% | 17,85% | 17,64% |
| TOTAL | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

## Discussion

The Nosocomial infections represent a major public health problem in the world nowadays, as they cause a heavy morbidity and a significant lethality.[11]

The hospital environment constitutes an ecological niche for microorganisms of various origins, which can come from patients, health care staff or visitors, from which a nosocomial infection can be developed. This contamination varies qualitatively and quantitatively from one establishment to another, within the same establishment according to the services, the patients, the care practiced and the survival capacity of the microorganisms in the environment.[8]

The microbiological monitoring of surfaces represents one of the principal axes in the policy for the fight against nosocomial infections.[12] In our study, a total of 167 microbiological samples were carried out and analyzed in the context of the microbiological control of the surfaces in the provincial hospital of Sidi Kacem. 92% of positivity rate was detected with only thirteen (n = 13) samples were negative. Other studies have shown a similar contamination percentage for example:

Meunier et al (2005) reported a positivity rate of 87%.[13] Saouide El Ayne et al (2014), in Kenitra (Morocco), reported a positivity rate of 92.85%.[12] El Ouali Lalami et al (2016) in the city of Fez found a percentage of 96.29%.[4] Touijer (2014) revealed a contamination percentage of 97.92% in a study realized in four hospital services in the city of Fez(Morocco).[14] Méité et al (2010) noted a lower positivity rate in Abidjan (Ivory Coast) than the one we found at 46.7%.[15]

In our study, the most contaminated points were the floor, the wrists, the walls, the beds, the bedside tables, the light switch and the trolleys of care have also presented a very high contamination rate. the reason why these objects cause contamination is that first, they are commonly used by health care staff, patients and visitors, and secondly, its related to not adequate of the cleaning and disinfection practices. we noted a visible lack of cleanliness as well as the presence of blood stains both on tables surfaces and on some mattresses.[10]

The analysis of our results has shown a predominance of coagulase negative staphylococci with a percentage of 29%, and Bacillus sp with 27%, followed by S. aureus with 18%, Klebsiella pneumoniae (13%). Finally, Pseudomonas aeruginosa with 3%, Enterobacter cloacae (3%), E. coli (3%), acinetobacter sp (2%), Serratia rubidaea (1%), and Proteus vulgaris (1%). The classification according to gram coloration has shown a predominance of Gram

(-) with a percentage of 70%, while the Gram (+) with 30%. Many studies have found similar results to ours namely:

The study of Saouide el ayne et al at El Idrissi Kenitra-Morocco hospital: which found out the following results: Bacillus 27%, and coagulase negative staphylococci at 26%, followed by Staphylococcus aureus (20%), Klebsiella pneumoniae (16%), Pseudomonas aeruginosa (5%), Enterobacter cloacae (5%) and Proteus vulgaris 1%.[12]

El Ouali Lalami et al. they found out the following results: Bacillus sp (30%) and coagulase negative staphylococci (24%). whereas Staphylococcus aureus, Aeromonas salmonicida and Lactobacillus, they respectively represent (9%) (7%) (6%) of the total flora analyzed.[4] A study in the hospital in Fez city, where bacterial species were isolated with variable proportions: Acinetobacter baumannii (33%), Pseudomonas aeruginosa (26%), coagulase-negative staphylococci (17%) and Klebsiella sp 8%.[14]

In 2005, Meunier et al. reported that Bacillus sp bacteria are omnipresent in the environment, and their resistance against bio-cleaning is strong, which can be explained by their ability to sporulate, and are constantly present in the environment. The same authors reported that the detection of bacteria such as coagulase-negative staphylococci, S. epidermidis, S. aureus, on the surfaces analysed, reflects a good human activity on the sites.[13],[4]

According to many studies, the most frequent germs responsible for the nosocomial infections are: Escherichia Coli (a frequent germ in digestive tract), Staphylococcus Aureus (a frequent germ in skin and nostrils), Pseudomonas aeruginosa, Acinetobacter (especially A. baumani), and Klebsiella pneumoniae. The particularity of these germs is that they have acquired a strong resistance to antibiotics, such as MRSA (staphylococcus aureus resistant to methicillin) or EBLSE (betalactamase producing enterobacteries with extended specter).[16],[17],[18]

In our study, the repartition of germs by services has shown a predominance of bacterial strains in the intensive care unit with (19%), and in the emergency department with (17%). The other services of medicine and surgery with (15%), maternity (14%), pediatrics (12%), and the central block (8%). In this context, other studies have found out similar results:

El Ouali Lalami et al found a predominance of bacterial strains in the emergency room with (19%), the central block (17%), the neonatal unit (16%) and the kitchen (14%). The Trauma service, intensive care unit, surgery and cardio-gastrological unit contain respectively 9%, 9%, 8% and 8%.[4] Saouide el ayne et al have shown a predominance of bacteries in the intensive care unit (28%), trauma unit (11%), emergency (10%) and operating block (8%).[12]

Amezian K. et al. have noticed that the intensive care units had the highest prevalence of nosocomial infection with 24.8%, followed by the pediatric service with 11.3%.[18] El Rhazi K. et al at Hassan II University Hospital in Fez, found that surgical site infections were the most frequent.[17]

All these studies have shown the presence of pathogenic bacteria in the hospital surfaces, which can cause infections for patients, health care, and visitors. From this analysis, we can conclude that the microbiological monitoring of the hospital environment is a strategic element in the fight against nosocomial infections.

The results obtained in our study, approved by previous studies, impose the implementation of a quality approach to risk management based on the following principles:

Ø Implementation of a cleaning and disinfection protocol for surfaces.

Ø professionals training in hospital hygiene and the care quality.

Ø Information, education and communication (IEC) for patients and visitors on hygiene rules.

Ø Formalize and standardize the microbiological monitoring of surfaces.

Ø detection of resistant strains by using antibiotic susceptibility testing of isolated bacteria.

Ø Testing the disinfection products on the isolated strains

## Conclusion

This study is realized with the aim to reducing the rate of nosocomial infections, and ameliorating the quality of care, our results have shown that the surfaces of the hospital services represent an ecological niche for pathogenic microorganisms, which can be the cause of hospital infections, which make it necessary to carry out a periodical microbiological control of the surfaces. the global rate of contamination is 92%, with a variation between the services. the principal bacterial strains isolated are represented by: coagulase negative Staphylococcus 29%, Bacillus sp 27%, Staphylococcus aureus 18% and Klebsiella pneumoniae with 13%.

The most contaminated surface points were the floor, wrists, walls, beds, bedside tables, light switch and care trolleys.

This work demonstrates the importance of preventive actions in the fight against nosocomial infections, thus the importance of establishing a global risk management approach based on simple preventive measures such as the formalization of cleaning and disinfection protocols and procedures, the training of healthcare professionals in hospital hygiene, and patient awareness.

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**Ethical Clearance** : Nil

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# Predicting Pandemic Curve Distribution Using Statistical Models

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## Abstract

This article discusses the distribution of pandemic in the world and pandemic curve in Jordan and how the science of probability and statistics predict when active cases tend to zero by determining the shape of epidemic curve and relating it to a special probability distribution that has specific measures and properties. At the beginning of the outbreak of any virus in a society, reliable data describing it and its spread will be rare, hence researchers set up statistical models that have the ability to predict the spreads' shape, where the prospected people hosting such viruses will go to and the likelihood of transmitting it to places they travel. Those models use known statistical measures that estimate the probability of disease transmission from infected people to others. In addition, the factors related to roads and people's movement, taking into consideration, public health interventions, such as wearing masks, closing places of people's aggregations like schools, universities mosques and churches and quarantine make difference in numbers of infected people. The fundamental differences between the "Spanish flu" that attacked the world a hundred years ago and "Coronavirus" the world facing since the beginning of the current year 2020 is the amount of huge data concluded from scientific studies and reports related to virology and epidemiology.

***Keywords****: Coronavirus, Pandemic Curve Distribution, Exponential Distribution.*

## Introduction

Nowadays, it is possible to track the increasing number of scientific searches day after day using available search engines that may serve as indicators of the imminent emergence of a specific epidemic in a certain region.

Data Science mainly has three packages of skills, science and knowledge, starting with algebra, statistics and probability, where such data related to the nature of the field we are studying are displayed, analyzed and decisions are taken based on obtainable data. Lots of researches working on collecting, showing and analyzing genomic data for the evolution of "Coronaviruses" helped in predicting the next mutation of the virus. Such researches considered as sources of people where such researches facilitate data and raise up levels of understanding of the epidemic.

Typically, the lack of data is worse than having some inaccurate data. Ali Abdel-Hadi, a professor of mathematics and founder of the Doctoral Sciences Program at the American University in Cairo, says: "The main data must be available first, for data science to play its role, specifically in two spatio-temporal scales, and then the basic variables, such as injuries, recoveries and deaths, are added" [1]. Statistical models can be used to gauge the effectiveness of government actions and measures. It can also be used to explore "what if?" Scenarios.

### Material and Methods

A statistical function which describes likelihoods and possible values that any random variable can take within a given interval (era) is a probability distribution that we depend on in our study. Such interval is bounded by the minimum and maximum values, but precisely number of factors (measures) force possible value to be plotted on the probability distribution. Such measures are: mean, median, mode, standard deviation, interquartile range, skewness and kurtosis. The most common distribution is the "Gaussian-normal" distribution which has a bell shaped curve. Now to interpret data generated by some phenomenon, we dictate its probability distribution which is called the probability density function or simply (pdf). Our methodology in this article depends on the probability distribution the pandemic curve has which leads to determine the passage of the pandemic and track when it increases and decreases and hence, when it tends to zero.

## Findings

The notion of data science unifies each of statistics, informatics, data analysis.

Probability and statistics besides data, scientific methods and processes used to get extract knowledge about the spread patterns of viruses help in giving alarm if viruses spread reached its maximum, hence such methods are necessity in each country. Phenomena like viruses spread have to be understood and analyzed. Everything concerning science changes since the impact of information technology is becoming huge.

### Spanish Flu

In 1918, the Spanish flu which is the most severe disease outbreak in human history, reached remote areas days before global air travel, and caused between 20 and 50 million deaths worldwide. The epidemic began in Spain after huge religious celebrations in Madrid in May and after few days, the disease spread an unprecedented spread. The second epidemic wave came in September during harvest celebrations and it was more mortal than the first one where governments at that time were easing prevention procedures. The end of the epidemic happened because the societies began to form general immunity that help each one to resist such epidemic and that generates the so-called herd immunity. "According to the results of seasonal influenza, we see that influenza the is the second most studied virus in the world, after HIV" [2]. If we take a look at the figure below, we notice that The curve of the epidemic displays, with its two parts, ascending and descending, is the movement of community members in which the epidemic is spreading between three different sections:

Susceptible, Infected and Recovered.

### The Stages of Epidemic:

1) This stage starts with the entry of the epidemic virus into a community of healthy susceptible individuals and since it is a virus with an epidemic spread, each infected person will transmit the infection to more than one case per day.

2) If the infected person transmits the infection to two other people, then on the next day the number of cases will be two, then on the following day it will be four, then eight, the next will be 16, then 32, and so on according to the "geometric sequence" $2,4,8,16,32,\ldots = 2^n 2,4,8,16,32,\ldots = 2^n$ that has a base (ratio) 2 as shown in figure 1 below [11], where "the exponential function with unending succession of numbers is increasing".

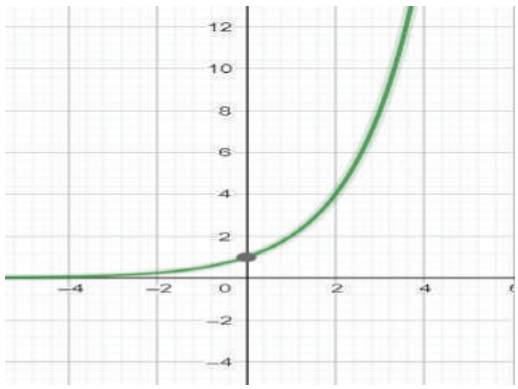



**Figure 1**

The geometric series of this sequence is: $\sum_{n=0}^{\infty} 2^n = 2 + 4 + 8 + \cdots + .. = \infty$ because it is a divergent (endless) series not convergent.

"When numbers of infected people start to descend, we realize that we left the summit of the curve and we are going gradually to the final stage of the epidemic wave where the geometric series became convergent" [5].

Increasing numbers of recovered people who passed the disease successfully or received vaccinations forces the epidemic curve to decrease gradually. According to probability distributions, we are talking about "log-normal distribution" which is "a continuous probability distribution with curve" shown in figure 2 below [4] where this curve represents a single epidemic wave. Such distributions have symmetrical or forms a bell shaped and 68% of data falls within one deviation about the mean and 95% of data falls within two deviations.

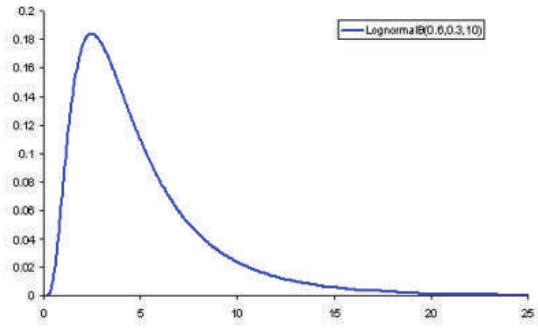



**Figure 2**

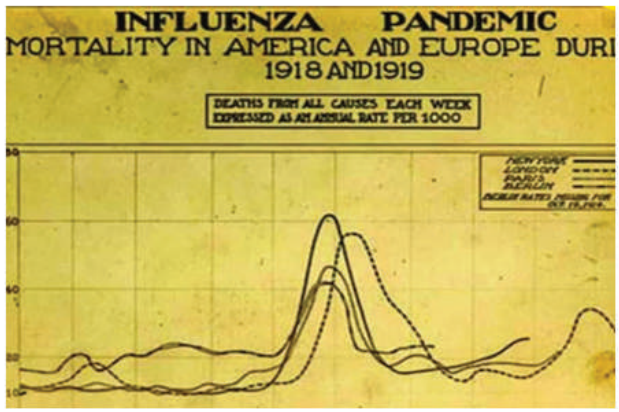
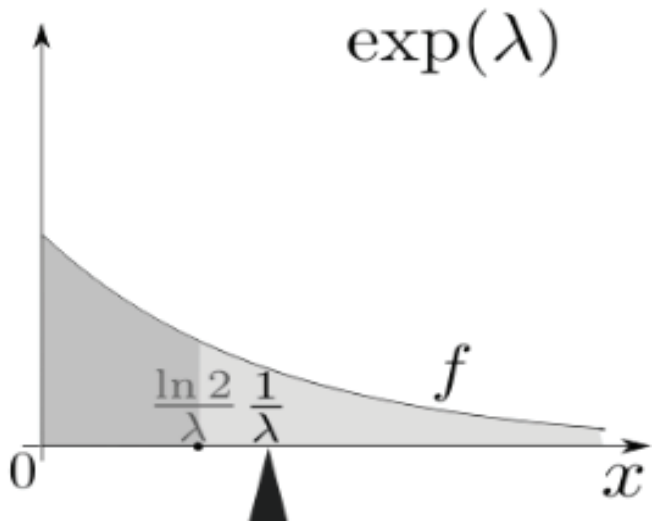



**Figure 3**

**Figure 3 [12] shows the "spread of Spanish Flu in 1918-1920".**

**Exponential Distribution**




The exponential distribution model is vastly used for products with constant failure or arrival rates. The most

featured thing when taking about it is the exponential probability density function (pdf) in which independent events occur at a constant rate. "Such distribution is used to model the time between independent events that happen at the average with a constant rate" [6].

"A random variable (denoted by $X$) is a role that associates to each outcome $\omega$ of an experiment a real number (denoted by $X(\omega)$), and "the probability density function (denoted by pdf $f(x)$) is a role that associates to each real number $X(\omega)$ a probability $P[X = x]$

$$f(x) = \begin{cases} \lambda e^{-\lambda x} & if\ x > 0 \\ 0 & if\ x \leq 0 \end{cases}$$

Where $\lambda > 0$ is a rate parameter" [7].

The expected value of a random variable X that represents number of infected people is given by $E(X) = \frac{1}{\lambda}$ and the standard deviation from the average $E(X)$ is given by $\sigma = \frac{1}{\lambda^2}$ " [8]. These two measures give us an idea that the pandemic curve is decreasing considering $\lambda$ a constant that takes different values .

Explaining the Curve

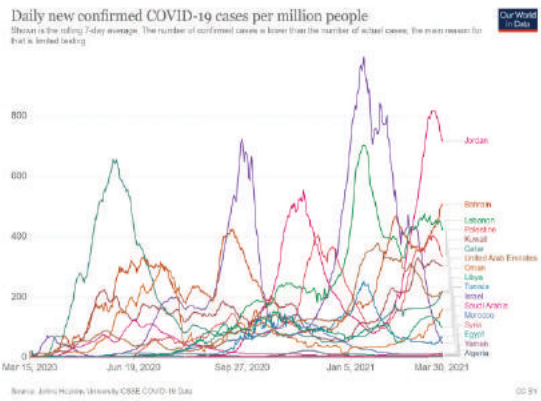



**Figure 5**

"The equation of the pandemic curve might not be familiar; its parameters are understood. The best description of it is given by: $f(x) = ke^{-\frac{(t-m)}{2w^2}}$ $f(x) = ke^{-\frac{(t-m)}{2w^2}}$

where $f(t)f(t)$ is the number of active cases per day, $tt$ is the time, $mm$ represents the day on which maximum $kk$ occurs where a constant $k\,k$ is the maximum number of active cases recorded per day, $w$ is the width of the curve at half $k$, and is given by:

Full Width at Half Maximum (FWH$k$) divided by $2\sqrt{2ln2}$ " [2].

We obtain "pandemic curves that display and analyze pandemic path from WHO statistics" [10] Such curves imply at different stages of the pandemic comparisons across countries and display different policies in many regions.

According to Jordanian Ministry of Health, "the pandemic curve after two waves till April, 2021 is shown in figure 6 "[13]

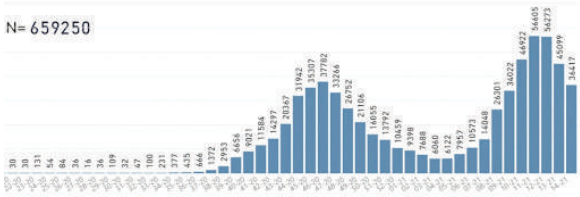



**Figure 6**

We notice that an extreme decline in the epidemiological curve in Jordan after witnessing a second wave of pandemic and The government has taken severe procedures to limit the spread of the epidemic and to treat the infected people and isolate them till they get well.

**End of Epidemic**

Typically, we found that the shape of the epidemic curve takes the exponential distribution and the spread of the epidemic is according a geometric sequence, limit of this distribution as time approaches infinity is zero, mathematically translation of this is:

$$\lim_{t \to \infty} ke^{-\frac{(t-m)}{2w^2}} = 0$$

According to a writer Gina Colata in the "New York Times" [3], historians noticed that epidemics have two types of endings: the first one is the medical end that occurs when infection and death rates decrease. The second one is the social end that occurs when the fear of disease disappears.

Before the Spanish flu vanished, it killed 20 million to 50 million people around the world, old and young and soldiers, in the midst of World War I. After it swept the world, the Spanish flu virus faded away and evolved into a different form of the milder flu that occurs every year. The pandemic is also socially over. After World War I ended, people were ready for a new era and they were eager to put the disease and war behind them.

**Comparison with other Diseases**

Deaths of Corona virus exceeded 3 million people around the world and 11381 in Jordan. These numbers are fatal compared to other viruses, nevertheless number of its victims till this moment is much less than the victims of Spanish flu. As the pandemic continues to spread, the death toll provides a "reference point" for comparing Corona with other viruses in the past and present. Twelve years ago, there were more between 151,700 and 575,400 people who are victims of the SARS virus that was the first coronavirus to cause panic in the world, but the total number of its victims did not exceed 774 people. Corona is compared with the deadly seasonal flu, even though the such flu scarcely appears in the headlines. "Seasonal influenza kills more than half million people annually", according to the WHO [PERDUE, NGUYEN]. Death toll caused by Corona exceeds that which is caused by the Ebola hemorrhagic fever, Ebola appeared in 1976 its last outbreak between 2018 and 2020 killed around 2300 people. Over forty years, a seasonal spread of Ebola killed 15,000 people throughout Africa. AIDS that was detected at the beginning of eighties of the last century is the deadliest disease among modern epidemics, as 33 million people have died around the world as a result of the disease that strikes the immune system, but till now, no effective vaccine has been discovered to prevent it. The death toll caused by hepatitis B and C viruses is so high, exceeding one million deaths every year, the majority of which are in poor countries.

## Conclusion

According to the distribution of the curve, we notice that after a steady increasing of active cases, the numbers started to decrees for the main reasons:

"Strict government procedures in dealing with the epidemic, people commit to social distance, people are becoming aware of the importance of vaccines and all of us try to adapt to this epidemic" [9].

**Decision**

At first, the spread pattern of Coronavirus was according a geometric series that is strictly increasing, and we noticed that the curve's distribution of the attacking virus is according log-normal distribution as implied in figures, then after a steady increasing of active cases of infected people and reaching the peak of a wave, the distribution of the curve representing active cases became exponential which means that the curve starts to decrease with a limit zero after a long time. The end of this pandemic is either socially or taking a long time and giving a huge number of vaccines.

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**Declaration of Competing Interest**

Authors declare that they have no

conflicts of interest to disclose.

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**Ethical Clearance:** Taken from Applied

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# Steeping Tin Leaves (*Ficus carica*) Improves Sperm Quality of Male Mice (*Mus musculus*) Exposed to Lead Acetate

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## Abstract

**Introduction**: Lead can induce lipid oxidation in cell membrans, thus forming free radicals. The process of imbalance of free radicals and antioxidants will disrupt the normal function of cells, causing cell death and decreased sperm quality. **Purpose**: The purpose of this research is to explain the mechanism of steeping tin leaves (*Ficus carica*) increase sperm quality in male mice (*Mus musculus*) exposed to lead acetate. **Methods:** This type of research was true experimental design with posttest only control group design with the number of replications of 10 male mice, the experimental unit will be distributed proportionally to 5 groups. Steeping tin leaves will be given with a dose of Pb + Tin Leaf 1.664 mg (P1) and Pb + Tin Leaf 3.328 mg (P2), while for lead acetate dose 0.5 mg and quercetin dose 0.7 mg. The analyzed variables included spermatozoa motility, spermatozoa morphology, and spermatozoa concentration. Data analysis was conducted including the Shapiro-Wilks normality test, and the homogeneity test used the Levene test. If the data were not homogeneous, the group average test would use the Brown-Forsythe test, then continued with a different test for each group using the Post Hoc Games-Howell test. If the Levene test data were homogeneous, the group average test would use Oneway Anova. **Findings**: The results showed that giving of steeping tin leaves with a dose of Pb + Tin leaves 3.328 mg (P2) is able to increase spermatozoa motility and spermatozoa morphology. **Conclusion**: The steeping tin leaves increase sperm quality.

***Keywords:*** *tin leaves (Ficus carica), sperm quality, motility, morphology, concentration*

## Introduction

Male reproductive function has declined since World War II in many countries. Several studies suggest that the incidence of testicular cancer, hypospadias, and cryptorchidism has increased and sperm quality has decreased in the last 50-60 years [(1)]. Several studies have identified men in North America, Europe, Asia and Africa, who show that there has been a significant reduction of 57% in mean sperm concentration over the past 35 years [(2)]. Rolland, et al [(3)] also reported a 32% decrease in the number of spermatozoa in men from 1989 to 2005. Rolf et al [(4)] reported that as many as 14% of men over the age of 40 had reproductive tract infections and lower sperm counts. than a 20 year old man.

Common causes of male reproductive disorders are impaired sperm production, blocked sperm transport system, health conditions and environmental conditions, as well as heavy metal contamination. [(5)] Some chemicals in the environment are known as Endocrine Disrupting Chemicals (EDCs), which are chemicals. which can interfere with endocrine function in the body [(6)]. Lead has the strongest influence on endocrine disorders in humans [(7)].

The human body naturally has a defense system against free radicals, namely intracellular endogenous antioxidants consisting of enzymes synthesized by the body such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [8]. If the production of ROS exceeds the existing antioxidant capacity, it will lead cells to oxidative stress, apoptosis or necrosis [9]. Therefore, the body needs an important substance, namely antioxidants that are able to capture these free radicals, so that these radical compounds become stable and cannot cause oxidative stress (imbalance between pyroxides and antioxidants) [10]. The antioxidant mechanism can occur through the binding of metal ions, oxygen scavenger, hyproperoxide decomposition into non-radical forms, absorbing ultraviolet light or deactivating singlet oxygen. A preventive defense system, the formation of ROS compounds and free radicals is inhibited by binding to metals or damaging their formation. This metal binding system occurs in the extracellular fluid. On the other hand, in intracellular fluid, ROS compounds and free radicals are damaged by enzymes [11].

Antioxidants of flavonoid compounds can donate hydrogen atoms to free radicals so as to produce stable low-energy radicals that come from flavonoid compounds that lose hydrogen atoms [12].

*Ficus carica Linn* is a plant that is rich in polyphenol antioxidants such as flavonoids, especially in fruit and leaves to prevent free radical damage, besides being a source of minerals, high in fiber, low in sodium and free of fat or cholesterol [13].

## Material and Methods

### Making Tin Leaf Steeping

Tin leaves were obtained from cultivating the tin plant "Bumi Tin" in Ngrawe Hamlet, Morosunggingan Village, Peterongan District, Jombang Regency, Indonesia. Tin leaves taken were 3-6 pieces below the shoot and dark green. Plant identification was carried out at the Indonesian Institute of Sciences (LIPI), Purwodadi Botanical Garden. A total of 70 plucked tin leaves were cleaned, washed, dried and kept out of the sun for 1 week. Then mashed in a blender to obtain 104.5 g of dry tin leaf powder. Diluted simplicia 104.5 g of dried tin leaves with 1400 ml of water, then bring to a boil and obtain a solution of 950 ml of tin leaves. After that, freeze dry was placed for 2x24 hours and obtained 18 g of tin leaf extract.

### Preparation of Experimental Animals

Adult male mice were taken from mice stock developed by outbreeding at the Center for Veterinary Medicine, Surabaya, with the criteria of being healthy, 2-2.5 months old, 30-35 g body weight, then adjusted to the environment for 1 (one). week, given to eat pellets at the place to eat and given a drink. After that, mice were grouped randomly into 4 (four) groups, namely:

a. KN group: The normal control group consisted of 10 mice without steeping tin leaves and without any exposure to lead.

b. Group K: The negative control group consisted of 10 mice without steeping tin leaves and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW.

c. Group P1: The treatment group consisted of 10 mice which were given tin leaf infusion at a dose of 1.664 mg / 0.01 kgBW per day and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW

d. Group P2: The treatment group consisted of 10 mice that were given tin leaf infusion at a dose of 3.328 mg / 0.01 kgBW per day and exposed to lead acetate at a dose of 0.5 mg / 0.01 kgBW

### Spermatozoa Motility

The sperm motility examination was done by dropping 1 drop of sperm suspension on a glass object, then examining it under a microscope with a magnification of 100 times. In each field of view the individual movement patterns of each spermatozoa

were observed. With a hand counter, out of 100 spermatozoa, the percentage of each movement pattern was calculated, especially progressive motion. Movement of spermatozoa was observed and categorized as follows [14]:

a. +++ = If the sperm is moving fast and straight forward (forward motion is very good)

b. ++ = If the motion is slow or difficult to advance straight or move not straight (weak motion)

c. + = If not moving forward

d. N = If the sperm does not move (Necrozoospermia)

**Spermatozoa Morphology**

Spermatozoa shape was called abnormal when there is one or more abnormal spermatozoa parts (head, midpiece, circular tail, small head, double tail), and the result was expressed as a percent. Normal mouse spermatozoa consist of a head (caput) that forms a hook-like tip, a short middle piece, and a very long cauda. The results obtained are calculated in value by the formula,

$$\frac{a}{a+b} \times 100\%$$

Information: a = number of normal morphology b = number of abnormal morphology [15]

**Spermatozoa Concentration**

Calculation of the spermatozoa concentration was done by taking spermatozoa from the cauda epididymis. Spermatozoa were put into the counting chamber of the haematometer until the room was evenly filled. Then count the number of spermatozoa in one of the counting rooms and then determine the dilution to be carried out and the number of squares to be counted. Calculation of spermatozoa concentration (million / ml) can be seen in table 1.

**Table 1. Spermatozoa concentration formula**

| No | Number of boxes counted | Spermatozoa concentration formula |
|---|---|---|
| 1 | 5 | n x 10,000 x 50 x 5 x 0.5 |
| 2 | 10 | n x 10,000 x 20 x 2.5 x 0.5 |
| 3 | 25 | n x 10,000 x 10 x 1 x 0.5 |

## Statistical Analysis

The data obtained were processed with the SPSS 17.0 for windows program. The data analysis in this study included the normality test using the Shapiro-Wilks test, and the homogeneity test using the Levene test. For non-homogeneous data, the Brown-Forsythe test was used to determine the mean difference between groups. To find out the difference in the mean between groups, a comparison test was carried out using the Post Hoc Games-Howell test. For homogeneous data, the mean differences between groups were tested by Anova.

## Findings

Figure 1 shows that the highest spermatozoa motility was in the normal group, namely 2.25, followed by the Pb + Tin leaves 3.328 mg (P2) group at 1.55, followed by the Pb + Tin leaves 1.664 mg (P1) group at 1.22, and the lowest at the Pb + Aquabidest group of 0.62. Statistical analysis showed that there was an effect of infusion of tin (*Ficus carica*) leaves on the sperm motility of male mice ($p < 0.05$). The results of the analysis showed that the spermatozoa

motility data were not normally distributed and homogeneous, then Anova test was performed, which showed differences in spermatozoa motility between groups (p <0.05).

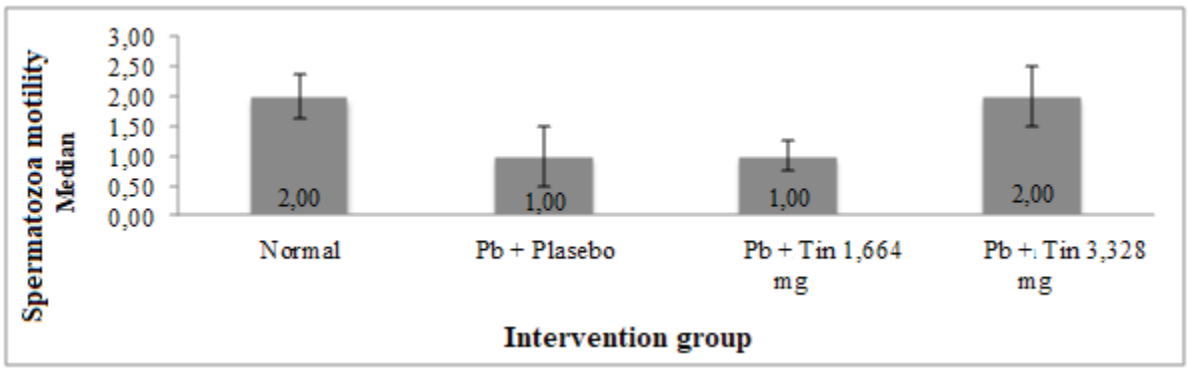


**Figure 1. Mean spermatozoa motility in cauda epididymis of male mice (*Mus musculus*)**

Figure 2 shows that the highest mean spermatozoa morphology was in the normal group, namely 77.00, followed by Pb + Tin Leaves 1.664 mg (P2) at 69.11, followed by Pb + Tin Leaves 1.664 mg for 62.66, and the lowest at the Pb + Aquabidest group of 45.12. The analysis showed that the spermatozoa morphology was normally distributed and homogeneous between groups. Anova test results showed that there were differences in spermatozoa morphology between groups (p <0.05).

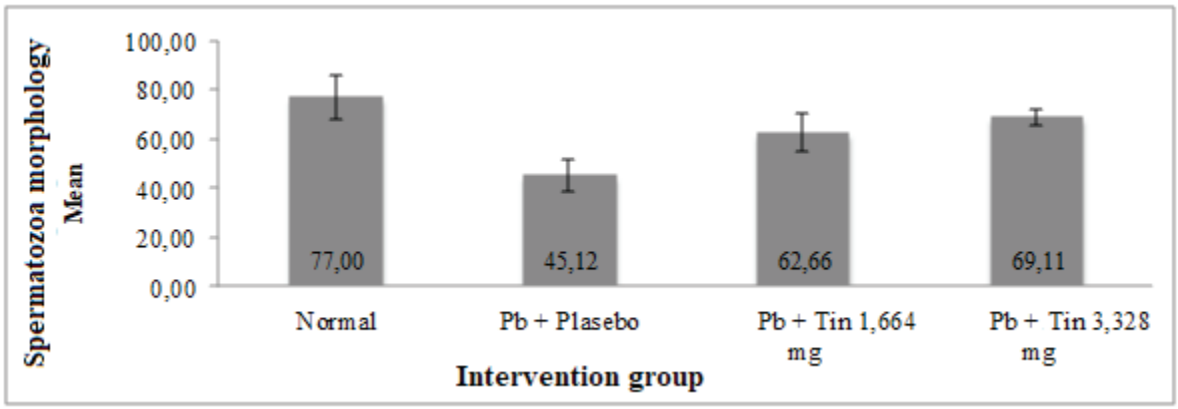


**Figure 2. Mean spermatozoa morphology in cauda epididymis of male mice (*Mus musculus*)**

Figure 3 shows that the highest mean spermatozoa concentration was in the normal group, namely 2.31, followed by the Pb + Tin Leaves 1.664 mg (P1), followed by the Pb + Tin Leaves 3.328 mg (P2) group of 1.44, and the lowest was the Pb + Aquabidest group of 1.26. The analysis showed that the spermatozoa concentrations were normally distributed and homogeneous between groups. Anova test results showed that there were differences in spermatozoa concentration between groups (p <0.05).

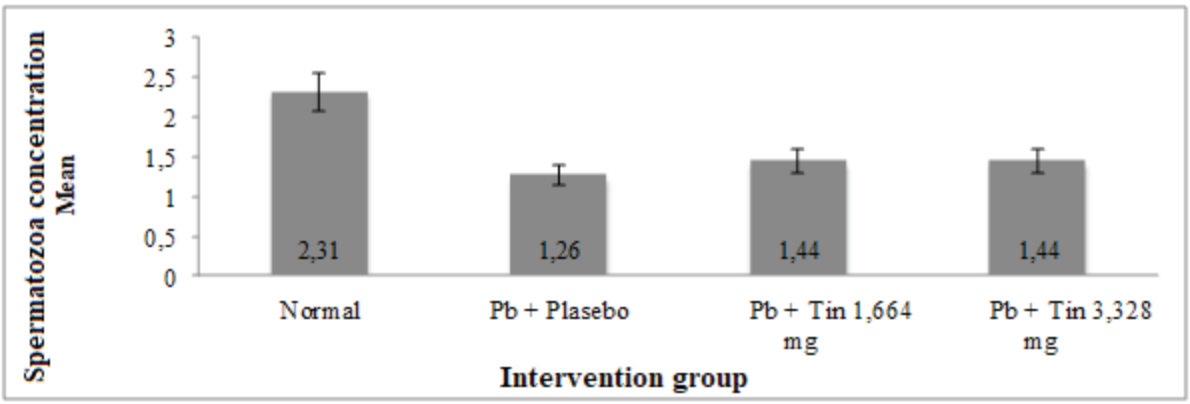


**Figure 3. Mean spermatozoa concentration in cauda epididymis of male mice (*Mus musculus*)**

## Discussion

### Spermatozoa Motility

Lead as a free radical can interfere with the activity of ATP-ase in the cell membrane. ATP-ase is located in the middle of the tail and functions to maintain internal homeostasis of the cell membrane [16]. Spermatozoa motility is highly dependent on ATP which is produced from oxidative phosphorylation in the mitochondral sheath. The movement of spermatozoa requires a certain amount of ATP which is used to move the flagellum apparatus. Impaired mitochondrial respiration function can lead to decreased motility and fertility [17].

In the biochemical system there is a balance between prooxidants and antioxidants, so that the body's tissues are protected from damage due to ROS [18]. When there is an increase in ROS levels, the body will respond by producing CAT, HPx and SOD enzymes to neutralize ROS. However, there is still some ROS left, especially if the ROS production is excessive. To reduce the remaining ROS, it is necessary to provide additional antioxidants such as vitamin C, vitamin E, uric acid, polyphenols (flavonoids), and others in order to minimize the effects of these ROS [19-21].

Polyphenol compounds have activity as antioxidants, as direct scavenger of free radicals. There are several components in tin leaves, and it is reported that caffeoylmalic acid (CMA) is the most abundant polyphenol in tin leaves, which exhibits antioxidant activity similar to vitamin C or catechins. Other antioxidants such as ubiquinone and beta carotene are fat-soluble antioxidants that will trap radicals on the lipoprotein plasma cell membrane. Apart from fat-soluble antioxidants, there are also a variety of water-soluble antioxidants such as ascorbic acid, uric acid, and polyphenol derivatives of plant origin [22].

### Spermatozoa Morphology

Lead has a tendency to catalyze oxidation reactions leading to the formation of reactive oxygen species (ROS). ROS can inhibit the production of sulfhydryl antioxidants, inhibit the production of heme antioxidants, damage nucleic acids, and inhibit DNA repair. ROS also induces lipid peroxidation reactions in cell membranes [23]. Oxidative damage to spermatozoa cell membrane lipids will change the fatty acid composition of the spermatozoa cell membrane, resulting in increased membrane permeability and damage to the spermatozoa membrane resulting in low spermatozoa membrane integrity [24]. Damage to the spermatozoa plasma membrane will result in disruption of the active transfer of substances that are a source of spermatozoa such as glucose, amino acids and fatty acids. As a result of the disruption of this mechanism, the spermatozoa will lack energy so that their vitality will decrease, as well as their motility.

Damage to the plasma membrane will also disrupt the balance of ions which are essential for spermatozoa [25]. In addition, lipid peroxidation can destroy the structure of the lipid matrix in the spermatozoa membrane and cause loss of motility and damage to the integrity of the spermatozoa membrane, decreased levels of ATP that reduce viability, causing axonal damage and increasing morphological defects in the mid-piece [26].

Tin leaf steeping contains polyphenols and tannins [27]. Tannin compounds have a role in phenolic compounds that produce antioxidant effects and protect lipids [28]. Polyphenol compounds can also increase the work of antioxidant enzymes in the body such as GSH which can convert $H_2O_2$ molecules and lipid peroxide into $H_2O$. The GSH enzyme in the cytoplasm will act on the phospholipid membrane which is oxidized by free radicals [29].

**Spermatozoa Concentration**

Every day hundreds of millions of spermatogonia cells are formed into spermatozoa in the testes through the process of spermatogenesis. Some of the spermatogonia cells are successfully processed into viable spermatozoa and intake, while some others fail to process, so they remain immature, defective or undergo apoptosis [18, 20]. As a result, the remaining spermatozoa concentration in the ejaculate is only half, or even less, with deformities or motility disorders, so it is called oligoasthonoteratozoosperm (OAT). Such failure of spermatogenesis is mainly due to oxidative stress, as a result of increased ROS or decreased antioxidants. Under normal circumstances, certain levels of ROS are needed to promote spermatozoa function such as hyperactivation, capasitation, acrosome reactions, and fertilization [30].

Tin leaves contain phenolic or polyphenol compounds which consist of several other types of compounds, namely simple flavonoids, phenolic acids, complex flavonoids and colored anthocyanins. Polyphenol compounds are able to inhibit oxidation reactions by donating one electron to an unpaired electron in free radicals so that the number of free radicals is reduced. Phenolic compounds include a variety of compounds derived from plants, which have the same characteristic, namely aromatic rings containing one or two hydroxyl groups. Flavonoids are good reducing compounds, inhibiting many oxidation reactions both enzymes and nonenzymes [31]. Flavonoids act as good reservoirs for hydroxy and superoxide radicals, thus protecting membrane lipids against damaging reactions [32].

To maintain balance so that hemeostasis is maintained, both the reproductive tract and the ejaculate are equipped with a system to counteract the increased production of excessive ROS. A system consisting of enzymatic and non-enzymatic antioxidants functions to maintain an optimal and sensitive balance between antioxidants and prooxidants [33].

## Conclusion

Steeping tin leaves (*Ficus carica*) has the potential to increase spermatozoa motility, prevent spermatozoa morphological damage, and increase the concentration of spermatozoa in the cauda epididymis of male mice (*Mus musculus*) exposed to lead acetate.

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**Ethical Clearance** : This research has received ethical approval from the Health Research Ethics Commission, Faculty of Public Health, Universitas Airlangga, with number: 514-KEPK.

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# Causes of Post Surgery Disputes between Plastic Reconstructive and Aesthetic Surgeons with Patients

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## Abstract

Advances in science and technology and the ability, skills, and soul of the art of Plastic Reconstructive and Aesthetic Surgeons can change human organs according to the patient's wishes to improve one's appearance to become more beautiful or handsome. Aesthetic plastic surgery is not performed to treat physical disabilities, in contrast to reconstructive plastic surgery, which aims to treat physical disabilities. However, misunderstandings or failures can occur because Plastic Reconstructive and Aesthetic Surgeons are also human, so it does not give satisfaction to the results expected by the patient. Not infrequently, the misunderstanding peaked, resulting in a dispute between the doctor of Plastic Reconstructive and Aesthetic Surgeons with the patient. This research method is normative juridical research empirical. The research specification in this study is descriptive-analytical. Research conducted using secondary data sources that include primary legal materials in norms, basic rules, laws, and regulations. The results of this study explain that Plastic Reconstructive and Aesthetic Surgeons should not give an appointment or guarantee success on the surgical efforts that have been done because there are other factors beyond their power as a doctor. The doctor should explain the risks and complications that may occur. In addition, before surgery, it is recommended that a complete agreement be made with the patient, and the patient understands and understands the risks or possibilities that can occur postoperatively in the future. The doctor and patient agreement are made in full and detailed in written form before medical action is carried out by involving a notary public to ensure legal certainty, fairness, and benefit.

***Keywords:*** *Plastic Reconstructive and Aesthetic Surgeons, Patient, Dispute.*

## Introduction

Health is a human right that must be protected and is one of the elements of welfare that must be realized as referred to in Pancasila[1]. Pancasila is the basis of creating the articles contained in the Law of the Republic of Indonesia year 1945, reviewed from the sociological aspect, aesthetic plastic surgery in Indonesia, to improve the quality of life and benefit from science and technology for the welfare of patients or users of medical services contained in Article 28 C paragraph (1) of the Constitution of the Republic of Indonesia year 1945. Then, from the philosophical aspect, the decision to perform aesthetic plastic surgery is a human right by Article 13 of the Law of the Republic of Indonesia Number 39 of 1999, it is based on considerations that affect the atmosphere of spirituality, namely physical and

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psychological suffering, where aesthetic plastic surgery is considered to be able to treatsufferers, namely by increasing confidence[2].

The desire to be beautiful, handsome and young, is the desire of every human being[3]. Advances in science and technology and the ability, skills, and soul of the art of a Plastic Reconstructive and Aesthetic Surgeons can be used to change one's organs according to his wishes to improve one's appearance to support his work. Aesthetic plastic surgery is not performed to treat physical disabilities, in contrast to reconstructive plastic surgery, which aims to treat physical defects, such as lip clefts, while aesthetic plastic surgery enhances or enhances appearance[4]. This is greatly embraced by perfectionists. which caused them to want to achieve their wishes by going to the Plastic Reconstructive and Aesthetic Surgeons to perform surgery on him.

Plastic surgery is a series of procedures designed to restructure damaged or injured parts of the body, where the above types of operations include reconstructive plastic surgery[5]. While the surgery that changes the body part is less attractive to be better by the patient's wishes, to support his appearance, including aesthetic plastic surgery. To fulfill his wishes, the aesthetic plastic surgery patient went to the Plastic Reconstructive and Aesthetic Surgeons to perform surgery on him.

Plastic Reconstructive and Aesthetic Surgeons should be careful in providing medical services to avoid unwanted things[6]. However, misunderstanding or failure is often inevitable because the Doctor is also human, no matter the efforts that are fought, God is the determinant. Not infrequently, the misunderstanding peaked, so there was a dispute between the two sides. It is this failure that causes disagreement. Therefore it is necessary to look for what factors cause the dispute to arise[7].

## Research Methods

This research uses empirical normative juridical research method, a study conducted using secondary data sources that include primary legal materials in the form of norms, basic rules, laws, and regulations—then coupled with secondary legal materials, namely legal materials used to support primary legal materials[8]. In contrast, empirical approach methods are carried out by conducting interviews in the field. Data collection is conducted by studying documents from literature, collecting, reviewing, and processing literature data related to juridical aspects, aesthetic plastic surgery, and treaty notation, as well as interviews with the Chairman and members of the Indonesian Association of Plastic Surgeons (PERAPI), Nurses, Notaries, Aesthetic Plastic Surgery Patients. Data obtained from this study, researchers conduct studies or analysis of opinions from experts in various literature and writings, namely by studying, analyzing, and interpreting every data that has been collected Techniques of data collection in this study is conducted through two ways, namely field research and literature research and data analysis in this study are conducted qualitatively.

## Results and Discussion

The legal relationship between Plastic Reconstructive and Aesthetic Surgeons and their patients is that since the patient came to the Plastic Reconstructive and Aesthetic Surgeons expressed his desire to perform surgery to change the organs as desired[9]. The patient is a healthy patient who wants the desired "results," so the legal relationship is verbintenis resultaat because it appoints an outcome. However, because the medical procedure is invasive, which is highlydependent on a person's unpredictable condition, the therapy given to the patient is an "effort" of healing (*inspanning verbintenis*) so that the agreement becomes the right effort to achieve a certain result (*inspanning & resultaat verbintenis*)[10].

Doctors performing specific medical actions require medical approval or informed consent[11]. The patient will approve if he has been fully explained about the medical treatment to be completed. By the

autonomy of the patient, they have the right to decide their choice freely. If the patient refuses medical treatment, then the patient must sign the informed refusal[12]. For aesthetic plastic surgery patients, a complete agreement is required, as it appoints a specific result. To create this agreement to be used as a guideline, it must be regulated in the laws and regulations.

In today's global era, the tendency to appear attractive to both men and women is an essential factor. This appearance is a concern, among others, for people who work as celebrities, presenters. Even today, the tendency to appear attractive has penetrated all circles. This is evident from the results of the author's interview with the Plastic Reconstructive and Aesthetic Surgeons that the patients are not only from celebrity circles but also from socialites, officials, academics, homemakers, and even laypeople who come from labor to household assistants have begun to take advantage of the expertise of Plastic Reconstructive and Aesthetic Surgeons. One of them is due to the promotion in the mass media that promotes the success of this aesthetic plastic surgery[9].

One way patients can be more attractive, they go to the Plastic Reconstructive and Aesthetic Surgeons. This causes people to be attracted to Plastic Reconstructive and Aesthetic Surgeons because of the number of patients interested in aesthetic plastic surgery to look beautiful, handsome, attractive, and charming[2]. Therefore, Plastic Reconstructive and Aesthetic Surgeons become very important to avoid unwanted things and always pay attention to the prudence in providing services. However, even if it has been done carefully, any action or work done by everyone is certainly successful, and sometimes it does not work, especially when it is medical matters related to the condition of one's body that can not be predicted[13]. Especially when it comes to surgical actions that have to do with anesthesia or anesthesia and one's resistance to medication, it is fraught with risks.

The medical action performed by the doctor in this study is Plastic Reconstructive and Aesthetic Surgeons, basically always resulting in 2 possibilities of success and failure. The failure of a Plastic Reconstructive and Aesthetic Surgeons to perform medical procedures is caused by several things, among others, caused by overmatch (force majeure), the second is caused by reconstructive plastic and aesthetic surgeons performing medical procedures that are not by the standards of the medical profession or the standards of the medical profession.

Health services have a distinctive characteristic that is different from other services, patient ignorance of treatment, lack of bargaining power, and selectability of other service products. Especially when it comes to special skills, such as aesthetic plastic surgery, patients usually do not know what risks will occur. Therefore a detailed explanation is required, which must be notified before the operation is performed. For this explanation to be forgotten, it should be noted and is a mutually known agreement.

Aesthetic plastic surgery is a service that pays attention to the final result, so patient satisfaction becomes one of the barometers of service quality because the patient's dissatisfaction can be a problem. However, do not forget about the previous explanation that the condition or condition of a person's body is unpredictable. This needs to be a form of cooperation between patients and Plastic Reconstructive and Aesthetic Surgeons. Patients should convey precise information to the doctor and listen well to the explanation from the doctor so as not to cause misunderstandings. According to the author, the patient's expectations for Plastic Reconstructive and Aesthetic Surgeons are the same as the expectations of other patients, namely:

a. Reliability: the provision of service promised immediately and satisfactorily.

b. Responsiveness: help and provide services with Responsiveness without distinguishing Tribe, Religion, Race, Class of patients.

c. Assurance: guarantee of safety, safety, comfort.

d. Empathy: good communication and understanding of the needs of patients.

In medical actions performed by Plastic Reconstructive and Aesthetic Surgeons, the cause of the dispute is due to several things:

a. Changes in the pattern of the doctor's relationship with the patient.

Previously, the relationship pattern was paternalistic, where the patient always adhered absolutely to what the doctor instructed but has now changed into a way of partner relationships between doctors and patients. The doctor should consider the patient's opinion in choosing to determine which surgery the patient is in. Because if the result is not by his wishes, then the patient feels harmed, so there will be a dispute. According to the researchers, especially for aesthetic plastic surgery, because there is no medical indication, if there is no agreement or conformity between the doctor and the patient, the Plastic Reconstructive and Aesthetic Surgeons may refuse not to perform the surgery.

b. Lack of cooperation between patients and Plastic Reconstructive and Aesthetic Surgeons.

In the healing process, the patient must comply with the advice and instructions of the Plastic Reconstructive and Aesthetic Surgeons, such as the patient must maintain cleanliness and should not be wet, the control must be timely, and so on.

c. Lack of information and communication between Plastic Reconstructive and Aesthetic Surgeons with patients so that it can be a trigger for disputes.

Many Plastic Reconstructive and Aesthetic Surgeons judges that communicating with patients is not an easy task because it is considered difficult. After all, patients lack knowledge about medical problems. Akibanya raises a misunderstanding between the doctor and the patient because the patient does not understand the term medicine. Otherwise, the patient is reluctant to ask because of embarrassment.

d. Discrepancies between the patient's expectations and the final result obtained.

The patient's expectations are too high, so the patient's dissatisfaction with the doctor occurs. Patients do not know that there are other factors beyond the doctor's power that can affect medical efforts, such as physical condition, endurance, quality of medication, and compliance of the patient to obey the doctor's advice. These factors can result in the best efforts or medical actions becoming meaningless at all. Therefore, it can be said that the results of a medical procedure are full of uncertainty and cannot be taken into account mathematically.

e. Differences in perception.

According to the authors in this study, the perception is that patients consider the most important is the result. They do not understand that although the result is the goal, the alliance between the doctor and the patient remains inspanning verbintennis. This is a misperception of the patient regarding the object of the agreement made with the doctor. The patient only understands that this study is a special medical action for aesthetic plastic surgery if the doctor fails in his medical effort. If the surgery results do not match what the patient expects, the patient will sue the doctor by calling it malpractice.

f. The cost of plastic surgery and maintenance is expensive.

The patient feels that he has incurred a high cost, but if the result is not as expected, the patient feels dissatisfied that he demands compensation from the doctor.

Any medical treatment performed by a doctor, both diagnostic and therapeutic, always carries risks, whether or not this risk arises regarding health care.

Therefore, it is necessary to distinguish between medical risk and medical malpractice. If the patient has been treated by the standard procedures of medical services but fails, the patient is injured or dies, this is a medical risk, but if the patient in treatment fails or suffers a wound or death, as a result of the doctor performing services below medical standards, it means that there is medical malpractice

Medical risks that can occur in medical services or medical actions are:

a. inherent risks, such as hair loss due to cytostatics (cancer cell killer drugs)

b. hypersensitivity reactions, such as a distorted immune response (immunity) to the entry of foreign bodies (drugs) that are often unpredictable beforehand.

c. complications that occur suddenly and unexpectedly before.

Thus it can be concluded that the physical disability or death of the patient is not always a doctor's negligence but also a risk that may occur in medical actions performed by the doctor.

Distinguishing between malpractice and medical risk can be seen from the element of negligence. This negligence must be proven that there is a connection with the disability or death of the patient. If this element of failure does not exist, it means that the defect or death of the patient is not the result of malpractice but is a medical risk that may occur in the course of treatment. So if the doctor acts with medical risk, then the doctor does not have to be responsible for the actions performed. In contrast to medical malpractice, if the doctor performs a medical action that causes the onset of medical malpractice, then the doctor must take responsibility for his actions legally[14].

Disputes arising between Plastic Reconstructive and Aesthetic Surgeons and aesthetic plastic surgery patients have always been linked to suspected medical malpractice[15]. Settlement of medical malpractice disputes in Indonesia, taken through 2 lines, namely litigation (judicial) and non-legal lines (out of court), but most of the path taken either through lawyers or not with lawyers is through mediation or resolved by themselves peacefully. The settlement of medical malpractice through litigation holds the Plastic Surgeons of Reconstruction and Aesthetics accountable for performing aesthetic plastic surgery[16]. Doctors may be penalized. Three aspects of the law used to determine malpractice:

1) Deviation from medical professional standards.

2) Mistakes made by doctors, both in the form of deliberateness and negligence.

3) The consequences are caused by medical actions that cause material or non-material, physical (injury or death) or mental harm.

According to the researcher, the doctor's awareness of legal obligations to himself and others in carrying out his profession must be carried out correctly. Especially to be done and what should not be done by Plastic Reconstructive and Aesthetic Surgeons. If the patient sues the doctor with a lawsuit against the law, the patient must show the doctor's guilt due to negligence in carrying out his professional obligations causing harm to the patient. Losses that occur must be explained as a result of negligent doctor's actions, or in other words, there is a clear causal relationship, and there is no justification

A doctor can be declared guilty and must pay compensation if the losses incurred. There is a close relationship with the mistakes made by the doctor. In determining the mistakes of doctors, we must refer to professional standards. So that in the implementation of medical practice, acts against the law can be identified by the actions of doctors who are contrary or not by professional standards applicable to the development of the profession in the field of medicine.

Researchers found the rules on plastic surgery are incomplete, contained in Article 68 paragraph

(1) of the Law of the Republic of Indonesia No.36 of 2009 on Health, that the installation of implants or medical devices into the human body can only be done by health workers who have expertise and authority and are carried out in health care facilities. Ideally, the Article coupled with the sentence of Medical Devices must have a medical device distribution license from the Ministry of Health of the Republic of Indonesia as evidenced by a Circulation License Number. While paragraph (2) mentions the provisions on the terms and procedures for installing drug implants or medical devices and stipulated by a Government Regulation, this Government Regulation contains health care recipients and implant users. Medical devices must mention the requirements of the desired medical devices, among others brand, size and other characteristics that the Plastic Surgeon Reconstruction and Aesthetics must agree, and proven by a letter of the agreement under the hand authorized by a Notarial Or Notarial Certificate. This agreement involves a Notary Public to have a force that can be held accountable because the deed made by a Notary public is an authentic deed in which the form and procedure are stipulated by law.

## Conclusion

Based on the research that has been done, it can be concluded that several things can cause disputes between Plastic Reconstructive and Aesthetic Surgeons with patients. In addition, the Plastic Reconstructive and Aesthetic Surgeons do not guarantee the success of the operation, and the doctor also explains the risks and complications that may occur. So to resolve the dispute, there must be good ethics between the two parties to fix it properly an not harm each other. One way to prevent this by making a special agreement that can guarantee legal certainty, fairness and benefit, is in the form of a deed under the hands or a notarial deed. In case of dispute can be resolved by mediation. Researchers advise that risky matters can be discussed first and better addressed in writing and included as clauses in the agreement. In connection with the medical measures taken by Plastic Reconstructive and Aesthetic Surgeons prone to lawsuits, it is necessary to provide legal protection for doctors to maintain a sense of comfort and safety in work.

**Ethical Clearance :** Data taken from the books, websites and use of legal provisions only.

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# Evaluation of Biomarkers in Workers Exposed to Air Pollutants in Oil Refineries

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## Abstract

Air pollution is nowadays a complex problem due to industrial prosperity. Among the chemical industry, oil refineries have been identified as major emitters of a wide range of pollutants, the workers of the oil refinery are exposed to a great variety of toxic compounds. Air pollution is the main environmental cause of human disease and death. Therefore, it is necessary to develop early warning signals or biomarkers that convincingly reflect adverse biological responses towards anthropogenic environmental toxins even at minute concentrations. This work aims to study the effects of exposure to the air pollution of oil refinery, on the parameter blood, TNF-a, antioxidant glutathione peroxidase(GPx), and oxidative stress malondialdehyde (MDA), in workers of the oil refinery. Results showed that exposure to air pollution in the oil refinery lead to( a significant increase in levels Hb, WBC, Lymphocyte, TNF-a, and MDA, decrease levels of GPx) $P<0.05$. And non –significant in( RBC, HCT, Neutrophils ) $P>0.05$ in workers, compared to healthy control.

***Keywords:*** *air pollution; oil refinery workers; oxidative stress*

## Introduction

Air pollution is a phenomenon in which(liquid or solid) particles and gases contaminate the atmosphere and such contamination can impact the population[1]. In other words, an air pollutant is any gas or particulate that may be harmful to life, the environment, and/or property when its concentration is high enough. Among the chemical industry, oil refineries have been identified as major emitters of a wide range of pollutants [2]. The workers of the oil refinery are exposed to a great variety of toxic compounds [3]. The most important gases emitted from oil refineries, which are considered air pollutants are hydrocarbons (HC), CO, NOx, H2S, CH4, CO2, SO2, and particulate matter(PM) [4,5]. Environmental contaminants create harmful conditions for living organisms, including humans. This represents the growing interest in early warning tools for the detection of adverse biological responses to toxins in both humans and wildlife. Molecular and cellular biomarkers of pollution meet this requirement. pollution biomarker is an alteration in a biological response occurring at molecular, cellular, or physiological levels which can be related to exposure to or toxic effects of environmental chemicals [6].

Air pollutants stimulate the immune system to activate leukocytes and macrophages[7]. Cytokines are a group of proteins of low molecular weight secreted by the cells of the immune system. they regulate the inflammatory and immune response. these include,pro-inflammatory(TNF-a)[8]. Oxidative

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stress is a biochemical imbalance in which the production of reactive oxygen species exceeds the natural antioxidant capacity. In the body, this imbalance may occur after exposure to pro-oxidant air pollutants. Reactive oxygen species cause tissue damage and dysfunction in the presence of oxidative stress by targeting and denaturing structural and functional molecules (lipids, proteins, carbohydrates, DNA, etc.)[9]. Chemical components of air pollution exposures that cause oxidative stress and subsequent inflammation may be responsible for associations of cardiovascular morbidity and mortality with pollutant gasses associated with airborne particulate matter combustion. One feasible approach is to measure systemic oxidative stress in the blood for health effects. It is important to measure the genes and/or protein expression of endogenous antioxidant enzymes when measuring oxidative stress, since they may modify the relationship between biomarkers of oxidative stress and air pollutants [10].

## Materials and Methods

A total of 70 Iraqi individuals (males) in Basra (Southern Iraq) were included in this study, 40 individuals working in the Shuaiba refinery in Basra exposed to air pollutants during work(Exposed group). their ages ranging between 18-55 years. The other 30 individuals are non-exposed to air pollutants( not work in the refinery), they were considered as (Control group), this group ages ranging between 18-55years.

Blood parameters determined from complete blood count that was performed by hematology analyzer Sysmex. Total serum tumor necrosis factor-alpha (TNF-a) was determined by the use of a total TNF-a ELISA(enzyme-linked immunosorbent Assay) kit(Sunlong, china). The level of GPx in serum was estimated by using commercially available kit ELASA (Elabscience, USA). The level of MDA was determined by the Buege and Aust method [11].

Spss program (version 26) was used to elucidate the difference in parameters. The results were expressed as mean±SD. Comparisons were made between two groups using the T-test for categorical. The p-value of<0.05 was considered to indicate statistical significance.

## Results and Discussion

The means of red blood cell (RBC)in the exposed workers was 5.41 ± 0.5and in controls 5.21 ± 0.4, Hematocrit (HCT) in exposed workers was 43.58±3.9% and in controls were 44.08± 3.1, mean corpuscular hemoglobin (MCH) in exposed workers was 28.45 ± 2.5 and in controls was 28.43 ± 1.9, and Neutrophil count in exposed workers was 3.87±1.7 while in control 4.24±1.3. These results showed that the values of previous parameters within the normal range with insignificant differences between exposed workers to air pollution and controls. However, hemoglobin in exposed workers was 15.33 ± 1.4 g/dL and in controls was 14.64 ± 1.1, WBC in exposed workers was 7.64± 2.1(10^3/uL) and in controls was 6.68 ± 1.4, and Lymphocyte(LYM) in exposed workers was 2.87± 0.7and in controls was 2.31± 0.4. All previous results showed a significant increase in exposed workers compares to control, as shown in table 1.

**Table 1: Mean and standard deviation of the blood parameters**

| parameters | Exposed (mean±SD) | Controls (mean±SD) | P-value | |
|---|---|---|---|---|
| Hb(g/dL) | 15.33±1.4 | 14.64±1.1 | 0.040 | S |
| RBC(10^6/uL) | 5.41±0.5 | 5.21±0.4 | 0.108 | NS |
| HCT% | 43.58±3.9 | 44.08±3.1 | 0.568 | NS |

***Cont.*. Table 1: Mean and standard deviation of the blood parameters**

| | | | | |
|---|---|---|---|---|
| MCH(pg) | 28.45±2.5 | 28.43±1.9 | 0.973 | NS |
| WBC(10^3/uL) | 7.64±2.1 | 6.68±1.4 | 0.041 | S |
| NEU(10^3/uL) | 3.87±1.7 | 4.24±1.3 | 0.348 | NS |
| Lym(10^3/uL) | 2.87±0.7 | 2.31±0.4 | 0.000 | S |

S:significant,NS:non-significant

**Table 2:level TNF-a in exposed and controls**

| Groups | Exposed | Control |
|---|---|---|
| Mean | 162.78 ng/L | 148.27ng/L |
| S.D. | 23.57 | 24.91 |
| P-value 0.015 | | |

**Table 3:level biomarker oxidative stress in exposed and controls**

| | Exposed Mean± SD | Control Mean± SD | P-value | |
|---|---|---|---|---|
| GPx pg/ml | 1273.0±423.08 | 1527.3 ±373.57 | 0.011 | S |
| MDA µmol/L | 1.34±1.02 | 0.73±0.7 | 0.007 | S |

This study's results demonstrated the existence of a significant correlation between occupational air pollution exposure to oil and gas activity in the environment and Hb, as shown in table 1.There was an increase in the level of hemoglobin(Hb) in the exposed compared to the control group, and this is the case agreed with other studies[12]. Hb is a major component of red blood cells that has a key role in oxygen and carbon dioxide transport in the body [13]. Some of the gases that pollute the air contribute to a rise in the blood hemoglobin level, such as CO. This gas enters the bloodstream and binds to the protein Hb. This can reduce the blood's ability to deliver oxygen to the body's tissues. As a means of compensation, the body can increase the erythropoiesis process, potentially increasing Hb production[14].

The current study showed that the level of white blood cell (WBC) was a significant increase in exposed workers compared with control. WBC are cells of the immune system involved in defending the body against both infectious diseases and foreign materials. Inhaling dirty air can trigger the releases of WBC into the bloodstream and can result in inflammation. Through this result, exposure to air pollutants may increase the number of WBC, this case agreed with another study [15].

The mean of LYM in exposed workers is significantly high as compared to controls. LYM are the type of WBC that is of fundamental importance in the immune system because the lymphocytes are the cells that determine the specificity of the immune response to infectious microorganisms and other foreign substances [16]. This result indicates that exposure to air pollutants affects LYM levels, and this is the case agreed with another study [17].

The serum level of TNF-a a significant increase $p<0.05$ in exposed workers with serum median levels (162.78±23.5 ng/L) compared to control group serum median levels(148.27±24.9ng/L), as shown in table 2.

In this study, the exposed workers showed a significant increase of pro-inflammatory biomarker TNF-a due to exposure to air pollution. The inhalation of toxic environmental gases and particles impacts the defense systems of the lung. Lung macrophages play a critically important role in the recognition and processing of any inhaled foreign material such as pathogens or toxic gases and particles. Alveolar macrophages and lung epithelial cells are the predominant cells that process and remove inhaled air pollutants from the lung. Cooperatively, they produce pro-inflammatory mediators when exposed to air pollutants [7]. Cytokines are mainly secreted by T helper cells (Th) and macrophages, they regulate the immune and inflammatory response and are primarily involved in the events of pathogenesis in air pollution-related diseases. They may also have the potential to be used as indicators for assessing the harmful effects of air pollution [18]. TNF-a is a major pro-inflammatory cytokine produced by macrophages and the generation and development of inflammatory reactions and related diseases[19]. The air pollutants can stimulate pro-inflammatory cytokine production by macrophages [20] Studies indicate that inhalation of air pollutants can result directly or indirectly in the formation of ROS, as a result, macrophages are activated and released large amounts of TNF-a [21]. Alveolar macrophages exposed to atmospheric particles increase both their phagocytic activity and increase their production of pro-inflammatory mediators such as TNF-a [22].

In this study, the Level of GPx significantly decreases in exposed workers $p<0.05$ with mean level (1273.0±423.08pg/ml) compared to control group mean levels (1527.3±373.57pg/ml), as shown in table 3.

GPx is an antioxidant that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides [23]. Antioxidant enzymes are proteins involved in the catalytic transformation of reactive oxygen species(ROS) and their by-products into stable nontoxic molecules, therefore, representing the most important defense mechanism against oxidative stress-induced cell damage. Antioxidants in the lungs are the first line of protection against ROS. Individual responsiveness to air contaminants could be influenced by the composition and quantity of antioxidants in respiratory tract lining fluids [24]. After air pollutants entering the lung, faces first the extracellular antioxidant mechanisms present in the respiratory tract lining fluid, which contains enzymatic antioxidants such as GPx [25]. The result of this study might suggest that the decrease of GPx levels in refinery workers exposed to pollutants might be a part of the antioxidant status to protecting tissues from the effects of free radicals. The enzyme GPx plays an important role in regulating the level of different peroxides by accelerating the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG) after removing the different peroxides (such as hydrogen peroxide, lipid peroxides, and organic peroxides). GPx is part of the cell protection system against oxidative stress. And its products, thereby reducing cell damage caused by an increase in free radicals. Therefore, the exposure of the body to oxidative damage leads to a significant decrease in the level GPx. A rise in free radicals triggers an increase in the use of endogenous antioxidants, resulting in a decrease in endogenous antioxidant levels in the body [26].

MDA is one of the end-products of the peroxidation of membrane lipids caused by ROS formation. It is considered a good marker of oxidative stress [27]. In this study, the Level of MDA significantly increases P<0.01 in exposed workers with a mean level (1.34±1.02µmol/L) compared to control group mean levels (0.73±0.7µmol/L) as shown in table 3.

This study showed a highly significant difference among the exposed group compared to the control group regarding the level MDA which was higher among the exposed group. This is in agreement with other studies which illustrated that air pollutants exposure has been associated with an increase in the overall formation of MDA[28]. Elevated MDA level was observed in exposed workers study, indicating that lipid damages were induced in subjects occupationally exposed to air pollutions [29]. Excessive formation of free radicals increases the process of lipid peroxidation, as evidenced by elevated levels of MDA, the end product of lipid peroxidation, in serum and tissues of exposed subjects [30].

## Conclusions

The exposure to air pollutants in refineries can cause markered alterations in hematological parameters and suggestive as useful tools to serve as a marker for biological control or monitoring of residents for the level of exposure to air pollutants. Inhalation of air pollutants can stimulate the immune system. Increase exposure to air pollutants can lead to oxidative stress for refinery workers. A decrease in antioxidant GPx in refinery workers' serum and an Increase in level MDA in refinery workers serum that considers good biomarkers to oxidative stress.

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# Extraction and Purification of Resveratrol from Grape Waste

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## Abstract

The current study aimed to extract and purify the phenolic compound Resveratrol from dried black grape residues, Vitis vinifera, which were purchased from the local market in Iraq. Resveratrol was extracted from sun-dried grape residues with 2.5 liters of 80% ethanol. Then the compound resveratrol was concentrated using a rotary evaporator and dried in very high freezing conditions after being filtered with animal charcoal. A partially purified material was obtained after a column chromatography process, where 9 grams were obtained for every 600 grams of grape residues as a result of these steps. Chemical tests were conducted to detect resveratrol, and they included: general tests for polyphenols, detection of unsaturated cyclic compounds, high-efficiency liquid chromatography (HPLC) method. The results showed the appearance of two peaks at a wavelength of 280 nm for the compound resveratrol extracted from grape residues, where the time of its appearance was at 4.267 /min compared with the standard compound resveratrol which was at 4.356 /min. The concentration of the resveratrol extract was 107.6 ppm. The total antioxidant activity of resveratrol extracted from grape residues and the standard compound resveratrol was estimated. The total antioxidant activity of resveratrol extracted from grape residues was 71.14%, while it was 62.21% for the standard compound resveratrol. The results showed that the resveratrol compound extracted from grape residues was purified in the dark to prevent the conversion of resveratrol from trans to cis. The study also shed light on the quantities of resveratrol contained in grape residues, and this depends on the extraction steps as well as the purpose of its applications.

***Keywords:*** *Resveratrol, grape waste, Antioxidants, Polyphenols, HPLC.*

## Introduction

Resveratrol (3,5,4-trans-trihydroxyestilbine) is a polyphenolic phytoalexin belonging to the stilbene family. It is a natural dietary plant compound that occurs main-ly in grape skin and seeds but is also found in wines and various other types of plant foods, especially pea-nuts, berries, and tea [(1)]. Resveratrol is synthesized by more than 70 species of plants in response to infection, stress, injury, bacteria or fungal infections, and UV-irradiation [(2)]. Synthesis of this molecule in plants is catalysed by resveratrol synthase in the phenylpropanoid pathway in a process similar to that of flavonoids [(3)]. Resveratrol possesses two phenol rings (monophenol and diphenol) bonded together by a double styrene bond and it exists in both cis and trans isomeric forms, Trans-resveratrol appears to be the more abundant and stable natural form [(4)]. This molecule has three hydroxyl groups which are involved in free radical scavenging and metal chelation [(5)]. The presence of hydroxyl groups also facilitates interaction with macromolecules.

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Resveratrol reveals a wide range of biological properties including anti-glycation, antioxidant, anti-inflam- mation, neuroprotective, anti-cancer, and anti-aging ac- tivity in various in vitro and in vivo experimental models [6]. For industrial purposes, resveratrol is generally obtained by chemical or biotechnological synthesis from yeasts [7]. Fresh grape skin contains (50-100) µg/g of trans- resveratrol [8].

[9]studied the effect of resveratrol extracted from black grapes and its derivatives on some physiological and histological characteristics of experimentally infected female rabbits with type 2 diabetes. This study was also used to evaluate resveratrol and its derivatives in female rabbits at a concentration of 1 mg / kg of body weight given orally for 42 days after induction of type 2 diabetes. Histological sections of the liver and pancreas were studied, and the statistical results showed a significant decrease in glucose, Urea, creatinine, cholesterol, triglycerides, asparagine transporter enzyme (AST). Resveratrol and its derivatives when used in the treatment of rabbits showed a statistically significant increase in high-density lipoproteins (HDL) and insulin in serum levels. [9] also studied the effect of the compound Resveratrol extracted from the peels of grapes Vitis vinifa on some cell lines in the laboratory in order to extract and purify the compound Resveratrol from the peels of the black grape plant. Where a partially purified substance was obtained after performing a column chromatography process and examining the purity of the resveratrol compound by high-efficiency liquid chromatography. The study included an ex vivo evaluation of the cytotoxic activity of the purified and partially purified substance and their effect on some normal and cancerous lines. The study showed a comparison of the cytotoxic effect of both partially purified and fully purified extracts in the three cell lines, and it appeared that different concentrations gave a difference in effect, and that the pure substance had a more inhibitory effect than the partially purified. The cytogenetic study of the completely purified extract on the division of normal human blood lymphocytes showed that Resveratrol inhibited the action of the cleaved substance (PHIA) and showed significant differences within the level ($P < 0.01$) for all concentrations as an anti-cell fission substance and anti-oblastogenesis in a significant manner. It is proportional to the concentration increase used and the exposure time.

## Materials and Methods

### Resveratrol extraction and purification

Resveratrol was extracted and purified according to the method approved by [5].

### Preparation of grape residue extract

Iraqi black grapes were collected from the local market and classified as belonging to the variety Vitis vinifera by Herbarium, Department of Life Sciences, College of Science, University of Baghdad.

### Preparation of grape residues

After the grapes were obtained, they were washed well, then they were mixed with an electric mixer for a period of (5-7 minutes). The mixture was filtered using a sieve lined with a fine cloth, discard the juice produced from it, while the remaining residues were taken in the strainer and dried solar, then put in bags of polyethylene. And the samples were preserved by freezing (at -20 ° C) until extraction steps were carried out for it.

### Extract preparation

Grape residue extract was prepared with a weight of 500 g of sun-dried grape residue and mixed with 2.5 liters of ethanol at a concentration of 80%, the mixture was left for 72 hours in a cool and dark place, then the extract was filtered using a vacuum filtration unit and then concentrated using a rotary evaporator at of 30-40 ° C. The concentrated filtrate was stored at -20 ° C for subsequent steps, as all the above steps were carried out away from direct light.

**Identification of Polyphenols**

Phenolic group (C6H5-OH) in phenolic compounds, which are colourless but attain colour due to oxidation, are soluble in 5% NaOH solution and insoluble in 5% sodium carbonate solution, and the phenolic groups in the molecule can be determined by the following general tests:

**Ferric Chloride Test**

The classic procedure for detecting phenolic compound is by means of the intense green, purple, blue or black colours, many of them give in solution when 1% aqueous or alcoholic ferric chloride is added [9].

**Liebermann Reaction**

Only those phenols which posses a free para position respond to this test. The test includes the additional 1 ml of conc. $H_2SO_4$ to the phenolic compound in a dry test tube and addition of a few crystal of $NaNO_2$, a blue green or blue- violet colour is immediately formed which changed to red on dilution [5].

**Detection of the Aromatic Ring**

The aromatic ring was detected in the extract of grape tailings by conducting the aluminum chloride test mentioned by [7,8] as follows: 0.1 gm of the extract of prepared grape residues was taken and 1 ml of chloroform was added to it, then aluminum chloride crystals were added to it. Anhydrous (ALCl3) and using a spoon added along the sides of the test tube, formed yellow colored aluminum chloride crystals which turned dark orange within a few minutes, with the appearance of a colorless chloroform layer detachment.

**Specific Test for double Bond:**

In order to find out unsaturated compound, the following test is applied [6].

**Bromine Decolourisation Test:**

0.1 g of unknown resveratrol in 2 ml carbon tetrachloride is added with shaking, then a drop wise of 5% solution of bromine was added. The discharge of reddish brown colour of bromine without evolution of hydrogen bromide represents a positive test for unsaturated compound [9].

**Isolation and Purification of Resveratrol:**

The following steps were followed for the isolation and purification of resveratrol: Acid hydrolysis, Liquid portion, Column chromatography (Partial purification), HPLC.

**Acid Hydrolysis**

Acid hydrolysis was done using 10% V/V conc. HCl for (10-30) min on a water bath. This step led to the hydrolysis of the glycosidic linkage and got the aglycone moiety, cool and filter.

**Liquid – Liquid Partion**

The filtrate was transferred to seperatory funnel. An organic solvent like chloroform was added in a quantity equal to the aqueous phase, with gently shaking and reapting the process three times. The chloroform layers were collected together and washed from the access acid with distilled water. The collected chloroform layers were evaporated to dryness under vacuum with a rotary evaporator at 30°C. The residue was green viscous alquest stored in dark umber vessels at –20°C untill use [9].

**Column Chromatography (partial purification) of resveratrol:**

a method was adopted [5]. To obtain the pure resveratrol compound, the partial purification of the concentrated grape tailings extract was carried out using using open glass column (2.5 x 30) cm filled with silca gel G60 special for column chromatography. The residue was dissolved in 1-2 ml methanol and the mobile phase is benzene: methanol: acetic acid, 20:4:1. The elutions were collected in 40 separated tube each

filled with 3ml eluent. All fractions were tested for $FeCl_3$ 1% solution as a colourimeteric method for polyphenols identification [6]. then the ethyl acetate solvent was used at a ratio of 1:1 to obtain partially purified resveratrol, then the purified sample was treated with charcoal to get rid of the color, then the extract was concentrated in a rotary evaporator and dried in an electric convection oven.

**Diagnosis of Resveratrol using High-performance Liquid Chromatography(HPLC):**

Resveratrol was diagnosed according to [9]. Resveratrol was detected in the sample obtained using a high-sufficiency liquid chromatography device of SYKAMN HPLC under the following conditions:

| | |
|---|---|
| Column type | C18-OSD a Zorbax Eclipse |
| Column length | cm x 4.6 mm 0.25 |
| Flow Rate | ml/min 0.7 |
| Wave length | nm 280 |
| Mobile phase | Mobile phase Methanol (A) and formic acid 1% (B) diluted with water v /v as follows: 0-13 minutes 40% of formic acid 1% and 14-20 minutes, 50% of formic acid solution |
| time Rotation | It is set according to the result obtained |
| Injected sample size | µl 100 |

**Estimation of total antioxidant activity**

The total antioxidant activity of natural and synthetic resveratrol was estimated according to the method used by [2].

## Results and Discussion

**Extraction and Partial Purification of Resveratrol:**

Since grape skins are one of the sources of resveratrol [5], mentioned that each gram of fresh grape skin contains 100-50 micrograms of pure resveratrol. Therefore, the process of extraction and partial purification of the compound resveratrol was carried out using 600 gram of dried black grape residues with 2.5 liters of 80% ethanol. Then the extract was filtered and concentrated in a rotary evaporator, then the acid hydrolysis process was carried out using 10% hydrochloric acid in a water bath for (10-30) minutes and extracted with the organic solvent chloroform, Then the resveratrol was separated using column chromatography by an open column filled with silica gel G60. The remaining filtrate was dissolved in 1-2 ml of methanol, the extract was added to the column and washed by the mobile phase (benzene, methanol, acetic acid, 1:4:20), After that, ethyl acetate solvent was used in a ratio of 1:1, then the purified sample was treated with charcoal, then the extract was concentrated using a rotary evaporator and dried in the oven, In this step, partially purified resveratrol was obtained, Then the sample was kept in opaque glass containers in order to preserve the resveratrol from oxidation , since the naturally occurring trans-resveratrol easily oxidized and converted to the cis – configuration by day or UV light and with the presence of heat, heavy metals and atmospheric oxygen [4]. The results of separation and purification showed the following:

**Resveratrol Extract**

Resveratrol was extracted from grape residues according to (6) using ethanol at a concentration of 80%, As this alcohol is a good solvent for primary extraction purposes, all extraction steps were carried out in conditions far from light. 9 g was obtained from every 600 g of grape residue used, this method is consistent with the method used by [5] when using 80% ethanol alcohol to extract resveratrol for 30 minutes at a temperature of 60 °C to prevent the enzymatic oxidation of the compound. [3] also indicated that resveratrol is hydrolyzed using hydrochloric acid at a concentration of 10% after being placed in a water bath for 10-30 minutes, and it dissolves easily in organic solvents such as chloroform.

**Purification of Resveratrol**

Since the grape skin contains numerous amounts of chemical compounds, therefore, the extract cleaning up is necessary by liquid-liquid partion technique for separation compounds according to the different distribution coefficients and the solvent affinity to solutes [6]. Ethylacetate is a good solvent for resveratrol taking up as viewed in many studies [7].

**Chemical Identification of Resveratrol:**

Table (1) shows the most important general phenolic tests for resveratrol, it was clear that the resveratrol compound was soluble in 5% sodium hydroxide solution, while it was insoluble in 5% sodium carbonate solutionWhen tested with 1% ferric chloride solution, it gave a positive result, which resulted in a green color. It also gave a positive result with Liebermann test, which resulted in a blue-green color. It also gave a positive result with the aluminum chloride test, which depends on the presence of the aromatic ring. It also gave a positive result with the bromine decolorization test, which indicates the presence of the double bond. These results are consistent with the findings of [8].

**Table (1): Tests for the general phenolic compound**

| Test | Result |
|---|---|
| %5 NaOH solution %5 | Soluble |
| 5% Sodium carbonate solution | Insoluble |
| 1% Ferric chloride solution | Green colour |
| Libermann reaction | + ve |
| Aluminum chloride test for the aromatic ring | + ve ( yellow to orange colour) |
| Bromine decolourisation test for the double bond | + ve (Discharge of reddish – brown colour |

**High Performance Liquid Chromatography (HPLC) for Resveratrol**

The HPLC method was used to analyze the resveratrol compound extracted from grape residues and compare it with the standard resveratrol compound according to what was mentioned in [9]. And through the scheme obtained for the compound resveratrol in Figure (1,2) and the results obtained in Table (2), Two peaks at 280 nm wavelength were observed for the compound resveratrol extracted from grape residues, and when compared with the standard resveratrol

compound, the presence of resveratrol was confirmed in the extract at a concentration of 107.6 ppm. It was also noted that the time of appearance of the extracted resveratrol compound was 4.356 minutes compared to the standard compound where the time of appearance was 4.267 minutes.

**Table (2): HPLC results for resveratrol extracted from grape residues and standard resveratrol at 280 nm wavelength.**

| Resveratrol | Retention time (min) | Peak area |
|---|---|---|
| Standard | 4.267 | 603.61 |
| Extracted | 4.356 | 1087.948 |

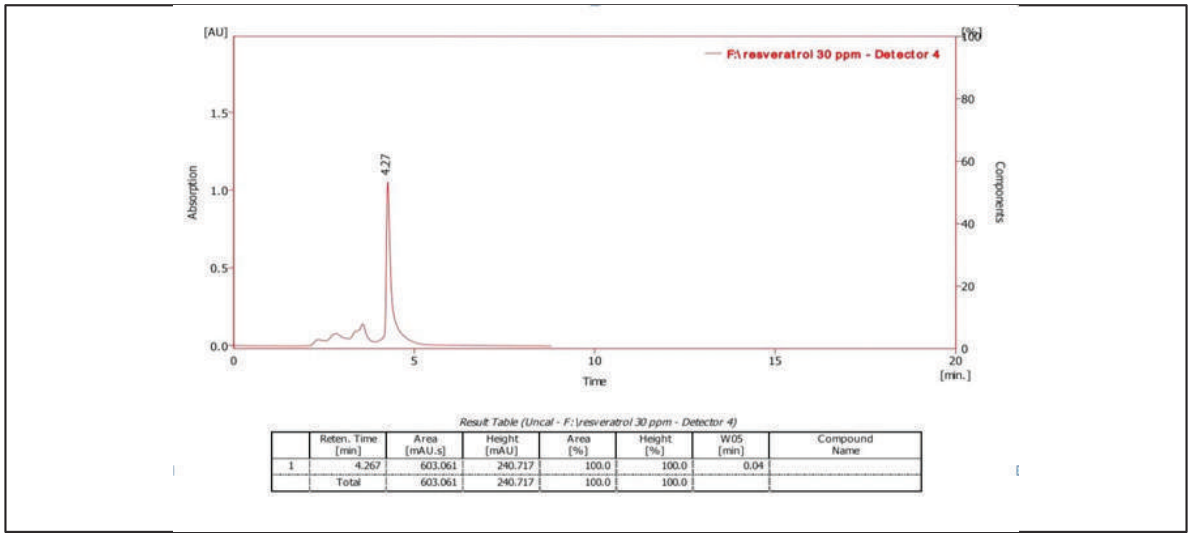


**Figure (1) Chromatographic analysis of resveratrol standard by HPLC technique.**

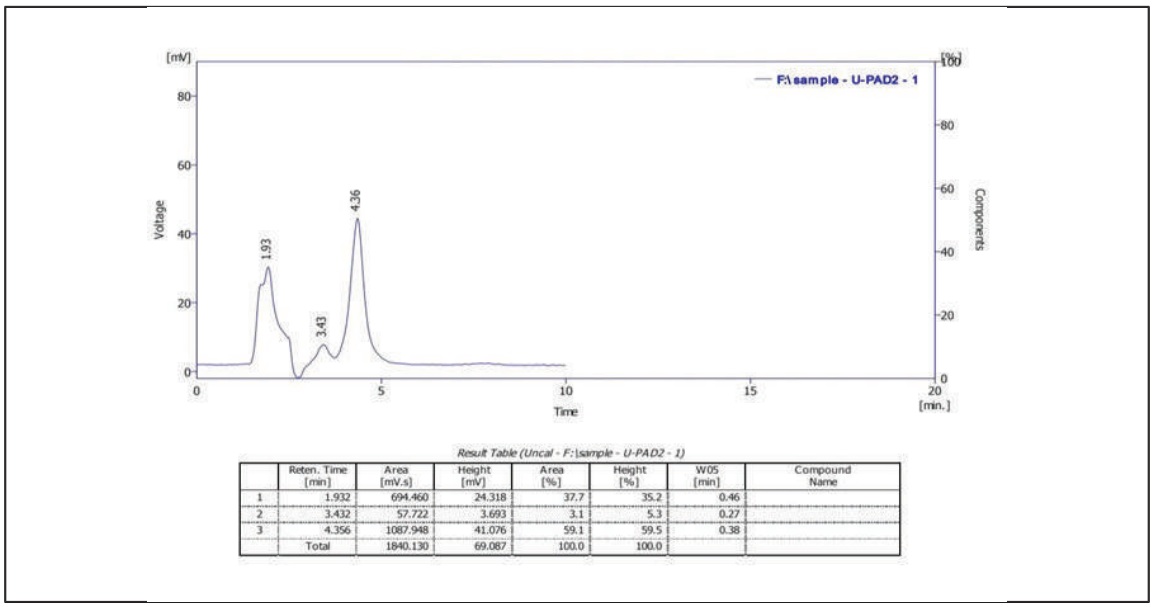


**Figure (2) Chromatographic analysis of standard resveratrol by HPLC technique**

Determination of the total antioxidant activity of Resveratrol

In order to detect the ability of resveratrol to show the antioxidant activity, the antioxidant activity of both resveratrol extracted from grape residues and synthetic resveratrol was estimated. The results showed that the resveratrol extracted from grape residues gave a higher antioxidant power compared to the synthetic resveratrol, as the percentage of the total oxidative activity of the extracted resveratrol was 71.14%, while the percentage was 62.21% in the synthetic resveratrol compound. When comparing the antioxidant power of resveratrol extracted from grape residues, which was mentioned above, we find that it was good when compared with the antioxidant power of ascorbic acid as one of the powerful natural antioxidants. [9] reported that the antioxidant power of this acid was 87.33%.

## Conclusion

The current study concluded the possibility to extract and purify the phenolic compound Resveratrol from dried black grape residues, and its quantities depends on the extraction steps as well as the purpose of its applications.

**Conflict of Interest:** None

**Funding:** self

**Ethical Clearance:** Not required

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# A Comparative Study on the Diagnostic Value of Conventional Spin Echo Proton Density and Fast Spin Echo Proton Density Sequences of Magnetic Resonance Imaging in Diagnosis of Meniscal Tear

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## Abstract

**Background & Objectives:** Knee is the largest synovial joint in the body. Although FSE PD (fast spin echo proton density) imaging technique for the diagnosis of meniscal tear has replaced CSE PD (conventional spin echo proton density) technique in many MRI centers, several studies have questioned the effectiveness of FSE PD technique in diagnosis of meniscal tear. In this study, the diagnostic values of CSE PD and FSE PD techniques in evaluation of meniscus tear are compared.

**Materials:** In this study, using CSE PD and FSE PD techniques, MRI was performed on 67 knees of patients with suspected meniscal tear referring to the MRI Center of Imam Reza Hospital (Kermanshah, Iran). Diagnostic arthroscopy was also performed for all patients. The data were entered SPSS version 20, and finally the results of MRI reports of the two techniques were compared in evaluation of meniscal tear taking into account the arthroscopic findings as the gold standard.

**Results:** According to the results of this study, no significant difference was found in specificity and positive predictive value of the two techniques. However, the sensitivity and negative predictive values were significantly different, in such a way that the related values were 97% and 97% in the CSE PD technique and 69% and 73% in the FSE PD technique, respectively.

**Conclusion:** Due to low sensitivity and high false negative results, MRI using FSE PD technique is not recommended as an alternative for CSE PD technique in evaluation of meniscal tear.

***Keywords:*** *Meniscal Tear, Knee MRI, Spin-echo*

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## Background

Knee is the largest synovial joint of human body. The main proportion of body weight is on knee which makes knee thus relatively vulnerable[1]. The methods of diagnosis of knee injuries such as meniscal tear include physical examination, MRI as

well as arthroscopy. Since the first application for examination of meniscus in 1984, knee MRI has acquired remarkable development. Because of less reliability, physical examination cannot be used as the only diagnostic method for meniscal tear and diagnostic arthroscopy is less welcomed by patients due to being invasive and expensive. However, knee MRI is very welcomed at the present time thanks to non-invasiveness and high precision[2-5]. MRI is considered a great transformation in knee imaging. Meniscus tear which is diagnosed through pain and instability in knee is the most common indication for performance of knee MRI[6-7]. With its high negative predictive value, MRI can play an important role in avoiding unnecessary diagnostic arthroscopy[8-11]. MRI determines the main features of meniscal tear (location, shape, length and depth of laceration) that play a significant role in correct treatment of patients[7,12].

In addition to being multi-planar, other benefits of knee MRI include: lack of exposure of ionizing radiation on patients, usually intravenous contrast material is not required, there is no need for joint manipulation, and it is without pain and can be performed in a short time. Furthermore, unlike arthrography, there is no need to inject intra articular contrast material. However, arthrography MRI is used for evaluation of recurrent meniscal tear as well as for diagnosis of the remaining meniscus after surgery because MRI is singly unable to diagnose both cases[13-16]. Sagittal sections of CSE PD technique are typically used in MRI for evaluation of meniscal tear[7]. According to some studies, the results of the FSE PD technique in evaluation of meniscal tear are comparable with the results of the CSE PD technique[17-20]. However, some other studies have reached opposite conclusions and do not consider the accuracy of FSE PD technique equal to CSE PD technique in evaluation of meniscal tear[7,21,22]. Furthermore, some studies have suggested high sensitivity and specificity for the CSE PD technique and do not recommend FSE PD technique due to blurring in this technique regardless of less time needed for its performance[23-27]. Since less time is needed to perform the FSE PD technique compared with the CSE technique, several other studies have compared diagnostic values of FSE PD sequence and CSE PD techniques and have obtained different results[28,29]. Thus, considering the mentioned advantages and non-invasiveness of MRI and given that it is much cheaper than diagnostic arthroscopy[5] and there is controversy about the diagnostic accuracy of FSE PD sequence and CSE PD technique to examine meniscal tear, comparative study of diagnostic value of these sequences may be important so that CSE PD technique, which needs more time for performance, can be avoided for diagnosis of meniscal tear provided that the diagnostic value of FSE PD sequence is more or equal to that of CSE PD technique. Furthermore, FSE PD technique can be avoided in case its diagnostic value is low. Accordingly, the main objective of this study is to determine whether the FSE PD sequence has different sensitivity and specificity in diagnosis of meniscal tear compared with the CSE PD technique, and whether it can be recommended for evaluation of meniscal tear?

## Materials

This study is of diagnostic value. The patients in the study were suspicious about meniscal lesions and volunteered for arthroscopy and MRI. CSE PD and FSE PD sequences were performed for them before arthroscopy. With 95% reliability and 80% power and according to the results of other studies, the minimum of sample size (using sample size calculation formula to compare two ratios) was estimated 73 subjects to compare the two sequences. Three tools were used in this study: 1- Questionnaire, 2- MRI as a test the diagnostic value of which was evaluated, and 3- Arthroscopy as the gold standard. First, the questionnaires were prepared in two types, one containing personal information as well as the MRI results and the other personal information and arthroscopy result. After reporting the MRI by co-partner, final MRI report of patients was performed by an experienced radiologist in the field

of musculoskeletal. The questionnaires containing the results of arthroscopy were also completed by co-partner orthopedic specialist. These two specialists were unaware about the results of each other and a common code was considered for responses of orthopedist and radiologist. Then, the data were analyzed using SPSS version 20 and considering that the arthroscopy was gold standard, the false positive results, false negative results, true negative results and true positive results were calculated and accordingly the sensitivity, specificity as well as positive and negative predictive values of the two methods were compared.As it generally accepted, we considered a meniscal signal change as tear, only when this signal change has reached to meniscal surface or surfaces, otherwise, when the signal abnormality was confined to inner parts of the meniscus and was not disrupted the meniscal margins; we considered it as intra substance degeneration or degenerative changes and no tearing. (Fig 1 and 2)

Typing of the tears (vertical, horizontal, etc.) was not included in our study. And the only aim of our study was detection of tearing of any type.




**Grade I and II signal abnormalities are confined to inner parts of meniscus and consider as**

Degenerative changes. Type Grade III signal abnormality reach to meniscus surface and is a tear.

## Finding

119 cases of knee MRI were performed in our study. In 36 cases of which no evidence of meniscal tear and abnormal signal change was reported. Furthermore, 16 cases of degenerative changes were reported and ultimately 52 cases were excluded from the study and diagnostic arthroscopy was performed on 67 knees including 34 right knees and 33 left knees (totally 134 menisci). The range of participants ages was 13-68 years (mean age was 33 years) including 35 men (52.2%) and 32 women (47.8%).

The reports results of CSE PD technique obtained from 134 menisci MRI included 73 lacerations (61 medial menisci tears and 12 lateral menisci tears, and 61 healthy cases (6 medial menisci and 55 lateral menisci) respectively.

The results of MRI reports of FSE PD technique included 55 lacerations (43 medial menisci tears and 12 lateral menisci tears), and 79 healthy cases (24 medial menisci and 55 lateral menisci) respectively.

In conducted arthroscopy, out of 61 reported medial menisci tears in CSE PD technique, 54 were confirmed and out of 6 reported normal medial menisci in this technique 5 cases were confirmed, in such a way that in one case which was reported healthy in CSE PD technique, meniscal tear was diagnosed using arthroscopy. Besides, in performed arthroscopy, out of 12 torn lateral menisci diagnosed by CSE PD technique, 11 cases were confirmed and in one case which was reported as torn lateral meniscus, tear was not seen in arthroscopy. In addition, in 55 normal lateral menisci reports using CSE PD sequence, 54 cases were normal in arthroscopy, too, but there was a case of torn meniscus with false normal MRI report.

Out of 43 cases of medial menisci tears reported in FSE PD technique, 38 cases were confirmed in arthroscopy. Only 17 out of 24 cases of healthy medial menisci reported in FSE PD technique were confirmed with arthroscopy. Furthermore, in conducted arthroscopy, out of 12 cases of torn lateral menisci reported in FSE PD, 9 cases were confirmed and the remaining 3 cases were diagnosed as healthy. Furthermore, out of 55 healthy lateral meniscuses reported in FSE PD, 52 cases were confirmed and in 3 of them tears were observed in arthroscopy.

Finally, the MRI reports were considered as the presence of meniscal tear and lack of meniscal tear in any of the CSE PD and FSE PD techniques and the results were evaluated with arthroscopic findings and considering the true positive and false positive as well as true negative and false negative in both techniques, the sensitivity, specificity, positive predictive value, negative predictive value and validity of each of the techniques are presented. In the table 3 we summarized match results and area under the curves of the methods Arthroscopy and CSE. In the table 4 we summarized Match Results and Area under the Curves of the Methods Arthroscopy and FSE, as well as in the Diagrams 1 we presented results of ROC Curve Arthroscopy and CSE and in the Diagrams2 we presented results of ROC Curve Arthroscopy and FSE Results of MRI reports and arthroscopies are summarized. In the tables 1 we reported frequency distribution of results Arthroscopy and CSE. In the table 2 we reported frequency distribution of results Arthroscopy and FSE. Since the results of sensitivities, specificities, positive predictive values, negative predictive values, and the areas under ROC curves in both techniques are more than 0.5, the validities of both CSE and FSE techniques against the arthroscopy as gold standard are significant ($P<0.05$). Diagnostic value of CSE PD technique is higher than FSE PD technique because the differences of sensitivity, specificity, positive predictive value, negative predictive value, and the area under ROC curve are more than 0.5 and closer to 1, in this technique. In addition we presented 2 figures. In the Fig 1 we showed FSE image, an oblique tear is faintly visible in Posterior horn of medial meniscus and in the Fig 2 we showed CSE image, the same tear is clearly visible

**Table 1- Frequency Distribution of Results Arthroscopy and CSE**

| arthroscopy | | | | Diagnostic Method |
|---|---|---|---|---|
| Total | Healthy | Tear | Result | CSE |
| 73 | 8 | 65 | Tear | |
| 61 | 59 | 2 | Healthy | |
| 134 | 67 | 67 | Total | |

**Table 2- Frequency Distribution of Results Arthroscopy and FSE**

| arthroscopy | | | | Diagnostic Method |
|---|---|---|---|---|
| **Total** | **Healthy** | **Tear** | **Result** | **FSE** |
| 55 | 8 | 47 | Tear | |
| 79 | 59 | 20 | Healthy | |
| 134 | 67 | 67 | Total | |

**Table 3- Match Results and Area under the Curves of the Methods Arthroscopy and CSE**

| sig | Area Under the Curve | Negative Predictive Value | Positive Predictive Value | Specificity | Sensitivity |
|---|---|---|---|---|---|
| <0.001 | 92.53% | 96.72 % | 89.04 % | 88.06 % | 97.01 % |

**Table 4- Match Results and Area under the Curves of the Methods Arthroscopy and FSE**

| Sig | Area Under the Curve | Negative Predictive Value | Positive Predictive Value | Specificity | Sensitivity |
|---|---|---|---|---|---|
| <0.001 | 78.49% | 73.42 % | 85.45 % | 87.88 % | 69.12 % |

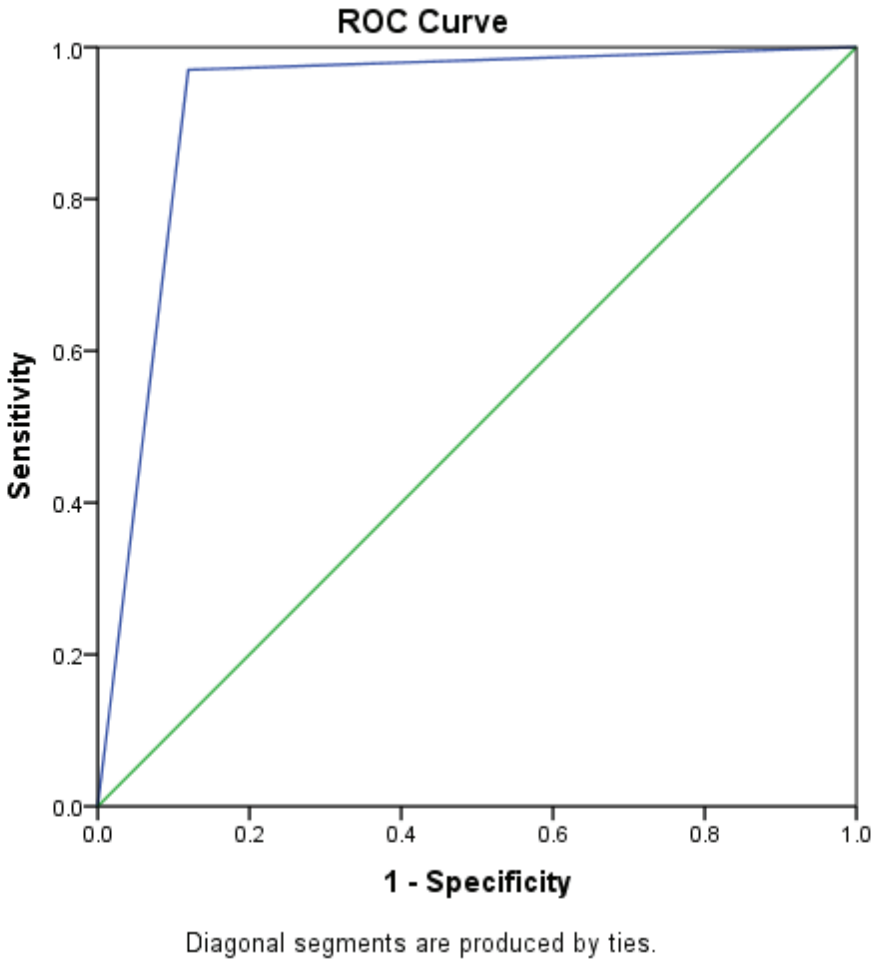


**Diagram 1- Results ROC Curve ARTHROSCOPY and CSE**

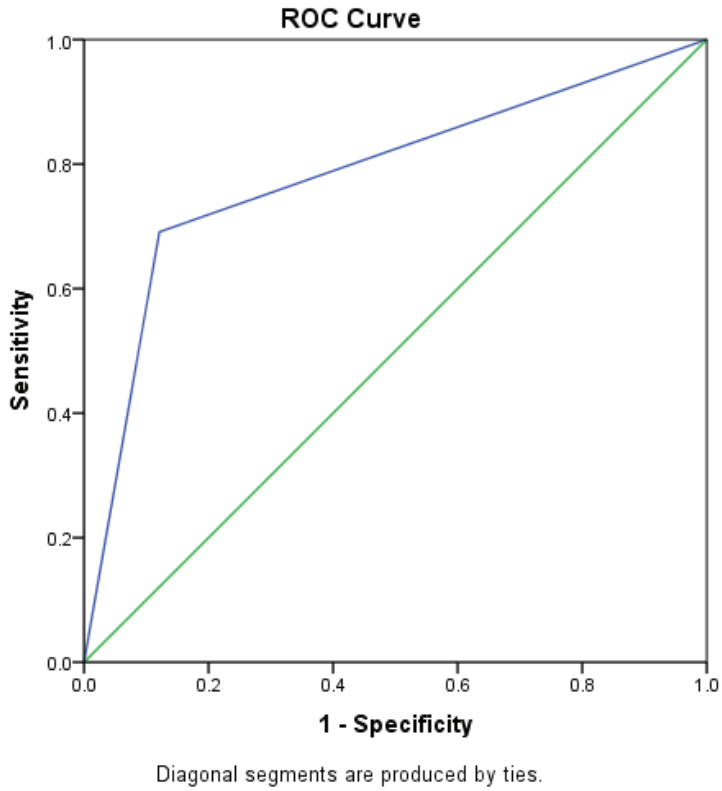


**Diagram 2- Results ROC Curve Arthroscopy and FSE**

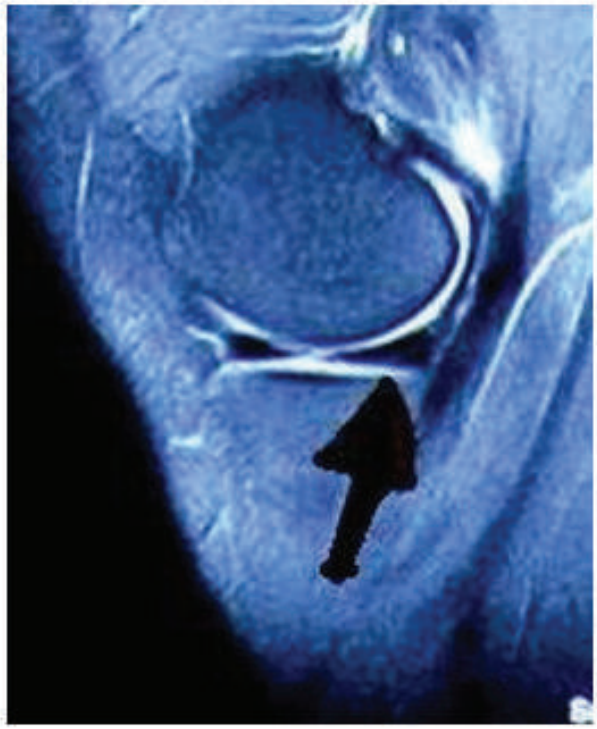

**Fig 1 - FSE image, an oblique tear is faintly visible in Posterior horn of medial meniscus**



**Fig 2 - CSE image, the same tear is clearly visible.**

## Discussion

The results of this study indicated that, the sensitivity, specificity, positive predictive value and negative predictive value in CSE PD technique are respectively 97%, 88%, 89%, and 96.7% and in FSE PD technique are respectively 69%, 87.8%, 85.4%, and 73.4% in comparison whit arthroscopy as gold standard. In addition, the validity of MRI using CSE PD and FSE PD techniques in diagnosis of meniscal tear is obtained 92.5% and 78.5% respectively.

As previously mentioned, MRI is a useful method in evaluation of meniscal tear[1,2,4,5,30]. Henning *et al.* first proposed the FSE technique[31]. In the spin-echo technique, after 90° pulse, magnetic field in homogeneity results in rapid protons de phasing thus by sending a 90° pulse, transverse magnetization is made but no image is produced, because of its rapid decay. Then the 180° pulse brings the protons in phase again and a strong signal is generated. In fact, the 180° pulse compensates magnetic field in homogeneity. In FSE technique or turbo spin-echo after a single 90° pulse, multiple 180° pulses are transmitted and finally multiple echoes are produced per one TR, instead of one echo in conventional spin echo. Since the multiple lines of phase encoding per one TR interval are obtained, thus the FSE technique can significantly reduce the imaging time[32]. Several studies have compared the two FSE and CSE techniques[7-11] and different results have been obtained. In this study, we investigated the diagnostic value of both CSE PD and FSE PD techniques in diagnosis of meniscal tear. The intensity of magnetic field of MRI machine in this study was 1.5 Tesla and similar to many previous studies. The age range of participants of this study

was 13-68 years (mean age of 33 years) and about 82.1% of meniscal tear diagnosed in arthroscopy was related to medial meniscus which was consistent with previous studies[33]. Since the results of sensitivities, specificities, positive predictive values, negative predictive values, and the area under ROC curves in both techniques were more than 0.5, therefore the validities of both CSE PD and FSE PD sequences are significant compared with that of gold standard arthroscopy (P<0.05). However, given the fact that if the mentioned values are greater than 0.5 and are closer to 1 thus the value of that diagnosis technique is more, the value of CSE PD sequence is higher than FSE PD sequence and the results are not in line with the findings of Escobedo *et al.* as well as that of Andrew *et al.* and Kojima *et al*[7,34,35]. As the result of Escobedo et al.'s study, the specificity was obtained 90% for both techniques and sensitivity was obtained 88% for CSE technique and 82% for FSE technique in diagnosis of meniscal tear. Ultimately, the FSE technique is considered an appropriate alternative for CSE technique in diagnosis of meniscal tear. In the study conducted by Andrew *et al.* - their study was retrospective reviewing CSE-T1 and FSE PD techniques - the diagnostic value of FSE-PD sequence was not considered less than CSE-T1 and the evaluation of meniscus using this technique is considered unnecessary. On the other hand, the results of our study are in line with the studies conducted by Rubin *et al.* and Anderson *et al*[21,36]. Similarly, in the Clyde *et al.*'s study, the sensitivity and specificity of CSE PD technique were 93% and 97% respectively and the sensitivity of FSE PD was 80%. In addition, there was almost 17% difference in CSE PD and FSE PD techniques and this difference was statistically significant[37].

## Conclusion

The FSE technique may significantly reduce imaging time. This decrease of MRI time and thus avoidance of artifacts due to patient movement have made advocates for FSE PD technique as an alternative for CSE PD. However, given the significant difference of diagnostic value of the two studied sequences as well as low sensitivity and negative predictive value in FSE PD technique, FSE PD sequence is not recommended for evaluation of meniscal tear.

**Conflict of Interest**: The authors declare that there is no conflict of interest

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**Availability of data and material:** The data used to support the findings of this study are available from the corresponding author upon request

**Ethical Clearance:** The Kermanshah University of Medical Science Ethics Committee endorsed the study. Objectives of the study were stated for all samples, emphasized the confidentiality of their

Specifics and responses, and informed written consent was obtained from all participants

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# A Study of Chronic Disease Management in Indonesian Primary Health Care

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## Abstract

Chronic disease management program in Indonesia(Prolanis) is a preventive and promotive program developed by Indonesian national health insurance. The initial survey showed thatonly 37.04% of primary health careperformchronic disease management programsoptimally. Prolanis can succeed and be sustained if health care services included health care teams effectively address patient needs as well as increasing the quality of health services.For this reason, physicians as key roles in primary health care should enhance their competencies to deliver high-quality care. This study aimed to identify the impact of physician competencies on patient satisfaction in Indonesian primary health care. This study used a cross-sectional designwith multistage random sampling which included 90 chronic disease patients in primary health care. The results revealed that the average physician competencies in providing health services to patients were 73.57%. In addition to this, as many as 73.64% of patients expressed satisfaction with physician skills. Lambda correlation test obtained a value of r = 0.76, so it was stated that the physician competencies had a strong influence on chronic disease patient satisfaction. It can be concluded that the more physicians master their competencies,the more confident patients seek treatment at primaryhealth care.

***Keywords:*** *Physician Competencies, Patient Satisfaction, Chronic Disease Management, Prolanis*

## Introduction

Chronic disease becomes an epidemic every year and affects 75% of the national health service budget. Studies explored about 10 major chronic diseases in Indonesia, such as cerebrovascular disease, heart disease, diabetes mellitus, tuberculosis, hypertension, chronic lung disease, liver disease, accidents, pneumonia, and a combination of diarrhea and gastroenteritis. Currently, the government focused the treatments on managing two major chronic diseases: diabetes mellitus and hypertensionthrough the Indonesian national health insurance[1,2].

Chronic disease management program in Indonesia (Prolanis) is a preventive and promotive program developed by Indonesian national health insurance in collaboration with primary health care services. Prolanis is a medical system that combines health management and communication services for a group of chronic disease patients. Thus, primary health care services are expected to be able to improve community health status through holistic and comprehensive services. Coordinating care and controlling costs of Prolanis through integrating system is needed across the entire range of chronic care services[3,4].

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The management of chronic diseases needs to be improved through holistic management by involving physicians and other health care teams working together through strengthening national health insurance financing[5].The initial survey showed thatonly 37.04% of primary health careperformthe programs optimally. The various obstacles faced by physicians in handling this case were the lack of control over the compliance of chronic disease patients in attending treatment regimens while at home, as well as the limited awareness of patients to immediately examine themselves when complaining symptoms of certain diseases[6-7].

Physician competence is a crucial component in providing effective public health care. Physicians should master the competency standards and meet patients' expectations. Physiciancompetency standards have been developed as guidelines to improve clinical practice and focused more on patient safety. These standards regulate the scope of activities relevant to day-to-day practice and ensure benefits for both physician and patient.Physician is requiredto implement professional competenceby improving quality of health services. Patients' satisfaction could be widely used to evaluate health servicequality. Issues in physiciancompetence could contribute to medical errors and substandard health services quality. To evaluate physician competence, however, is challenging nowadays.In Indonesia, physicianis required to complywith medical practices based on Indonesian Physician Competency Standards (SKDI)[8].

Initial assessment is an essential part of chronic disease management. It could measure prior medical history and support effective treatment. It is therefore, physician should apply standardized assessment and interview protocols.Therapeutic interactions carried out by physicians can increase mutual trust between physician-patient. Repeated interactions with physician allowed patients to develop positive attitudes and expectations based on the history of interactions[9-11].Further, physicians had difficulties in determiningaccurate diagnosesdue to the inability to explore patient complaints, as certain patients have poor acknowledgment of feelings. Communication competence and clinical skills are needed in carrying out the initial history. Feelings of anxiety, fear, or other negative feelings could develop into distrust between physician-patient. Additionally, effective communication techniques could promote patient involvement in decision-making process. Previous studies identified that half of the failures of diagnosing chronic diseases from the beginning could affect the complexity of the health services.Providing holistic and comprehensive approach in managing chronic disease could be significant methodwhen providing care[7,12,13].

Patient satisfaction is the main indicator of the standard of health facility, and the quality of health services.Additionally, patientsatisfaction with health services is very important because patients will adhere to treatment and intent to revisit health care facilitiesfor ongoingtreatment[14-15].Patient satisfaction can be determined by its quality of health services, equity to access to health services, promotive-preventive approaches, decision-making process, financing system, adequate information, waiting time of services.[14] Low patient satisfaction will have an impact on the number of visits; thus, it affects the quality of health services. Lack of professional attitude of physician also has an impact on reduced patient satisfaction. Furthermore,low satisfaction was related to lower trust to physicians, and in a long term, it had an impact on lower general life satisfaction. Measurement of patient satisfaction is an important element in providing better, more efficient and more effective services. Patients will be satisfied if the performance of the health services obtained is equal or exceeds their expectations and vice versa[16,17].

Prolanis can succeed and be sustained if health care services included health care teams effectively address patient needs as well as increasing the quality of health services. For this reason, physicians as key roles in primary health care should enhance

their competencies to deliver high-quality care. This study aimed to identify the impact of physician competencies on patient satisfaction in Indonesian primary health care.

## Materials and Method

This study was an observational analytic study with a cross-sectional design. The study was conducted at primary health care in East Java, Indonesia.Multistage random sampling was used to choose respondents in primary-level health facilities. 90 patients were chosen based on a diagnosis of chronic disease from medical records.The researchers asked the patients whether they agreed to participate in the study. Consent was signed after each respondent had been explained with regards to the study purpose. Ethical approval was gained from the Ethics Committee, Faculty of Medicine, University of Muhammadiyah Malang. Written approval from the study site was also obtained.

Questionnaires were given to 90 patients who were treated at the primary health care. Seven physician competencies were evaluated: professionalism, self-awareness and self-development, effective communication, information management, knowledge, clinical skills, and management of health problems. Patient satisfaction was measured based on the quality of health services performed by physiciansusing "Decree of the Minister of State for Administrative Reform Number 25/KEP/M. PAN/2/2004". There were five dimensions of health service quality: reliability, assurance, tangible, empathy, and responsiveness. The results of the validity test show r count >r table, where r = 0.370, so the questionnaires are valid. While the results of the reliability test using Cronbach's Alpha value of 0.770, it is considered reliable.Data were analyzed using the Lambda correlation test to see the relationship between variables. The instruments of physician competencies and patient satisfaction in primary health care as follows:

**Table 1: Instruments of Physician Competencies and Patient Satisfaction**

| **Physician Competencies** |
|---|
| ***Professionalism*** |
| Physician able to identify and manage patient complaints |
| Physician able to handle patient cases based on socio-cultural aspects |
| Physician able to pay attention to legal ethics in handling patient cases |
| Self-awareness and self-development |
| Physician able to listen to patient complaintsand find outthe reasons based on evidence-based practice |
| Physician able to look deeper into problems of each patient cases |
| Effective communication |
| Physician able to interact with patient effectively |
| Physician able to understand patient concerns, and opinions |
| Information management |
| Physician able to provide information about the disease to the patient |
| Physician able to use appropriate technology when providinghealth information to the patient |
| Knowledge |
| Physician know the patient's disease in detail |
| Physician know the management of chronic diseases at primary health care |
| Clinical skills |
| Physician able to assess symptoms of chronicdiseases |

***Cont...*** **Table 1: Instruments of Physician Competencies and Patient Satisfaction**

| **Physician Competencies** |
|---|
| Physician know standard precautions in the management of chronic disease |
| Management of health problems |
| Physician |
| Physician able to perform high-quality of chronic disease treatment |
| Patient Satisfaction |
| Reliability |
| Physician able to diagnose accuratelyand promptly on time |
| Patient feel better following treatment |
| Physicianexamine patientaround ± 15 minutes |
| Assurance |
| Patientis assessed by aphysician |
| Patient is examined carefully |
| Physician able to assess and treat patient holistic and comprehensively |
| No discrimination showed by physician |
| Tangible |
| Physician able to convince patient |
| Physician conduct inspection using high-quality tools |
| Physiciancharacteristics is well-known, using specific medical attributes |
| Empathy |
| Physicianable to provide attention to patients |
| Physician able to identify patient complaint |
| Physiciancan communicate well during patientcomplaints |
| Responsiveness |
| Physicianis responsive to manage patient complaints |
| Physicianis responsive to each patient complaint |
| Physiciancan communicate effectively during visite time |
| Physician provide adequateinformationto patients |

## Results and Discussion

The results of the study obtained data about the physician competencies, the level of patients' satisfaction and the influence of physician competencies on the level of satisfaction of chronic diseasepatients. These results can be shown in table 1 below:

**Table 2: Average Percentage of Physician Competency Domains and Patient Satisfaction**

(N = 90)

| Variables | Average Percentage (%) |
|---|---|
| **Physician Competencies Domains** | |
| Professionalism | 72 |
| Self-awareness andself-development | 70 |
| Effective communication | 78 |
| Information management | 71 |
| Knowledge | 74 |
| Clinical skills | 70 |
| Management of health problems | 80 |
| **Patient Satisfaction Based on Quality of Health Services** | |
| Reliability | 72 |
| Assurance | 74.2 |
| Tangibles | 72.8 |
| Empathy | 75.4 |
| Responsiveness | 73.8 |

**Table 3: Lambda TestResults for The Impact of Physician Competencies**

on Patient Satisfaction

| Hypothesis | Value | Error Standard | T[b] | Sig. |
|---|---|---|---|---|
| Patient satisfaction | 0.762 | 0.070 | 6.449 | 0.000 |

Based on table 2, physician competency related to the management of chronic diseaseswas the highest competency domain demonstrated by physicians in primary health care (80%). Meanwhile, the lowest competencywas in the domain of self-awareness, self-development, and clinical skills. The mastery of physicians' competencies in all domains has averaged 73.57%. From this study, it is known that the highest satisfaction of Prolanis patientswas in the dimension of empathy (75.4%) and the average patients' satisfaction in all dimensions was 73.64%. The results of Lambda correlation test in table 3 showed that physiciancompetencies could strongly influence the level of Prolanis patients' satisfaction with a correlation value of 0.76.

Rules and regulations with regards to physician competence in Indonesia were developed to improve the quality of care. Indonesian Physicians' Competency Standards (SKDI) is the standards'

reference for medical physicianin Indonesia. Based on the guidelines;physicians must have sufficient competence to be able to provide health services that in accordance with these regulations[18].

This study found that more than 70% of physicians in primary health careperform competencies in accordance with SKDI. Physician competencies in primary health care does not only assess patientcomplaints, but also includes providing motivation and encouragement so that patient can follow treatment effectively. Seven areas of physician competence in table 2 explained the need for holistic and comprehensive services to improve health services for Prolanis patients. Modification of a healthy lifestyle can be implemented gradually, started from developing emotional bonds between physicians and patients, empathetic attitudes of physicians in understanding patient problems. Then, physicians together with patients could find the best solutions to manage problems and being able to provide positive reinforcement on patient behaviour. The results of this study are also in line with previous study which stated that the quality of physician services in terms of technical abilities, interpersonal relationships and patient involvement positively influences the loyalty of outpatients in hospitals.Patient-centered carewas identified as a core element of health services quality. Previous study revealed that factors that influence patient-centered care were levels of education, occupation, and social class. This explained that low health literacy and socioeconomic statuswere still a great issuein most aspects of life[19-21].

The management of chronic diseases requires an increase in the quality of interactions between physicians and patients through five aspects: (1) leadership through promoting healthy behaviors, (2) knowledge by supporting changes in healthy behavior, (3) history skills, chronic disease management skills and (4) support from health teams and (5) foundations related through promotive, preventive and rehabilitative programs[19].Developing the ability to have a high self-awareness is part of the aspect of leadership that must be owned by a physician and this can be done through self-reflection, self-assessment and being able to receive feedback from others. This ability can build self-confidence and be able to be more consistent in decision making. So that physician can improve their communication skills effectively and be more open in addressing the problems faced by patients. Improving self-awareness could be done by spending time in self-evaluation and seeking feedback from others[22,23].In SKDI it has been stated that the competencyof physician includes the ability to carry out effective interpersonal communication, make an accurate diagnosis and conduct comprehensive management. This comprehensive management includes promotive, preventive, curative and rehabilitative efforts by involving patients in planning the treatment[24,25].

The results of the study in Table 2 showed that 73.64% of Prolanis patients expressed satisfaction with the quality of physician services. Health service quality is based on the RATER dimension which includes reliability, assurance, tangible, empathetic, responsiveness that is included in service quality. An empathetic attitude has the highest value of 75.4%. When physician provide services, empathy is done by giving full attention to the patient's problems and identifying the patient's needs in accordance with the results of the analysis carried out[26]. SERVQUAL was used to measure patients' perceptions of health services. It is a rating scale that includes several items that are concise with its reliability and validity, better understanding of service expectations and consumer perceptions which, as a result, improve the quality of health services. Previous study showed that five components of SERVQUAL: reliability, assurance, tangible, empathy and responsiveness were significant indicators to evaluate patients' satisfaction in the hospital[27-28]. This study showed that physician competencies have a strong influence on the patient satisfaction with a correlation value of 0.76. Thus, the more physicians master their competencies, the more confident chronic disease patients seek treatment at

primary health care.

## Conclusion and Acknowledgement

Managing chronic disease in Indonesia has been a challenging task for decades. Health professionals, such as physicians should be able to perform their competencies based on national standards to enhance chronic disease management programs in primary health care.It can be concluded from this study, physician competencies have a strong influence (r = 0.76) onpatient satisfaction.Providing high-quality of health services could encourage chronic disease patients to visit to primary health care, reduce hospital revenues, and minimize complications. We would like to thank all respondents as well as primaryhealth care in East Java for their contribution to this study.

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# Diabetic Ketoacidosis in Pregnancy: A Case Report

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## Abstract

A 31-year-old pregnant woman complained of vomiting, shortness of breath, fever, general weakness, painful and frequent urination with sign of hypertension, tachycardia, Kussmaul breathing. Laboratory studies showed neutrophilia, leukocytosis, hyperglycemia, hypokalemia, ketonuria, metabolic acidosis, low C-peptide, low Thyroid Stimulating Hormone, high FT4 and Staphylococcus in blood culture was diagnosed with Diabetic Ketoacidosis, pregestational Type 1 Diabetes Mellitus, hyperemesis gravidarum, 14 weeks of pregnancy, suspect Urinary Tract Infection, hypokalemia, hypertension, and subclinical hyperthyroidism. Besides, the patient blamed the fetus on causing the disease and financial problem so that the patient also was diagnosed with episode of moderate depression. The patient received KVT1 diet therapy, fluid replacement therapy, insulin therapy, correction of hypokalemia, correction of acidosis metabolic antibiotic and anti-hypertension. The therapies aimed to prevent maternal and fetal morbidity. Supportive psychotherapy therapy, relaxation therapy, and family psychoeducation were also needed to improve patient's compliant. The patient was also suggested to consult for family planning and glucose control before conception.

***Keywords:*** *Diabetic Ketoacidosis, Pregnancy, Diabetes Mellitus, Type 1 DM*

## Introduction

Diabetic ketoacidosis in pregnancy (DKP) is a serious condition causing several challenges. Incidence of Diabetic Ketoacidosis (DKA) among pregnant patients who have pregestational type 1 DM is 1.73%. DKP is related to maternal complications, such as acute kidney failure, acute respiratory distress syndrome, cerebral edema, coma and even death.(1) In recent years, the maternal mortality rate in DKA is less than 1% with a reported fetal mortality rate of 9-36%. Perinatal morbidity such as preterm labor, hypoxia, and acidosis remains high. (2) DKP requires immediate medical attention to prevent maternal and fetal morbidity and mortality. (2) (3)

Pregnancy is associated with physiological changes leading to DKA. (4) Pregnancy causes respiratory alkalosis associated with decreased compensation in bicarbonate levels. This impairs the capability of buffering so that pregnant women tend to have DKA. Relative insulin resistance in pregnancy along with increased lipolysis and increased free fatty acids. Increase of human placental lactogen, progesterone, and cortisol impair maternal insulin sensitivity. (5)

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A case of a DKP with T1DM are reported to discuss about proper treatment to avoid severe complications.

## Case Report

### History Taking

A woman, 31 years old, complained of nausea, shortness of breath, fever, painful and decrease in urination one week before admission.

Past medical history taking found DM since 19 years old. The last dose of insulin used is subcutaneous Aspart injection 34 units twice a day. During the first pregnancy in 2017, the patient also experienced the same complaint several times hospitalized and finally miscarried at 3 months of gestation. The patient had a history of hospitalization 4 times with the same complaints and treated in the intensive care unit (ICU).

At that time, the patient was on second pregnancy with a gestational age of 13-14 weeks. The insulin consumed was Aspart 6 units 3 times a day 15 minutes before eating and glargine 10 units in the morning and 18 units at night.

Frequently changed the insulin dose by herself, her compliance was not good. The patient also complained that she wanted to end her pregnancy because she thought that the fetus caused illness and economic problems.

### Physical Examination

The patient was compos mentis with general weakness. She weighed 50 kg and 160 cm in height. The body mass index was 19.53 kg/m$^2$. The vital sign examination found blood pressure (BP) of 90/60 mmHg, heart rate (HR) of 110 beats per minute with regular rhythm, Kussmaul breathing with respiratory rate (RR) of 26 times per minute, axillary temperature of 37.2$^o$C, and peripheral oxygen saturation of 99% using nasal cannula.

Physical examination showed normal head, neck, thorax, lung, heart, abdomen and extremity

Obstetrical examination found fundal height of 3 fingers above symphysis, fetal heart rate (FHR) of 148 beats per minute, no contraction. Vaginal examination showed no opening.

### Workup

Laboratory study found leucocytosis (18,330/ µL), neutrophilia (81.9%). Blood gas analysis (BGA) showed metabolic acidosis (pH 7.28; pCO2 21; pO2 205; BE -16,8; HCO3 9.9). Urinalysis found ketonuria (+3), proteinuria (+1), Leukosituria (10/ hpf) and Nitrituria (+), C-peptide of <0.01 ng/ml on the second day, and Procalcitonin 0,42 ng/ml, TSH 0.017mU/l, FT4 1.66ng/dL on the seventh day. Blood culture found bacteria *Staphylococcus haemolyticus* and urine culture did not found any germs.

Electrocardiogram (ECG) found sinus tachycardia rhythm (112x/min) with normal axis. Chest X ray was within normal limit.

### Diagnosi

According to the results of history taking, physical examination and workup examination, the patient was diagnosed with DKA, Pregestational T1DM, Hyperemesis gravidarum, G2P0010 13-14 weeks, suspect Urinary Tract Infection (UTI), hypokalemia, hypertension, Episode of moderate depression, and subclinical hyperthyroidism.

### Treatment

The patient was given central venous access to facilitate fluid administration. Diet planned was 2100 kcal of KVT1 diet. The fluid therapy given was 2 liters of NaCl 0.9% within 2 hours intravenously, followed by 80 dpm per 4 hours, 30 dpm per 18 hours. The insulin given to the patient was short-acting pump 1.5 IU per hour intravenously continued by 1 IU per hour on the second day and basal bolus regimen on the seventh day with adjusted dose according to the

BSL. Other treatment given were ceftriaxon 1 gram twice per day intravenously, 10 mg metoclopramide 3 times per day intravenously, vitamin B6 1 tablet once per day, 400 mcg folic acid twice per day, Calcium 500 mg twice per day. Premixed KCl 25meq and sustained-release potassium 3x600 mg orally were given on the second day due to hypokalemia. Sodium bicarbonate 250 mEq was given on the fourth day due to metabolic acidosis. Paracetamol 3x500 mg orally was given due to fever on the fourth day. Methyldopa 2x250mg orally was given due to hypertension. Phosphomycin injection 1x3000 mg was given intravenously on the seventh day based on the blood culture results. Spironolacton 1x50 mg orally was administered on the thirteenth day.

The patient was consulted to the Department of Psychiatry and diagnosed as episodes of moderate depression with somatic symptoms and was given supportive psychotherapy therapy, relaxation therapy, and family psychoeducation. Psychopharcologic treatment was postponed.

The patient was self-discharged against medical advice and suggested to visit endocrine outpatient unit. Patient was given subcutaneous detemir injection 18 units in the morning, subcutaneous aspart injection 10 units 3 times a day 15 minutes before meals, sustained release potassium 3x600 mg orally, Calcium tablet 1x500 mg orally, sodium bicarbonate 3x1 tablet orally, methyldopa 3x250 mg orally, folic acid tablet 3x1 orally.

One day after discharge, the patient's condition worsened and eventually miscarried and curetted. Eventually, the patient's condition improved and she was discharged.

## Discussion

ADA defines DKA as a triad of ketonemia or ketonuria, hyperglycemia and acidosis. [6] [7] The criteria for diagnosis of DKA are polyuria, polydipsy, nausea, vomiting, Kussmaul breathing, weakness, dehydration, hypotension to shock, awareness disturbed to coma accompanied by hyperglycemia (more than 300 mg/dL), hyponatremia (125-135 mEq/L), normal or hyperkalemia, normal magnesium, normal chlorine, hypophosphatemia, slightly increased creatinine, hyperosmolarity (300-320 mOsm/ml), Ketonemia (more than 3 mmol/L), low serum bicarbonate (less than 20 mEq/L), metabolic acidosis (pH less than 7.35; PCO2 20-30 mmHg), anion gap more than 10 mEq/L, glucosuria and ketonuria (more than 2+). [8] [9]

DKA can be triggered by specific factors such as prolonged vomiting, hyperemesis gravidarum, hunger, infections, non-compliance with the insulin use, beta sympathomimetic agents, steroid use, insulin pump failure and conditions such as diabetic gastroparesis. [5]

The patient in this case complained of nausea, vomiting, shortness of breath, fever, general weakness weaker, loss of appetite, painful and decreased urination. The patient had hyperemesis gravidarum, starvation, possible infection and inadequate use of insulin, which might trigger DKA. Physical examination obtained hypotension, tachycardia and Kussmaul breathing. Laboratory test found, hyperglicemia, metabolic acidosis, anion gap, ketonuria and hyperosmolality. Therefore, the patient was diagnosed with DKA.

According to the American Diabetes Association (ADA), DM is diagnosed based on several criteria, including HbA1C $\geq$ 6.5% standardized by the Diabetes Control and Complications Trial (DCCT) and is certified by the National Glycohemoglobin Standardization Program (NGSP) or fasting BSL $\geq$ 126 mg/dL or BSL 2 hours after an oral glucose tolerance test $\geq$ 200mg/dL or classic symptoms of hyperglycemia or hyperglycemia crisis with random BSL $\geq$ 200 mg/dL. [6]

Family history taking, measurement of autoantibodies to Langerhans islets, and measurement of plasma or urine C-peptide concentrations help to distinguish T1DM and T2DM.

C-peptide measurements represent a better alternative index of insulin secretion and residual ß-cell function. (10)

In this case, the patient was a woman, 31 years old, with classic clinical symptoms of hyperglycemia, history of DM since 12 years ago and consumption of insulin injection. BSL was 380 mg/dl, HbA1C was 7.4% and C-Peptide was 0.01 ng/ml. Therefore, the patient was diagnosed with T1DM.

One of the life-threatening complications of DM is DKA. The incidence of DKA has increased by 30% in the last few decades. DKA can occur in 15-20% of adult patients with type 2 DM. The mortality rate due to DKA reaches 5%, especially in patients with concomitant diseases.

Pregnant women have more risk of developing DKA than non-pregnant women. Pregnancy creates condition of respiratory alkalosis associated with decreased compensation in bicarbonate levels. This impairs capacity buffering so that pregnant women tend to have DKA. Relative insulin resistance in pregnancy along with increased lipolysis and increased free fatty acids. Increase of human placental lactogen, progesterone, and cortisol impair maternal insulin sensitivity. (5) Vomiting and use of betamimetic drugs also lead to DKA. Pregnant women with DM on chronic corticosteroid therapy have high risk of developing DKA due to increased serum glucose and risk of infection. (2)

Severe maternal dehydration and acidosis in DKA reduces uteroplacental perfusion. Severe maternal electrolyte disturbances result in maternal and fetal cardiac arrhythmias. Changes in fetal heart rate is a sign of acidosis due to maternal metabolic acidosis. (11)

DKA in pregnancy is considered as an emergency so that it has to be managed in high care unit (HCU) or ICU. The care provider team usually consists of obstetricians, endocrinologists, obstetric anesthesiologists, and trained nurses/midwives. There are 6 main management aspects that must be done simultaneously, including intravenous fluid therapy, intravenous insulin therapy, electrolyte correction, evaluation of the need for bicarbonate administration, identification and treatment of all trigger factors and monitoring. (3)

The goals of DKA management therapy include replacement of body fluids and salts, suppression of lipolysis and gluconeogenesis using insulin, overcoming triggering factors and restoring the normal physiological state. DKA therapy consists of 2 phases: phase I (emergency phase) and phase II (rehabilitation phase). The border between those phase is blood glucose of 250 mg/dL. (12)

Phase I DKA consists of fluid replacement, insulin rapid acting, potassium correction, acid-base correction, and antibiotics. Rehydration using isotonic fluid (ringer lactate or NaCl 0.9%) 2 liters in 2 hours, then 8 followed by 80 drops/minute in 4 hours, then continued with 30 drops/minute in 18 hours, and then with 20 drops/minute for 24 hours. This hydration formula is called "2-4-18-24" formula. Insulin is given using the formula "minus 1" for intravenous bolus or "times 12" for continuous infusion. However, insulin administration is delayed in hypokalemia. If the BGA showed pH $\leq$ 7.2 or HCO-3 <12 mEq/L, bicarbonate is given 50-100 mEq/500 ml in 24 hours intravenously. If pH $\leq$ 7.0, bicarbonate is given 50 mEq IV in 10 minutes, then 50 mEq is given in 2 hours.

Phase II consists of fluid maintenance, electrolyte maintenance, insulin and nutrition. If the patient is able to eat and ketosis is resolved, insulin is given with the formula of "times 2" subcutaneously. (13)

The targets of BSL in DM with pregnancy are fasting BSL of 80-110mg/dl, 1-hour post-prandial BSL of 100-155mg/dl, HbA1c of <7% without the risk of frequent hypoglycemia. (14) Upon admission, DKA patients require insulin infusion and close monitoring of electrolyte and BSL with subsequent transitioning to subcutaneous insulin and oral nutrition.

No recommendations exist regarding the appropriate timing for initiation of oral nutrition. Early reinstitution of oral nutrition did not result in worsening of DKA complications and was associated with improvement in ketoacidosis, hypokalemia and hypophosphatemia. Finally, on-demand oral nutrition reinitiated within the first 24 h of admission has the potential to shorten ICU and overall hospital lengths of stay. (15) However, the patient may meet difficulties in oral nutrition in the first 24 hours, including difficulty in BSL monitoring and insulin dosing, altered mental status predisposing to aspiration, and worsening of nausea, vomiting, and abdominal pain.

The patient was given 2100 kcal of KVT1 Diet. Fluid therapy given was 2 liters of NaCl 0.9% within first 2 hours, continued by 80 dpm in 4 hours, and 30 dpm in 18 hours. Other therapies given were short-acting insulin pump 1.5 IU/hour, continued by 1 IU/hour, and basal bolus regimen. Other therapies given are ceftriaxon 2x1gr intravenously, metoclopramide 3x10mg intravenously, sodium bicarbonate 250 Meq, NaCl 0.9% 500ml/24 hours, vitamin B6 1x1 tablet orally, folic acid 2x400 mcg orally, calcium 2x500 mg orally.

Sodium bicarbonate has been assigned to pregnancy category C by the FDA. Some authors recommend bicarbonate administration during severe acidemia (pH less than 7) or in patients complicated by cardiac dysfunction, sepsis, or shock. Administration of bicarbonate may be associated with profound alkalosis or worsening acidemia secondary to increased partial pressure of carbon dioxide, leading in turn to impaired fetal oxygen transfer. However, further research is required to assess the potential risks and benefits of such therapy. (5) (16)

Metabolic acidosis and insulin deficiency cause extracellular potassium movements, causing total body potassium levels decrease in spite of normal or increasing serum potassium level. The total potassium deficit is around 3-5 mEq/kg. Insulin therapy lowers causes potassium to return to the intracellular compartment. Thus, potassium replacement must begin when the serum concentration is less than 5.2 mEq/L to maintain levels of 4-5 mEq/L. Giving 20 to 30 mEq of potassium per liter of fluid is sufficient for most patients, but lower doses are needed for patients with acute or chronic kidney failure. In patients with serum potassium levels less than 3.3 mEq/L, insulin can cause severe symptomatic hypokalemia leading to impaired insulin secretion, muscle weakness and cardiac arrhythmias. Potassium replacement must begin 10 to 20 mEq/h and insulin therapy must be postponed. (7) Serum potassium monitoring is done every 2-6 hours according to the clinical and patient response. Indications of potassium replacement are serum potassium <3.3 mEq/L, or serum potassium <4 - 5 mEq / L accompanied by urine output > 50 mL/hr with the addition of 20 - 40 mEq potassium every 1 liter of infusion fluid. (4) (17)

Potassium sustained release has been assigned to pregnancy category C by the FDA. There are no human data related to use of potassium sustained release during pregnancy, and animal reproduction studies have not been conducted. Potassium supplementation that does not lead to hyperkalemia is not expected to cause fetal harm. (18)

The decision to terminate is individualized which must be based on evaluating the clinical status of the mother to ensure safe delivery, gestational age of the fetus, and fetal heart rate. The most DKP aims to maintain the fetus and mother in stable state to continue the pregnancy with complete DKA resolution. (3)

The patient had TSH of 0.017 mU/L and FT4 of 1.6ng/dL, showing subclinical hyperthyroidism. In pregnancy, high human chorionic gonadotropin (hCG) levels supresses TSH. The syndrome of gestational hyperthyroidism is defined as transient hyperthyroidism, limited to the first half of pregnancy, characterized by normal or borderline elevated FT4 or adjusted TT4 and suppressed or undetectable serum TSH. This causes thyrotoxicosis in pregnancy. It is diagnosed in about 1–3% of pregnancies, caused by a

TSH-like effect of the hCG and self-limiting disorder, resolving spontaneously. [19]

Many people with T2DM fail to achieve glycaemic control promptly after diagnosis due to clinical inertia related to the physician (50% relative contribution), the patient (30%) and the healthcare delivery system (20%). Potential solutions require a multiple approaches involving fundamental changes in medical care due to multiple factors leading to clinical inertia. [20]

In this case, patient compliance is low and strict examinations are rather difficult. Likewise, correction of hypokalemia should be more aggressive, especially patients already with central venous access. But electrolyte testing every 6 hours is also rather difficult.

Girls with T1DM should consult about metabolic control and family planning prior to conception in order to prevent DKP. [10]

## Conclusion

DKP may lead patient to maternal and fetal morbidity and mortality. Pregnancy also lead the patient to insulin resistance and decrease ability to physiologically buffer metabolic acidosis, which worsen patient with DM. Prompt treatments, including fluid replacement, insulin therapy, potassium correction, acidosis correction, and management of triggering factors are needed to prevent morbidity and mortality. Besides, family planning and metabolism control before conception are required to prevent women with DM from falling into DKA.

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# In Vitro Antibacterial Activity of Waste Palm Cooking Oil Against *Staphylococcus Aureus*

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## Abstract

**Background:** *Staphylococcus aureus* is a Gram-positive coccus which acts as a pathogen causing a wide range of infectious diseases. In recent years, several strains of *S. aureus* have been found to show resistance to several antibiotics. Waste cooking oil may be considered as an alternative antibacterial product, as it contains long-chain fatty acids whose antibacterial effectiveness against *S. aureus* has been known for years. In addition, oxidative biocides produced during the frying process have many targets for antibacterial activity in the cell and affect almost every biomolecule. Nonetheless, there is no literature that is able to prove the antimicrobial effects of the waste palm cooking oil. **Objective:** To examine the in vitro antibacterial effect of waste palm cooking oil against *S. aureus.* **Method:** a two-fold serial dilution method to set the minimum level of both inhibitory and bactericidal concentrations. **Conclusion:** This study showed that waste palm cooking oil did not show antibacterial effects against *S. aureus,* indicating that waste palm cooking oil is not possibly to be applied as an antibacterial agent against *S. aureus*.

***Keywords:*** *Staphylococcus aureus; waste palm cooking oil; minimum bactericidal concentration; minimum inhibitory concentration; antibacterial*

## Introduction

Infectious diseases are the top cause of health problems in developing countries, including Indonesia, and infection is associated with morbidity and mortality[1] .*Staphylococcus aureus* is one of the bacteria that can cause various kinds of infectious disease, including meningitis, pneumonia, endocarditis, and necrotizing fasciitis, but it most often makes soft tissue and skin get infected[2]. In the last decade, it was recorded that there was a significant increase of infection cases caused by methicillin-resistant *Staphylococcus aureus* (MRSA), affecting the cost of treatment and infection control. In recent years, several strains of *S. aureus* have been found to have developed resistance to antibiotics[3]. In 2014, the World Health Organization (WHO) reported data from 85 member countries on antibiotic resistant by *S. aureus*, revealing that antibiotic-resistant *S. aureus* was identified in over 20% of cases in the WHO region, even exceeding 80% of cases in some reports[4].

Therefore, it will be attracting to have the alternative products promoting antibacterial activity while affecting less side effects either in animals or humans. They are also needed since the bacteria start to be resistant to the antibiotics currently used. One of the alternative products with antibacterial activity being considered is waste palm cooking oil, which contains long-chain fatty acids[5]. For many years, it has been acknowledged that long-chain fatty acid promotes antibacterial activity. In Indonesia, many myths about healing wounds are still believed by many Indonesians to this day, and

one of these myths is that applying cooking oil to a body part that has a wound or burn can promote healing. In South Africa, linoleic acid and oleic acid are antibacterial components commonly utilized in the wound healing process of male circumcision[6]. In addition, long-chain unsaturated fatty acids have been considered bactericidal against imperative pathogenic microorganisms, in this case, including MRSA[7]. This study aims to evaluate the antibacterial activity found in the waste palm cooking oil against *S. aureus* by considering the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) for this substance.

## Materials and Methods

### Waste palm cooking oil

Waste palm cooking oil with various frying time was collected randomly from various households and food vendors in Banyuwangi, Indonesia. The waste palm cooking oil to be used in this study was prepared by dissolving waste palm cooking oil in Mueller Hinton Broth (CM0405, Oxoid, UK) and emulsifying with Tween 80 to ensure the sufficient dispersion into an emulsion. The tested sample shows that the last solvent concentration did not surpass 1%; as a result, bacterial viability was not affected by the presence of solvents[8]. The mixture was prepared using a vortex.

### *S. aureus* strains

*S. aureus* was provided by the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga. This study used *S. aureus* concentration of $1\times10^8$ CFU/ml, 0.5 McFarland standard.

### Minimum inhibitory concentration and minimum bactericidal concentration

The antibacterial activities of the waste palm cooking oil were determined by using two-fold serial dilutions. The waste palm cooking oil were diluted with Mueller Hinton Broth to the following concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% (v/v). To each tube, 1 ml of bacterial suspensions and 1% Tween 80 as an emulsifier was added. In this study, the positive control comprised Mueller Hinton Broth medium with bacteria that have been tested, while the negative control comprised Mueller Hinton Broth with waste palm cooking oil and 1% Tween 80 which was incubated for 24 hours at 37°C. MIC can be defined as the lowest antimicrobial agent concentration inhibiting visually 99% development of microorganism. To determine the MIC, tube visual turbidity was investigated before and after the incubation process. Meanwhile, the experiment was performed in three circles in order to make sure the tested bacteria values.

Once MIC has been determined, the isolates from each tube were streaked on a nutrient agar plate (NAP) (CM0003, Oxoid, UK) using a sterile wire loop and incubated at 37°C for 24 hours. In this case, the absence or presence of bacterial development in agar plates before and after incubation can be used to determine the MBC. The lowest antimicrobial agent concentration that kills 99.9% of the early bacterial population indicates the MBC endpoint.

## Results and Discussion

### Bacterial strain

Colony identification was performed using the blood agar and Gram staining methods. The bacterial colonies were thawed and grown for two days on blood media agar at 37°C. Gram staining was used to see the shape of bacteria through observation under an light microscope using 1000 x magnification.

### Contamination test of waste palm cooking oil samples

The contamination test was performed by streaking the study samples onto the NAPs and incubating at 37°C for 24 hours. Of the six samples collected randomly, two samples were contaminated with *Bacillus subtilis,* as demonstrated using the Gram staining method. This study used the four uncontaminated samples.

Minimum inhibitory concentration and minimum bactericidal concentration tests

Both MIC and MBC of waste palm cooking oil against *S. aureus* were determined using a serial dilution method. After the process of incubation at 37°C for 24 hours, the MICs were determined, as shown in Table 1. In this study, the MIC samples did not show a significant difference in turbidity and clarity before and after treatment. Thus, the MIC of waste palm cooking oil against *S. aureus* was absence.

Table 2 shows the MBC test results indicating the absence or presence of bacterial growth which is denoted as + or –, respectively. Suspensions from the tubes were streaked on a NAP and incubated at 37°C for 24 hours. *S. aureus* growth was obtained at all waste palm cooking oil concentrations. Thus the MBC of waste palm cooking oil against *S. aureus* was absence.

**Table 1. Minimum inhibition concentrations (MIC) of waste palm cooking oil after 24 hours.**

| **Dilution of waste palm cooking oil.** | | **Concentrations % (V/V)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sample No.** | **Set** | **1.00** | **0.5** | **0.25** | **0.125** | **0.0625** | **0.03125** | **0.0156** | **0.0078** |
| 1 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 2 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 3 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 4 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |

Negative sign (-) indicates there was no turbidity or absence of growth, while Positive sign (+) indicates there was turbidity or presence of growth.

**Table 2. Minimum bactericidal concentrations (MBC) of waste palm cooking oil after 24 hours**

| **Dilution of waste palm cooking oil.** | | **Concentrations % (V/V)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample No. | Set | 1.00 | 0.5 | 0.25 | 0.125 | 0.0625 | 0.03125 | 0.0156 | 0.0078 |
| 1 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 2 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 3 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |
| 4 | Set 1 | + | + | + | + | + | + | + | + |
| | Set 2 | + | + | + | + | + | + | + | + |
| | Set 3 | + | + | + | + | + | + | + | + |

Positive sign (+) indicates the presence of growth, while Negative sign (-) indicates the absence of growth

When the development of bacteria at various concentrations of waste palm cooking oil was evaluated after 24 hours, both MIC and MBC of *S. aureus* were absence at any concentration, indicating that waste palm cooking oil has neither bacteriostatic nor bactericidal activity (Table 1 and 2).

Although various bacterial infections have been for years treated with antimicrobials, its repetitive use may increase antimicrobial resistance[9]. Thus, alternative products with antibacterial activity have attracted attention. One of the alternative products being considered for this purpose is waste palm cooking oil because it contains long-chain fatty acids that have potential effectiveness against bacteria. The objective of this study is to assess the antibacterial effects of waste palm cooking oil against *S. aureus* using MIC and MBC tests, which are commonly used to assess the antibacterial effects[10]. The MIC is considered the lowest antimicrobial concentration which is able to prevent the obvious growth of microorganism after the incubation for 24 hours. Furthermore, MIC tests are believed to be the ultimate procedure to decide the organism vulnerability to antimicrobial agents[11]. The MBC, on the other hand, is considered the lowest antimicrobial concentration which is able to prevent the development of an organism and is determined by assessing the clear microbial inhibition zone (the zone without microbial growth) in the media by visual observation[11]. The MIC and MBC are used to determine potential antimicrobial resistance and to make rational decisions in determining how successful an antimicrobial treatment is likely to be[11].

The bacterial strain selected for this study is *S. aureus*, a non-motile, Gram-positive coccus[12]. Furthermore, it is one of the pathogens which is able to be source of various infectious diseases[13]. In addition, in recent years, several strains of *S. aureus* have been found to show resistance to several antibiotics[3].

Waste palm cooking oil contains some long-chain fatty acids, namely: oleic (28.64%), palmitic (21.47%), linoleic (13.58%), stearate (13%), palmitoleic (7.56%), and other compositions (8.04%)[5]. It is obvious that, for many years, long-chain fatty acids have antibacterial activities. They serve as the main additive ingredient of antimicrobial food which prevent the development of undesirable microorganisms[14]. Long-chain fatty acids (C>16) have bactericidal effects for pathogenic microorganisms, including MRSA**.** Linoleic acid and oleic acid, for example, show antibacterial activity as well as synergistic effects on *S. aureus* inhibition[6]. Linoleic acid inhibits bacterial enoyl-acyl carrier protein reductase (FabI) which is an encouraging target for antibacterial drugs since it is an important element of bacterial fatty acid synthesis[15]. Meanwhile, oleic acid was reported as a killer agent of *S. aureus,* and it has a natural protective effect against the primary adhesion stage in *S. aureus*[16]. Oxidative biocides, including hydrogen peroxide and chlorine, are the result of repeated frying processes and have multiple targets in cells and approximately in each biomolecule, including enzyme inhibition, oxidation of scavenger and thiol groups, peroxidation and disruption of membrane layers, disruption of energy production, disruption of protein synthesis, nucleoside oxidation, and, ultimately cell death[17].

In this study, the MIC and MBC of waste palm cooking oil were determined using an in vitro serial dilution method, and this substance was found to have no antibacterial effect at any concentration. Thus, the hypothesis of this study was rejected. This is the first study in the literature to assess the antibacterial effect of waste palm cooking oil against *S. aureus*. Another study evaluated the antimicrobial effect of palm kernel oil and palm oil with the agar diffusion method and obtained results similar to ours: Their results of this study show that the five microorganisms tested, including *S. aureus,* did not show any inhibition[18]. This could be because palm oil contains a diverse combination of fatty acids. Furthermore, it is presumably considered that these compounds make interaction since there is an action mechanism which finally decrease the antibacterial activity of their oils. In addition, the antibacterial activity of palm oil decreased because the release from triglycerides was incomplete during cleavage by lipase[8].

In conclusion, waste palm cooking oil showed no antimicrobial activity against *S. aureus*. However, this study has a limitation: because the sampling was done randomly, the composition of each waste palm cooking was not known.

## Conclusion

Based on the study result, the MIC of waste palm cooking oil against *S. aureus* was absence because there was no significant difference in clarity and turbidity at any concentrations. And, the MBC was also absent, because *S. aureus* at any concentration continued to grow. Thus, waste palm cooking oil has no antibacterial activity against *S. aureu*s. Further research is needed by using a variety of bacteria and different types of oil to determine the inhabitation which potentially found in the waste palm cooking as an antimicrobial.

**Ethical Clearance:** This experimental study protocol had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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# The Implementation of Fraud Prevention on the National Health Insurance at Salewangan Maros Hospital, Indonesia: A Qualitative Study

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## Abstract

Background: Various fraud control methods are designed to overcome potential fraud that occurs by every element that involved in the National Social Health Insurance System in Indonesia. This study aims to analyze how the implementation of fraud prevention efforts that have been carried out by the National Health Insurance (JKN) Fraud Prevention Team which was formed at the SalewanganMaros Regional Hospital. Method: This study used a descriptive qualitative approach with data collection techniques through in-depth interviews and observation. The informants were the Head of the Service Division, the Chair of the Hospital Medical Committee, the Head of the Nursing Sub-Division, the Head of the Finance Sub-Division, and the Casemix team.**Results:**Efforts to increase the fraud prevention is still weak, efforts to detect and resolve fraud are already underway, but efforts to detect fraud have not been continuous, detection through observation at service locations is not routinely carried out, while detection efforts through data analysis still rely on service data feedback obtained from BPJS, as well as monitoring and evaluation by the Fraud Prevention Team at SalewanganMaros Regional Hospital has been implemented but is still very poor.Conclusion: Fraud prevention efforts must be carried out comprehensively and involve all individuals. There must be an automated integrated system at each service point so that fraud prevention can be optimized.

***Keywords:*** *Fraud Prevention, Indonesia, National Health Insurance*

## Background

Indonesia's health coverage program, the National Health Insurance (JKN) program, is administered by Health Care and Social Security Agency (BPJS). A membership comprising 222.8million people in April 2021, the program is one of the biggest health insurance in the world [1]. Fraud can be seen in all insurance types including health insurance. Fraud in health insurance is done by intentional deception or misrepresentation for gaining some shabby benefit in the form of health expenditures [2, 3]. In the United States, which is a developed country, reported by the General Accounting Office (GAO) in 1990, claims for indications of fraud were recorded at around US $ 100 billion or 10% of the total health costs per year [4].

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Further, based on a survey conducted by the Association of Certified Fraud Examiners (ACFE) in 2010, fraud on Health Care was in fourth place at 7.3%, and Indonesia was in third place with the highest number of cases out of 30 countries surveyed[5]. Fraud on Health Service Providers (PPK), especially Hospitals can be caused by Hospital dissatisfaction with INACBG rates (Indonesia Case Base Groups), an application used by hospitals to file claims to the government) and unpreparedness of Information Technology systems in Hospitals. In addition, the motivation to seek "economic gain" could lead PPK to commit fraud[6].The causative factor itself consists of high unmet needs and low salaries received which can result in employees taking actions that have the potential to be fraudulent[7, 8].

In addition, if aninternal control of the company's is work weakly, the possibility of errors and fraud is increasing[9, 10]. As an example fraud can result in losses for Manggala Regional Hospital in Indonesia because claims for services that have been performed cannot be paid [6]. A research was conducted by in-depth interviews in Indonesia, the interviewed informants had knowledge regarding the control of potential fraud at dr. AchmadMoechtar Bukit Tinggi, the results of the interview were the factors in the potential for fraud at dr. AchmadMoechtar as follows; differences in payment systems, the INA CBGs payment system is a prospective payment system, while the payment system for dr. AchmadMoechtar Hospital still uses the Governor Regulation Number 58 of 2015 on Health Service Rates for dr. AchmadMoechtarBukittinggi by using a fee-for-service payment system. Factors that can hinder the potential for fraud at dr. AchmadMoechtarBukittinggi, by implementing Standard Operational (SOP) and Clinical Pathway can prevent potential fraud, Based on in-depth interviews from informants, RSUD Dr. Low birth weight (LBW) and non-haemorrhagic stroke [9].

Furthermore, various fraud control methods are designed to overcome potential fraud that occurs by every element that involved in the National Health Insurance System in Indonesia. Through presidential regulation number 82 of 2018 and its derivate regulations, it is mandatory for Stakeholders, Health Department, BPJS,and Health Facilities which cooperating with BPJS to build a fraud prevention system and done systematically, structured, and comprehensive by engaging all human resources.

Taking into account the findings from the audit of the State Development Audit Agency(BPKP) at the SalewanganMaros Regional Hospital in 2018 with the result that there were 51 cases, including 6 cases of readmission and 45 cases of multiple claims (fragmentation, unbundling, cloning), even more there are problems with delays in claim submissions which is indicate that there a problems of governance system at SalewanganMaros Hospital in the year of 2020.

As mention in the Presidential Regulation number 82 of 2018 article 93 paragraph 3 states that BPJS, Health Department and Hospital must build fraud prevention system through, developing policies and guidelines for fraud prevention, developing an enhancement fraud prevention culture, developing the high quality and cost oriented health services and forming a fraud prevention team. Then as a form of obedience on that regulation, fraud prevention teams that was formed at the SalewanganMaros Regional Hospital based on the Director's Decree of the Fraud Prevention Team at the SalewanganMaros Regional Hospital in 2020, Number 197/24 / RSUD / 2020, the duties of the Fraud Prevention Team is Conduct early detection of JKN fraud based on data on health service claims conducted by the hospital. Disseminating new policies, regulations, and culture oriented towards quality control and cost control. Encourage the implementation of good organizational governance and clinical governance Increase the ability of Coder and Doctors and other officers related to claims Make efforts to prevent, detect and prosecute JKN fraud monitoring and evaluation.Based on the background above this study aims to analyze how the

implementation of fraud prevention efforts that have been carried out by the National Health Insurance (JKN) Fraud Prevention Team which was formed at the SalewanganMaros Regional Hospital.

## Methods

This is a descriptive qualitative study thatusedtypes of data such as opinions, facts, knowledge, with data sources from ordinary informants and key informants. Data collection techniques were used in-depth interviews with a structured interviews guideline. The data analysis technique was carried out in three stages; first, reducing the data, second describing the data, and third is making conclusions. Data analysis begins with preparing and organizing data (text data in the form of transcripts or documents) for analysis, then the next stage is done by reducing the data to themes through the coding process, and summarizing the code and the final stage presenting the data in the form of analysis.To ensure the validity of the data in this study, researchers used triangulation by validating information by looking at the consistency of information obtained by researchers through in-depth interviews. The data analysis using Nvivo 12 (a qualitative software).

## Results

**Table 1. The Empirical findings of the improvement of the fraud prevention culture**

| Respondent | Fraud Prevention Culture |
|---|---|
| FA | Claim verification control is not layered between casemix team and fraud prevention team<br>Dispute claim remain |
| IA | Delay in payment of services fees<br>No socialization of fraud prevention in the hospital and no technical meeting to discuss the teams duties |
| YU | The audit findings and result received and executed according to the provisions without discussing the prevention how should it not be repeated |

**Table 2. The empirical findings of the implementation of organizational governance and clinical governance**

| Respondent | Good Organizational Governance and Good Clinical Governance |
|---|---|
| IA | Claim submission to the Payer (BPJS) is delayed in process.<br>Consumable medical material less provide about 70% and it is mostly substitute which low quality below the standards |
| FA | Clinical pathway is not complete |
| SW | Medical record and Information system is still combining manual and digital process |

**Table 3. The empirical findings of the detection and settlement of fraud (fraud) at the hospital**

| Respondent | Detection and settlement of fraud |
|---|---|
| IA | Information System did not provide utilization data for analysis<br>Patients complaint did not fully conveyed |
| FA | Whistle blowing system did not provide by the management and fraud prevention teams. The Employee have no access and remain afraid of the action consequences due to the fraud potential reported |
| SR | Bed occupancy rate is not being analyzed to the amount of population and not customized with the length of stay of each patient. |

**Table 4. The empirical findings monitoring and evaluation carried out by the Hospital fraud prevention team**

| Respondent | Monitoring and evaluation |
|---|---|
| IA | Field observation direct to the services point's was not routinely implemented<br>There is no task details, technical instructions or implementation guidelines was made to help the teams. And no plan of action arranged.<br>Medical committee consisting of the specialist and subspecialist doctors was not attained the meeting to discuss fraud prevention due to limited facilitation. |
| FA | No person in charge to do the monitoring routinely<br>Some member of the teams have current positions and hard to manage time and people |

The fraud prevention team at SalewanganMaros Regional Hospital, which consists of management (structural) elements, the Hospital Internal Supervisory Unit (SPI), the Medical Committee and Casemix have tried to prevent fraud, but some things are still not optimally implemented. Referring to the research objectives and interview result, we found 4 major findings:

The first, efforts to increase the fraud prevention that are being carried out are still poor and not working well. The difficulty of managing schedules with the various activities of each team and the conditions of the COVID-19 pandemic that occurred in 2020 has made it even more difficult. Bringing together a team of structural elements with a team of functional elements. Cultural changes in fraud prevention have not been initiated by the management and fraud prevention team. Anti-fraud commitment signatures, anti-fraud posters and other new things have not been implemented. There are still several factors inhibiting the culture of fraud prevention, such as a lack of desire to change, indifference, busyness, and lack of communication. Fraud is considered taboo to discuss, because it damages self-image and organization. So it tends to be discussed in private and confidential[2].

Secondly, the implementation of good organizational governance and clinical governance in the hospital is quite good, but needs to be improved in several aspects. Efforts to apply the principle of accountability are shown by the availability of standard operating procedures, but clinical guidelines for all disease management are incomplete. This is slightly different in the application of the principles of openness and accountability related to filing claims and calculating medical services. Claims submitted late and not in accordance with the provisions of the submission routine. Meanwhile, some specialist doctors have difficulty calculating the action compared to the medical services received, and also complaints about the delay in distributing medical services[11, 12].

Thirdly, efforts to detect and resolve fraud are already underway, but efforts to detect fraud have not been continuous, detection through observation at service locations is not routinely carried out, while detection efforts through data analysis still rely on service data feedback obtained from BPJS. It still needs hard work from the fraud prevention team to sit down together to formulate structured preventive measures, create a whistleblower that is safe, reliable and confidential. Fraud settlement is carried out after the audit findings, by deducting claims, which will result in reduced operational costs, difficulties in financial management and even losses for the hospital. Therefore, it can also be seen that the higher the number of fraud findings, the lower the team's performance in preventing fraud[11].

Fourth, monitoring and evaluation by the Fraud Prevention Team at SalewanganMaros Regional Hospital has been implemented but is still very poor. Monitoring is carried out by officials in concurrent positions, there are no detailed tasks yet to carry out monitoring in any way. Meanwhile, the evaluation has not been carried out continuously. The difficulty in evaluating was due to the difficulty of managing time and gathering all teams to conduct regular meetings and evaluations.

In addition, the driving actors for the potential for fraud at the SalewanganMaros Regional Hospital are the lack of understanding regarding fraud, specialist doctors, verifiers, coders do not fully understand the use of the state budget that must be accounted for and patients are consumers who must be provided with quality, effective and efficient services. Complaints about real hospital rates with INA CBGs rates also become a rationalization for fraud. If there is a dispute of opinion regarding the determination of whether or not JKN fraud exists, the Provincial Health Office or District / City Health Office can forward the complaint to the JKN Fraud Prevention Team formed by the Minister.

## Discussion

According to Priantara (2013: 48), the fraud triangle consists of three conditions that are generally present when fraud occurs, namely: 1). Pressure to commit fraud (pressure). Pressure can be divided into 4 types, namely: financial problem, being involved in a crime or not in accordance with the norm, work-related stress and other pressures. 2). Opportunity or opportunity to commit fraud (opportunity); weak internal control system, poor organizational governance and a pretext to justify action (rationalization). Rationalization occurs because someone seeks justification for activities that contain fraud. Fraud perpetrators believe or feel that their action is not a fraud but is something that is their right, sometimes even the perpetrator feels that he has done a lot for the organization[13, 14].

Fraud prevention system according to Ministry of Health (Permenkes No. 36 of 2015) namely: 1).advanced level of healthcare facilities(FKRTL) compiles internal regulations in the form of good organizational governance and clinical governance. 2) FKRTL is able to develop health services that are oriented towards quality control and cost control through the use of effective and efficient management concepts, evidence-based information technology and forming the JKN Fraud Prevention Team at the FKRTL. 3) FKRTL is able to develop a JKN fraud prevention culture as part of organizational and clinical governance oriented towards quality control and cost control based on the principles of TARIK (transparency, accountability, responsibility, independence and fairness) [6, 15].

Based on Permenkes No. 36 of 2015, the JKN Fraud Prevention Team at the FKTP took action against JKN fraud and resolved the JKN dispute settlement based on a report or discovery. As stated in Article 16 Permenkes No. 36 of 2015, they are:

1). Transparency is the openness of information, both in the decision-making process and in disclosing information in accordance with the need for the

prevention of JKN fraud. 2). Accountability is the clarity of the function of the system structure and service accountability so that management is carried out effectively. 3). Responsibility is conformity or compliance in service management with the principles of a healthy organization in the context of preventing JKN fraud. 4). Independence is a condition in which an organization is managed professionally without conflict of interest and influence or pressure from any party that is not in accordance with the principles of a healthy organization in the context of preventing JKN fraud. 5). Fairness is a fair and equal treatment in fulfilling stakeholder rights arising from an agreement in the context of preventing JKN fraud[15].

Furthermore,Permenkes No. 36 of 2015, guidance and supervision of the prevention of JKN fraud at the Menggala Hospital has involved hospital supervisory bodies, hospital supervisory boards, hospital associations / associations, and professional organizations. One of the components in the supervision is to oversee compliance with the application of hospital ethics, ethics profession, and laws and regulations including Permenkes No. 36 of 2015.For complaints of alleged JKN fraud, it must include at least: the identity of the complainant, the name and address of the agency suspected of carrying out JKN fraud, and the reasons for the complaint (Permenkes No. 36 of 2015). With the JKN fraud complaint, the head of health facilities, the District / City Health Office and / or the Provincial Health Office must follow up on the complaint by conducting an investigation. The investigation was carried out by involving BPJS, the JKN Fraud prevention team at hospital[15].

## Conclusion

The fraud prevention teams at SalewanganMaros Regional Hospital, which consists of management (structural) elements, the Hospital Internal Supervisory Unit (SPI), the Medical Committee and Casemix have tried to prevent fraud, but some things are still not optimally implemented.

The driving factors for the potential for fraud at the SalewanganMaros Regional Hospital are the lack of understanding regarding fraud, specialist doctors, verifiers, coders do not fully understand the use of the state budget that must be accounted for and patients are consumers who must be provided with quality, effective and efficient services. Complaints about real hospital rates with INA CBGs rates also become a rationalization for fraud. Cultural changes in fraud prevention have not been initiated by the management and fraud prevention team. Anti-fraud commitment signatures, anti-fraud posters and other new things have not been implemented. There are still several factors inhibiting the culture of fraud prevention, such as a lack of desire to change, indifference, busyness, and lack of communication. Fraud is considered taboo to discuss, because it damages self-image and organization image. So it tends to be discussed in private and confidential.

**Recommendations**

The fraud detection need tools and big data analysis to be able to find where the place is over loss or fraud happened at SalewanganMaros Hospital. SalewanganMaros Hospital need to strengthen the hospital information system, digitalization of the patient registration and automation and implementation of e-medical records..

Lack of routinely monitoring is carried out by officials in concurrent positions, and there are no guidelines made or detailed tasks to carry out monitoring in any way. Meanwhile, the evaluation has not been carried out continuously. The difficulty in evaluating was due to the difficulty of managing time and gathering all teams to conduct regular meetings and evaluations.

The fraud prevention team needs to be more intense in holding meetings or compiling a work plan, formulating matters or findings submitted by the auditor, then discussing steps to prevent it to happening again. The prevention team determines future action

plans, establishes appropriate control mechanisms according to hospital conditions, then also establishes a path for complaints (whistleblowing system) of violations or fraud through media, correspondence or email.

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# Comparative Determination of Chlorpromazine in Pharmaceutical Injectable Veterinary and Human Formulations by Spectrophotometric and High Performance Liquid Chromatographic Methods

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## Abstract

**Background:** Chlorpromazine (CPZ) is a phenothiazine tranquilizer used in humans and animals. Rapid determination of the drug in pharmaceutical preparations is often needed. The aim of the study was to further examine and ascertain asimple spectrophotometric method in comparison with a high performance liquid chromatographic (HPLC) method for the determination of CPZ concentrations in injectable pharmaceutical formulations used in man and animals. **Methods:** Concentrations of CPZ in injectable pharmaceutical formulations for human and veterinary uses were determined by a modified spectrophotometric method and by an HPLC method with appropriate standard calibration curves. The spectrophotometric method of CPZ determinationwas conducted by diluting 0.1 ml of the veterinary (5%) or 0.3 ml of human (0.5%) formulations to 200 ml of 0.1N sulfuric acid. Four ml of the diluted CPZ samples or the standard solutions were mixed with 2 ml of 50% sulfuric acid. Then an aliquot of 0.2 ml of 2% ferric nitrate was added to the mixture. After 15 minutes, the absorbance was measured at 530 nm against water and vehicle blanks using a spectrophotometer. The HPLC method applied in the present study consisted of reversed phase gradient chromatography with a $C_{18}$ column and the eluent was (A) 50 mmol $NaH_2PO_4$, pH 2.5 and (B) acetonitrile - 50 mmol $NaH_2PO_4$, pH 2.5 (60:40 v/v), flow rate at 1 ml/min, with a gradient of 15-55% B in 10 min, to 100% B in 20 min, and 280-nm UV detection. From the calibration curves of CPZ standards, the following were calculated: Limit of detection limit (LOD) = 3.3 x SD/slope; Limit of quantitation (LOQ) = 10 x SD/slope, where SD is the standard deviation of response of CPZ concentrations of the calibration curves.Linear regression analysis and related coefficients of correlations were applied on the calibration curves. **Results:** The calibration curves of CPZ against water or vehicle blanks as determined by the spectrophotometric method or by the HPLC method were linear with strong correlations (r = and / or >0.99). Based on the calibration curves, limit of detection and limit of quantitation the spectrophotometric method were comparable to those of the HPLC one. The contents CPZ in human and veterinary injectable formulations as determined by the spectrophotometricmethod were in accordance (100.6% and 102%, respectively)to the concentrations claimed on the labels of the formulations.

**Conclusion:** The present results introduce a simple spectrophotometric method that could be used for routine measurement of CPZ concentrations in pharmaceutical formulations, with the added benefits of linearity, precision and cost effectiveness.

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***Keywords:****Chlorpromazine, colorimetric, HPLC, pharmaceutical preparation, phenothiazine tranquilizer*

## Introduction

Chlorpromazine (CPZ) is a neuroactive phenothiazine tranquilizer used in humans[1]and in various animal species.[2] The injectable formulation of the drug is applied clinically in veterinary practice on the basis of extra-label use with acceptable therapeutic efficacy and margin of safety.[2,3]Injectable aqueous veterinary formulations of CPZ (1 and 2.5%) have been described, with a modified spectrophotometric method to determine the concentration of the drug in these formulations.[4,5]

Aspectrophotometric method was originally described to determine CPZ in biological fluids after certain elaborate extraction steps.[6]Thereafter, the method wasreportedly furthersimplified and modified to be anadopted procedure under conventional laboratory conditions for rapid determination of CPZ in injectable veterinary formulations.[4,5]Other more elaborate analytical spectrophotometric[6-11]and high performance liquid chromatography[12-14]techniques are available for the determination of CPZas well as other phenothiazine derivatives in pharmaceutical formulations.However, no direct comparison was attempted between the designated spectrophotometric method of CPZ determination[4,5]and a standardized HPLC procedure.

The purpose of the present study was to further examine and ascertainthe previously described spectrophotometric method forCPZ measurement.[4,5] Then, to apply the present spectrophotometric method in comparison with a reference HPLC method[13]for the determination ofCPZ concentration in injectable pharmaceutical formulations intended for human and veterinary uses. Such a comparison has not been done previously.

## Materials and Methods

All chemicals and reagents used were of analytical grades obtained from well-known suppliers. A locally available injectable brand of aqueous CPZsolution (25 mg/5ml, 0.5%) was purchased from a local pharmacy. A veterinary injectable aqueous CPZ solution (5%) was prepared at the Veterinary Drugs Research and Production Center, Corporation of Research and Industrial Development, Ministry of Industry and Minerals, Baghdad, Iraq.

The concentrations of CPZ in the injectable pharmaceutical formulations were determined by a modified spectrophotometric method[4,5]and by an HPLC method[13]as described earlier with appropriate standard calibration curves. For the spectrophotometric determination of CPZ, the working reagents were0.1 N, 1 N and 50% solutions of sulfuric acid as well as ferric nitrate (2%) in 1 N sulfuric acid. The calibration curve of CPZ was prepared by dissolving CPZ powder in 0.1 N sulfuric acid at concentrations of5, 10, 20, 40 and 80 µg/4ml. The modified spectrophotometric method of CPZ determinationwas conducted by diluting0.1 ml of the veterinary (5%) or 0.3 ml of human (0.5%) formulations to 200 ml of 0.1N sulfuric acid. Four ml of the diluted CPZ samples or the standard solutions were mixed with 2 ml of 50% sulfuric acid. Then an aliquot of 0.2 ml of 2% ferric nitrate was added to the mixture. After 15 minutes, the absorbance was measured at 530 nm against water and vehicle blanks using a spectrophotometer (T 80, Biotech Engineering Management Co., U.K.). All determinations were done in duplicate at ambient room temperature.

The HPLC method applied in the present studyconsisted of reversed phase gradient chromatography with 250 x 4 mm Nucleosil $C_{18}$ column; the eluent was (A) 50 mmol $NaH_2PO_4$, pH 2.5 and (B) acetonitrile - 50 mmol $NaH_2PO_4$, pH 2.5 (60:40 v/v), flow rate at 1 ml/min, with a gradient of 15-55% B in 10 min, to 100% B in 20 min, and 280-nm UV detection at room temperature.[13] The external CPZ standard was calibrated between0.625 to 20 µg/ml.

From the calibration curves of CPZ standards, the following were calculated[15,16]asfollows:

Limit of detection limit (LOD) = 3.3 x SD/slope; Limit of quantitation (LOQ) = 10 x SD/slope, where SD is the standard deviation of response of CPZ concentrations of the calibration curves.

Linear regression analysis and related coefficients of correlations were applied on the calibration curves using the statistical package Past 4.03 (https://folk.universitetetioslo.no/ohammer/past)

## Results

The linear calibration curves of CPZ standards determined by the spectrophotometric methodare shown in figure 1 (a, water blank; b, vehicle blank) and in figure 2 by the HPLC method. The range of CPZ concentrations by the spectrophotometric method was between 5 to 80 µg/4ml (Figure 1), whereas that of the HPLC method was between 0.625 to 20 µg/ml (Figure 2). All the standard curves showed strong correlations with an r value = and / or> 0.99 (Figures 1a,b and 2).

The calculated LOD and LOQ of both methods are shown in table 1. In referenceto calculations of CPZ standard concentrations on the basis of µg/ml, as well as the estimated values of LOD and LOQ, the ranges of the calibration curves of CPZ determination by both methods were comparable to each other (Figures 1a,b and 2; Table 1).

The concentrations of CPZ in human and veterinary injectable formulations as determined by both methods are presented in table 2. The concentrations of CPZ determined by the spectrophotometric method were in agreement with of those of the HPLC method, andCPZ contents were in accordance (100.6% and 102%, respectively) with the concentrations claimed on the labelsof both formulations (Table 2).

**Table 1: Limits of detection and quantitation of the assays of chlorpromazine by spectrophotometric and HPLC methods**

| Variable | Spectrophotometry µg/4ml | HPLC µg/ml |
|---|---|---|
| Limit of detection | 4.0 | 2.8 |
| Limit of quantitation | 12.1 | 8.3 |

**Table 2: Percentages of chlorpromazine contents recovered from commercial human and veterinary formulations as determine by spectrophotometric and HPLC methods**

| Formulation | Concentration claimed on label (% w/v) | Spectrophotometry (% w/v) | HPLC (% w/v) |
|---|---|---|---|
| Human | 0.5 | 0.503 | 0.535 |
| Veterinary | 5 | 5.10 | 5.05 |

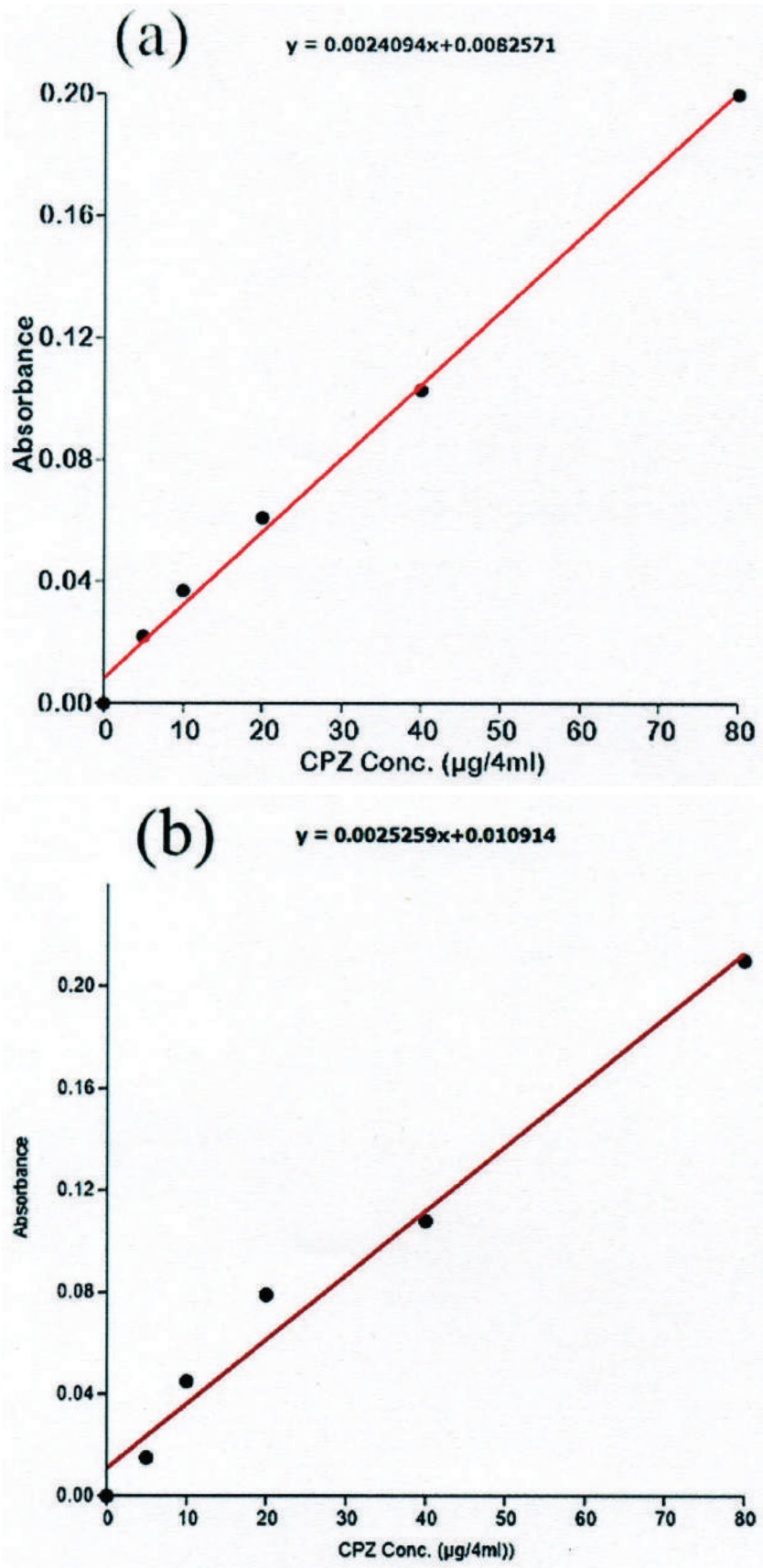


**Figure 1: Standard calibration curves of chlorpromazine (CPZ) with water (a) or vehicle (b) blanks determined spectrophotometrically at 530 nm, r = 0.998 and 0.99, respectively.**

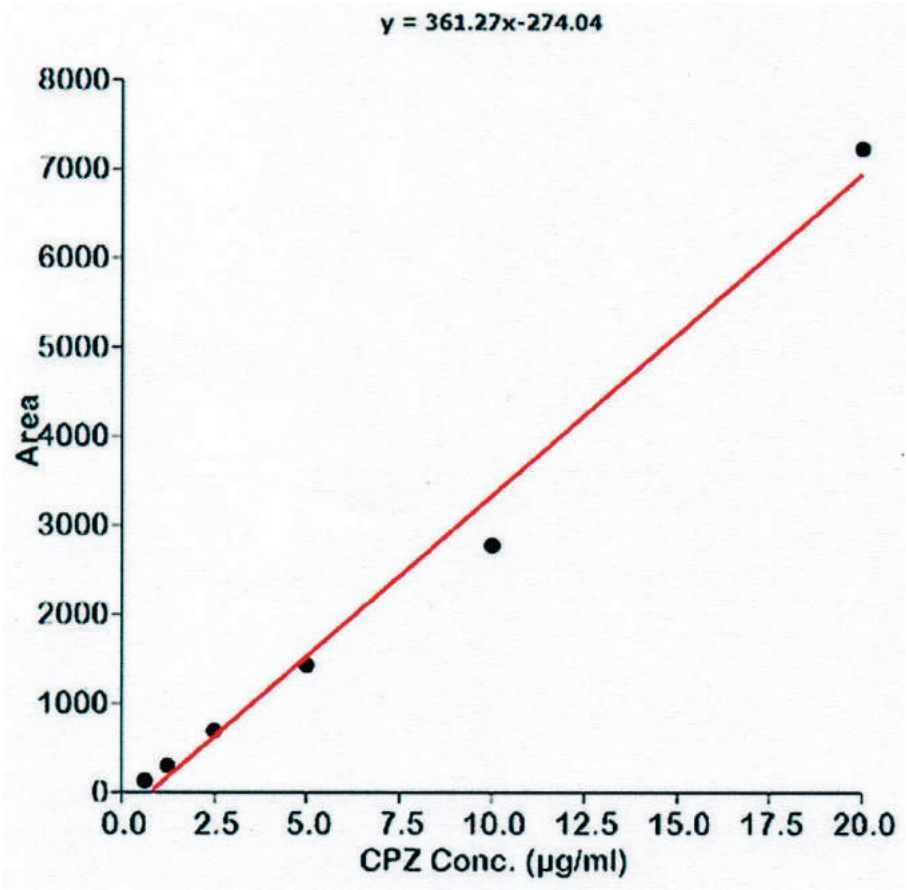


**Figure 2: Standard calibration curve of chlorpromazine (CPZ) determined by HPLC, r = 0.994.**

## Discussion

The present study introduces a useful, simple and accurate spectrophotometric method for determination of CPZ concentrations in pharmaceutical formulations in a manner comparable to a highly sensitive HPLC method.[13] The results further ascertain previous findings reported on the application of the present spectrophotometric method for the measurement of CPZ in aqueous pharmaceutical formulations.[4,5]The LOD and LOQ as determined by both spectrophotometric and HPLC methods are within the acceptable ranges for the drug assay.[4,5,15,16]

The contents of CPZ in both human and veterinary formulations of the present study as estimated by the spectrophotometric methodwere in accordance with those of the HPLC method. They were also in good agreement with the concentrations (100.6% and 102%, respectively) claimed by the manufacturers on the labelsof both formulations. These results of the modified spectrophotometric method for measurement of CPZ concentrations in aqueous formulations, support the findings reported earlier.[4,5] Furthermore, the added benefit of the present study was that the spectrophotometric method was very much comparable to a highly sensitive and standardized HPLC method[13], as there wasgood agreement between both methods. Further studies, however, are needed using the present simple spectrophotometric method on routine batch analysis of CPZ in pharmaceutical industries.

## Conclusions

A simple spectrophotometric method is presented herewith,possibly,for routine measurement of CPZ concentrations in pharmaceutical formulations, with the added benefits of linearity, precision and cost

effectiveness.

**Source of Funding-**Self

**Ethical Clearance-**The authors followed the institutional scientific research ethics as a well the research protocols and guideline of the Ministry of Industry and Minerals**.**

**Competing Interests:** The authors declare that they have no competing interests.

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# Protective Effect of Peppermint on the Toxicity Induced by Blue Green Algae in Poultry

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## Abstract

This study was done on 5 groups of poultry chicks from one day up to 45 days of age. We used blue green algae as source of feed additives added to the water and ration in addition to peppermint to evaluate the protective effect of peppermint against the toxicity induced by the blue green algae. The administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) and decrease in MDA and CAT. The algae showed histopathological alterations in the liver in form of congestion in the central vein, degeneration in the hepatic cords and circumscribed areas of lymphocytic infiltrations between the hepatic cords. While it was mild in heart tissues as it showed also lymphocytic infiltration and congestion of myocardial blood vessels. The biochemical parameters were corrected except the CK and LDH. The histopathological alterations were corrected by the addition of peppermint.

***Key words.*** *Blue green algae- peppermint -protective effect- liver, heart*

## Introduction

The cyanobacterium is wide distributed in most of water lakes in Egypt and lead to environmental contamination by the excreted cyanotoxin of the blue green algae (1 ,2).

The water toxins produced via the metabolism of the algae and cyanobacterium. The degree of toxicity depends mainly up on the signs which was occurred in the tissues. when it caused liver toxicity (dangerous) and mild when it caused dermatitis (3).

The toxic effect of blue green algae was discovered for the first time in Australia in sheep as it starts by nervous manifestations (4).

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The cyanotoxin are produced by the dead cyanobacterium cells when it consumed by another microorganism. the sun light changes the toxin to nanotoxic form which is the dangerous form and may lead to liver cancer (5). These toxins were toxic to all types of domestic and wild life including birds (6)

The blue green algae toxin includes microcystins (targeted for liver and heart), cylindrospermopsins, anatoxins and saxitoxins (targeted for nervous tissues and system. (7)

The microcystins toxicity may lead to death in severe hepatic intoxication in animals, birds and fishes (8)

Peppermint is a member of the Labiate family and one of the world's oldest medicinal herbs. (9). The Labiate family, rich in essential oil, has commercial and medicinal values. These herbs are widespread

throughout the world and are widely use in food, flavor, cosmetic, and pharmaceutical industries. [10,11]. The chemical components of peppermint are menthol, menthone, 1,8-cineole, methylacetate, methofuran isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate, and pulegone. Menthol is the main phenolic component in oil of peppermint, which has antibacterial activities. [12].

The peppermint has numerous properties which includes, antiseptic [13,14], disinfectant and antispasmodic action [15]. When it added to the ration, it made improvement in broiler meat production. [16]

The chemical composition of the peppermint varied according to the types of the cultivated land and the climatic condition in which the leaves grow up [17, 18,19].

## Material and Methods

5 groups of chicken were chosen for this experiment (cobb broiler chicks)

The blue-green alga dose was calculated according to [20]. The dose of peppermint 15g/kg was calculated according to [13].

Group 1 control group fed on normal growing ration without any additives

Group 2 fed on growing ration in addition to peppermint 15g/kg

Group 3 fed on blue green algae alone in food 13mg/kg daily

Group 4 fed on blue green algae alone in water 13mg /kg daily

Group 5 fed on blue green algae 13mg /kg in addition to peppermint 15g/kg in food daily

The chickens were raised for 45 days then slaughtered at the end of the experiments. The serum collected for biochemical analysis. The liver and heart tissues were collected for histopathological findings and antioxidants enzymes.

Preparation of algae extract:

Each sample of the marine algae was washed again with distilled water several times, followed by 5% ethanol to remove any epiphytes or any salt precipitates [21]. Samples were spread on white sheets and left for air drying. They were cut into small pieces and powdered in a mixer grinder.

Extraction of grounded algal samples was done using Ethanol: Dichloromethane (1:1) and stored in adark place. Remain extracts were filtered and concentrated in a rotatory evaporator at 35 °C. The weighted crude extracts were suspended in the dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml and stored in a refrigerator at 4ºC [22,23].

**Biochemical analysis:**

Determination of liver functions:

Biochemical parameters were measured in the collected serum samples; Alanine aminotransferase (ALT), aspartate aminotransferase (AST) are measured in serum according to the methods described by [24]

Antioxidant parameters were measured in liver tissues CAT activity was measured as described by [25], and Malondialdehyde (MDA) was measured by method described by [26].

Determination of heart creatinine kinase (CK) and lactate dehydrogenase (LDH ) parameters:

These parameter was measured according to [27] for CK and [28] for LDH. The biochemical analysis was done at the Central Lab of Faculty of Veterinary Medicine, Benha University.

**Histopathology**

The liver and heart tissues were fixed in neutral formalin, processed for preparing the slides. It was stained according to [29].

Statistical analysis: Statistical analysis of data was performed and expressed by means (± SE) using one way analysis of variance (ANOVA) to determine if there were significant differences between the control and experimental groups. $P \leq 0.05$

## Results

Biochemical parameters:

The results of biochemical parameters are summarized in Table ( 1 )

Effects on serum biochemical parameters:

The administration of peppermint alone (G2) did not cause any significant alterations in serum liver and heart enzymes (ALT, AST and LDH) except CK level was increased significantly compared to the control group (G1) while, the administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) in 3rd and 4th group (blue green algae treated group) compared to the control group (G1).While, treatment with peppermint and (G5) caused the decreased levels of ALT, AST, CK and LDH in 5th group compared to the (G3 and G4).

In regard to the route of administration of blue green algae (in food or water), there was no significant change in serum ALT, AST, CK and LDH levels between the two experimental routes (G3 and G4).

**Effects on malonaldhyde (MDA):**

The administration of peppermint alone (G2) caused non-significant decrease in MDA concentration while the administration of blue green algae alone in chicken food and water (G3 and G4) caused significant decrease in MDA concentration compared to the control group (G1). While, the administration of peppermint with blue green algae (G5) caused the lowest significant MDA level compared to the control and other treated groups.

In regard to the route of administration of blue green algae, the administration of blue green algae in water (G4) caused significant decrease in MDA concentration compared to the group administered blue green algae in food (G3).

Effects on catalase enzyme:

The administration of peppermint alone (G2) and blue green algae (blue green algae) alone in chicken food and water (G3 and G4) caused significant decrease in catalase (CAT) level compared to the control group (G1) while, the administration of blue green algae alone in chicken food and water (G3 and G4) caused significant decrease in CAT level compared to (G2). Moreover, the administration of peppermint with blue green algae (G5) caused the lowest significant CAT level compared to the control and other treated groups.

In regard to the route of administration of blue green algae, the administration of blue green algae in water (G4) caused significant decrease in CAT level compared to the group administered blue green algae in food (G3).

The heart CK and LDH were increased in all groups in compare to the (G1). It showed significant changes in (G5).

***Cont...*** **Table (1).**

**Table (1).**

| parameters | G1 (control) | G2 (peppermint) | G3 ( algae in food) | G4(algae in water) | G5 (alga +peppermint |
|---|---|---|---|---|---|
| ALT serum liver | 4.89c±0.17 | 4.74c±0.63 | 8.15b±0.46 | 6.40bc±0.30 | 6.16a±1.09 |
| AST serum liver | 186.30c±7.98 | 209.53c±9.23 | 249.33b±3.72 | 260.90ab±11.25 | 246.40a±10.22 |
| MDA tissue | 58.76a±2.34 | 47.13ab±2.07 | 41.036b±0.14 | 27.25c±3.50 | 21.13c±8.32 |
| Catalase tissue | 516.32a±23.27 | 346.13b±12.82 | 275.75c±33.12 | 210.30d±3.60 | 155.85d±10.89 |
| CK-heart serum | 1319.7c±131.59 | 2849.3b±118.97 | 3063.3b±78.17 | 3482.7b±202.09 | 4679.3a±412.34 |
| LDH-heart serum | 608.63c±44.40 | 728.53c±30.64 | 898.57b±46.26 | 929.83b±0.67 | 1140.7a±44.89 |

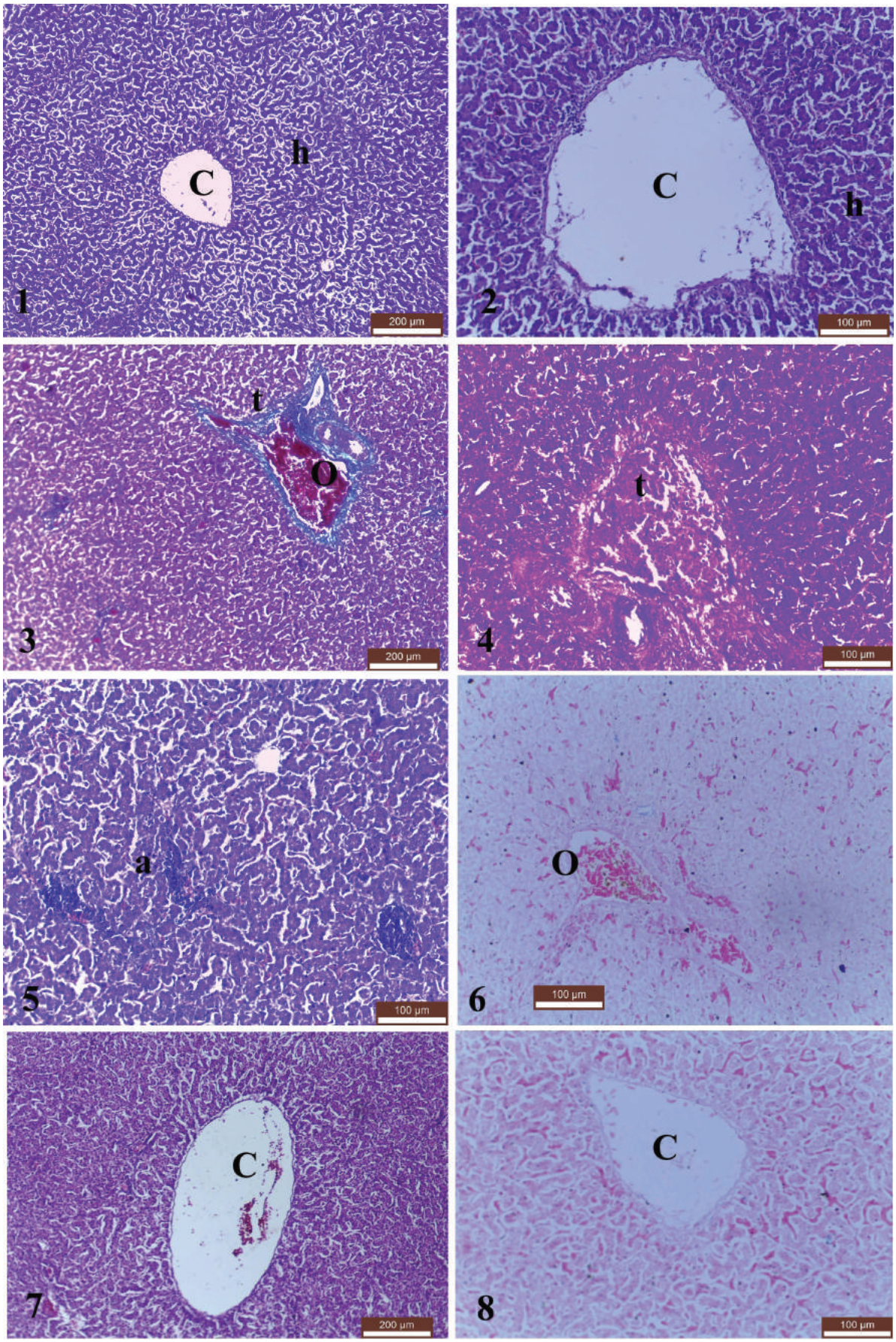
The mean values with different superscript letter within the same row differed significantly at ($P < 0.05$)

**Histopathological results**

The liver of the control group showed normal arrangement of the hepatocytes around the central vein (Fig.1). The hepatocytes arranged in the form of distinct cords (Fig.2). The blue green alga when added to the food and water made alterations in the architecture of the portal structure, in the form of congestion and edema of the central vein and hepatic vein (Fig.3). Loss of the architecture of the hepatocytes especially around the triad areas with proliferation of the fibrous tissues (Fig.4). Circumscribed areas of lymphocytic infiltrations between the hepatic cords were noticed in the portal lobules (Fig.5). PAS reaction was negative in the hepatocytes and faint positive in the CT fibers around the central vein (Fig.6). The addition of peppermint had protective effect on the alterations induced by the blue green alga as it improved the hepatic alterations (Fig.7). It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive (Fig.8).

The heart of the chicken showed myocardial muscles which was branched and anastomosing in nature (Fi.9). Fine collagen fibers were spread between the cardiac muscles (Fig.10). the addition of blue green algae made mild histopathological alterations in the cardiac muscles, in form of patches of degeneration in the cardiac striations (Fig.11) and congestion of the cardiac blood vessels in addition to lymphocytic infiltrations between the myofibrils of the cardiac muscles (Fig.12).

The uses of peppermint protect the myocardial muscles from the hazard effect of blue green alga in the form of well organized cardiac muscle (Fig.13), although some lymphocytes still persist betwen the myocardial fibrils (Fig.14).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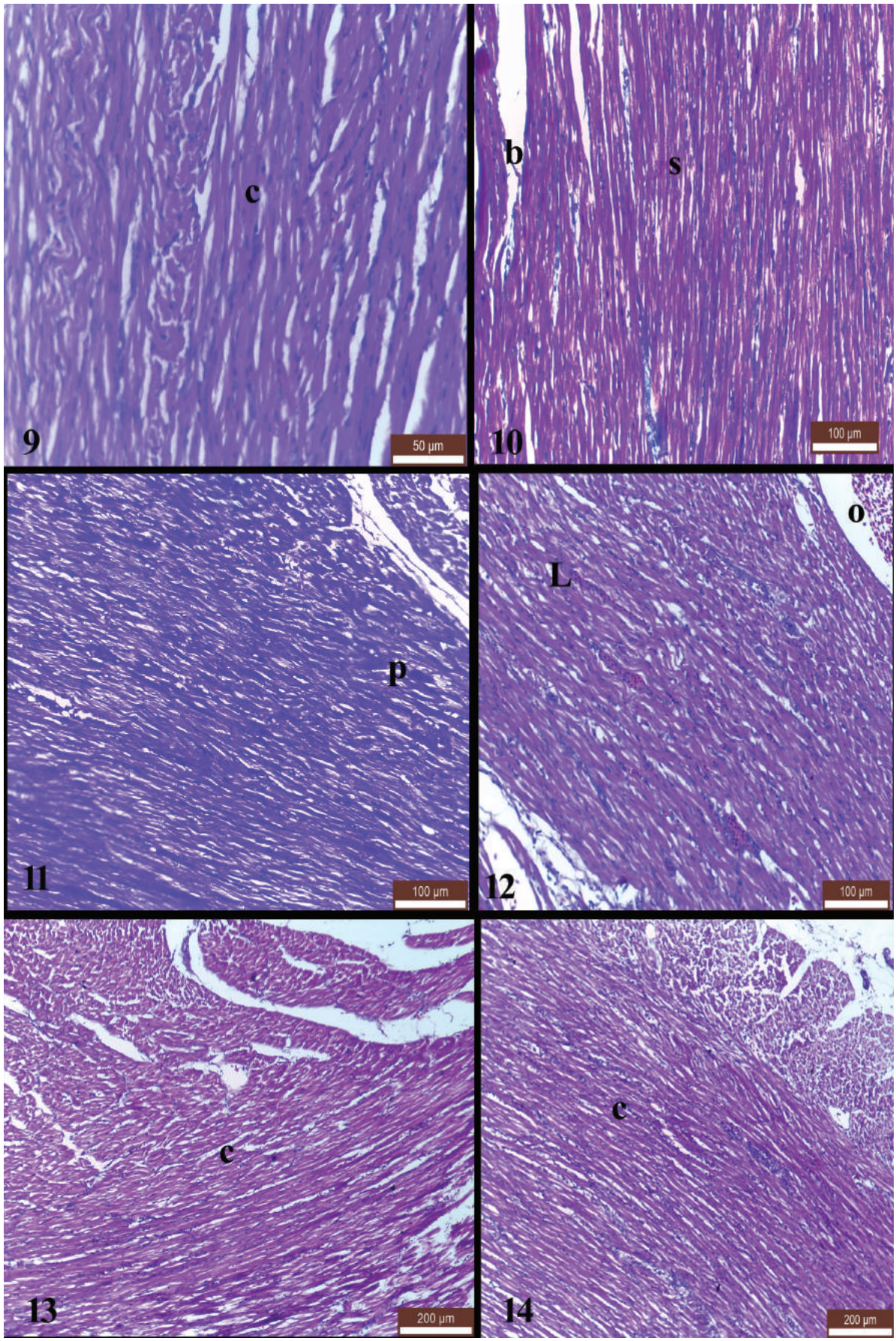
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The liver of the control group showed normal arrangement of the hepatocytes ( h) around the central vein (Fig.1). H&E scale bar 200. The hepatocytes arranged in the form of distinct cords around central vein ( c) (Fig.2) H&E scale bar 100. Alterations in the architecture of the portal structure in (G3), in the form of congestion and odema ( o) of the central vein and hepatic vein (Fig.3) Masson trichrome scale bar 200. proliferation ( t) of the fibrous tissues (Fig.4) H&E scale bar 100. Circumscribed areas of lymphocytic infiltrations ( a) (Fig.5) H&E scale bar 100. PAS reaction was negative in the hepatocytes and faint positive in the CT fibers (Fig.6) PAS scale bar 100. improve the hepatic alterations in group (G5) (Fig.7) H&E scale bar 200. It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive (Fig.8) PAS scale bar 100.

The heart of the chicken showed myocardial( c) muscles (Fi.9) H&E scale bar 50. Fine collagen fibers (s) were spread between the cardiac muscles (Fig.10) Masson trichrome scale bar 100. Mild histopathological alterations in the(s) cardiac muscles, in form of patches (p) of degeneration in the cardiac striations in group (G3) (Fig.11) H&E scale bar 100. Congestion of the cardiac blood vessels in addition to lymphocytic infiltrations ( L) between the myofibrils of the cardiac muscles (Fig.12) H&E scale bar 100.

well organized cardiac (e) muscle in group (G5) (Fig.13) H&E scale bar 200, Some lymphocytes still persist betwen the myocardial fibrils (Fig.14) H&E scale bar 200.

## Discussion

The blue green algae (cyanobacterium) was widely distributed in water. the cyanotoxin were the main cause of numerous veterinary problems in animal kingdom [30] and also in human [31]

Most cases of blue green algae toxicity occurred in summer where the water is worm. the algae grow and made a layer of pea green slime over the water surfaces. These slime was collected and introduced to the chicken as feed additive. These slime contain the cyanotoxin which cause liver toxicity and sever nervous manifestation. [32]

in this study, administration of blue green algae in chicken food and water caused hepato- and cardio toxicity indicated by significant increase in liver (ALT and AST) and heart enzymes (CK and LDH) compared to the control group. Also the administration of blue green algae alone in chicken food and water caused significant decrease in MDA concentration compared to the control group. These results suggested that blue green algae could exert hepato- and cardio toxic effect and/or radical scavenging activities with toxic dose used in this experiment. This hypothesis is supported by the findings of [7,33]

The blue green algae when added to the food and water made alterations in the architecture of the portal structure, in the form of congestion and edema of the central vein and hepatic vein. Loss of the architecture of the hepatocytes especially around the triad areas with proliferation of the fibrous tissues. The algae toxin caused extensive haemorrhage in the liver with initial perilobular distribution. This was accompanied by necrosis of hepatocytes .[34]. These findings were support our results in which congestion and odema were most prominent histopathological alterations in algae added groups

Circumscribed areas of lymphocytic infiltrations between the hepatic cords were noticed in the portal lobules. These results were supported by the findings of [20] who stated that the toxin was principally hepatotoxic causing massive hepatocellular necrosis and biliary hyperplasia in lethally affected chickens.

Peppermint (Mentha piperita L.) was used in this experiment to protect against the hepato and cardio toxic effect of blue green algae [35,36].

The administration of peppermint alone did not result in significant alterations in biochemical parameters compared to control chickens due to low dose which used in this experiment according to [37]

and [38] who reported that Peppermint leaves can be used as an effective nutritional bioagent up to 40 mg/kg in rats and 15 g/kg in chickens respectively.

Furthermore, the administration of peppermint with blue green algae improve the hepato- and cardio toxic effect for some extent due to low dose of peppermint which used in this experiment and avoid using excess of peppermint to avoid any hazard effects [38].

peppermint also improving feed efficiency which build up the immune system that face off the toxic effect of the cyanobacterium toxins.[39]. Moreover, the antiseptic property of peppermint prevents harmful bacterial growth in the digestive system that led to better digestion and absorption .[40].

. The antiseptic property of peppermint results from the presence of menthol. [41]. peppermint strengthened the stomach and intestinal slow motion because of alpha humlone. [42]

The addition of peppermint had protective effect on the alterations induced by the blue green algae as it improves the hepatic alterations. It also improved the histochemical reactions of the hepatocytes as it appeared faint PAS positive as it returned back to store glycogen in their cytoplasmic granules .[43].

Microscystin toxins had toxic effect on heart and cardiovascular system in human [44]. The blue green algae caused congestion in cardiac blood vessels and pathological alterations in myocytes. Similar results were support our findings. Also the CK and LDH showed significant increase [45]. The addition of peppermint has non significant effect on the correction of the heart enzymes although it made some histopathological corrections in compare to the normal histological structure

## Conclusion

The blue green algae were toxic to the chicken when added as feed additive. It had hepato and cardiac toxicity through histopathological alterations and elevation of (ALT,AST, CK, LDH) and decrease MDA. The peppermint when used as co treatment with the blue green algae in food or water made correction in the biochemical and histopathological alterations.

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# Studying of Some Physiological Parameters in Patients with Inflammatory Bowel Disease ( IBD ) in Al-Anbar Province

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## Abstract

This study was carried out to determine some hematological, parameters in patients with IBD. the total number were 100 blood samples, 60 samples from patients with IBD (cases), while the remaining 40 samples from persons without IBD (control), Ages ranged from 15 to 60 years. who attended general teaching hospital in Ramadi city in Al-Anbar governorate**.** a comparison in hematological parameters was carried between the two groups with each other showed that there was significant difference between patients and control with ($p<0.05$). and the means levels for hemoglobin HB (10.09 g/dl), (13.18 g/dl) \ there was significant difference between patients and control with ($p<0.05$). and the means levels for red blood cells RBC (3.194 X $10^6$ c/ $mm^3$), (5.291X $10^6$ c/ $mm^3$) \ there was significant difference between patients and control with ($p<0.05$). and the means levels for white blood cells WBC (12.39X $10^3$ c/ $mm^3$), (7.300X $10^3$ c/ $mm^3$) there was significant difference between patients and control with ($p<0.05$). and the means levels for \platelet (561.2X $10^3$ c/ $mm^3$), (258.4X $10^3$ c/ $mm^3$).

***Key words :*** *Hb, PLT, WBC,RBC,IBD*

## Introduction

Inflammatory bowel disease (IBD): a multifactorial immune disorder characterized by persistent intestinal relapsing inflammation of unclear etiology and characterized by an alternate duration of recurrence and alleviation, The major clinical symptoms of IBD are gastrointestinal diarrhea, bloody stool and weight loss ,Other autoimmune disorders, such as cholangitis, primary sclerosing psoria and ankylosing spondylitis, also occur in IBD patients [(1).] The blood tests used most often in IBD are levels of the three main types of blood cells (red cells, white cells, and platelets), The amount of white blood cells can indicate if there is any inflammation or infection in the body; More over PLT are small nuclear fragments derived from megakaryocytes, an increase in the number of platelets (small cells involved in blood clotting) can also be a sign of inflammation, As well as detect anemia by calculating the level of hemoglobin,Anemia is a common extraintestinal indicator of IBD affecting up to 75% of patients [(2).] Its pathogenesis is multifactorial, due to iron deficiency and also chronic inflammation, chronic blood loss is a common systemic complication of IBD, insufficient intake or absorption of nutrients and the effect of inflammation on the bone marrow and the handling/ transport of iron and can be one of the earliest signs of the disease[(3)] .With marginal contribution of folic acid and vitamin B12 deficiencies ;A growing body of evidence suggests that the neutrophilelymphocyte ratio (NLR) is a useful biomarker of systemic inflammation responses [(4)] . Leucocytes are a collection of cells involved in the immune defense mechanisms against

non-self antigens that challenge the immune system , The neutrophiles are mainly involved in a non-specific immune function that is phagocytosis, which is also shared by monocytes, but the latter are involved in antigen presenting, and are known as macrophages or antigen presenting cells; The lymphocytes are the cellular elements in the specific cellular and humoral immune responses [5] .

## Materials and Methods

**Patients and Controls :**

The study included (60)patients in different age(15-60) who were suffering of inflammatory bowel disease , who attended general teaching hospital in Ramadi city in Al-Anbar governorate during the period extended from the 1st of May 2020 to the 1st of January 2021. The samples of patients were selected According to the diagnosis of gastroenterologists. While control included (40) healthy persons in different age from (15-60) years. They were considered as negative control group as they did not show history of inflammatory bowel disease after investigation by gastroenterologists.

Ten ml of venous blood was collected from a suitable vein. Tourniquet was applied about (4-5) finger width above the selected venipuncture site and disinfected by 70% of Ethanol for 30 second, and allowed to dry completely, the bloode was divided into two type of tubes, the first one; 2.5 ml whole blood was dispensed in in tow tubes with ethylene diamine tetra acetic acid tube (EDTA-tube) and mixed gently, In second tube; Residual part of the blood sample was transferred to it (free of anticoagulation) and let to coagulate for serum separation by using centrifuge at (4000 rpm) for 5 min, The isolated serum was collected in a sterile clean white tube to be used for serological studies Then tubes were placed in a cool-box under aseptic condition and stored in the freezer at (-20$^{o}$C) until further processing [6].

**Diagnostic Methodes :**

The following diagnostic methods were used :

A method used the blood cells counter and diluent solution (Turke, s fluid) to calculate the total number of white blood cells The total WBC was calculated in the study samples using the following equation:

**WBCs/mm$^3$=Number Cells Counted x 50** [7] .

The blood cells counter and diluent solution (Hayem,s Solution) were used to calculate the number of the Red blood cells The total RBC was calculated in the study samples using the following equation:

**RBCs/mm3=Number Cells Counted X 10000** [7] .

Cyanomethemoglobin Method was used to measure Haemoglobin concentration in the blood sample, using Drabkin, s solution as a diluent. The concentration of Heamoglobin was determined in the study samples using the following equation:

**Haemoglobin (g/dl)=Absorbance(Test/ Standar) X Hb.Standar**[8] .

The blood cells counter and diluent solution (Ammonium oxalate) were used to calculate the number of platelets cells Platelets was calculated in the study samples using the following equation:

**Platelets/mm$^3$=Number Cells Counted X1000** [9].

## Results and Discussion

The total number of persons included in the study was (100) samples which included (60) patients were found to have inflammatory bowel disease (cases) and (40) normal persons as control.

**Distribution of Study Population According to Age groups:**

According to ages, study population was grouped

into 9 groups as described in figure (1). The older groups included the highest number of patient and the highest number of abnormal findings.

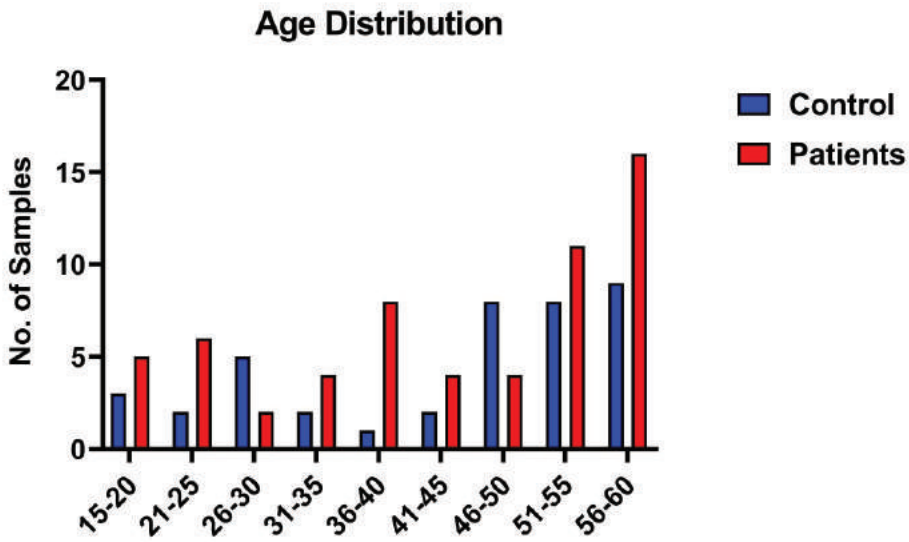



**Figure (1): Distribution of Study Population According to Age Groups**

The present study showed the prevalence of IBD in elderly more young persons.

The present study is consistent with the study of Stephanie *et al*., [10] which showed the Prevalence of IBD is increasing in all age groups, but particularly among the elderly.

**Distribution of Study Population According to Residency:**

Figure (2) illustrates the number of Urban patients was 47 (78%), and the number of Rural patients was 13 (22%).

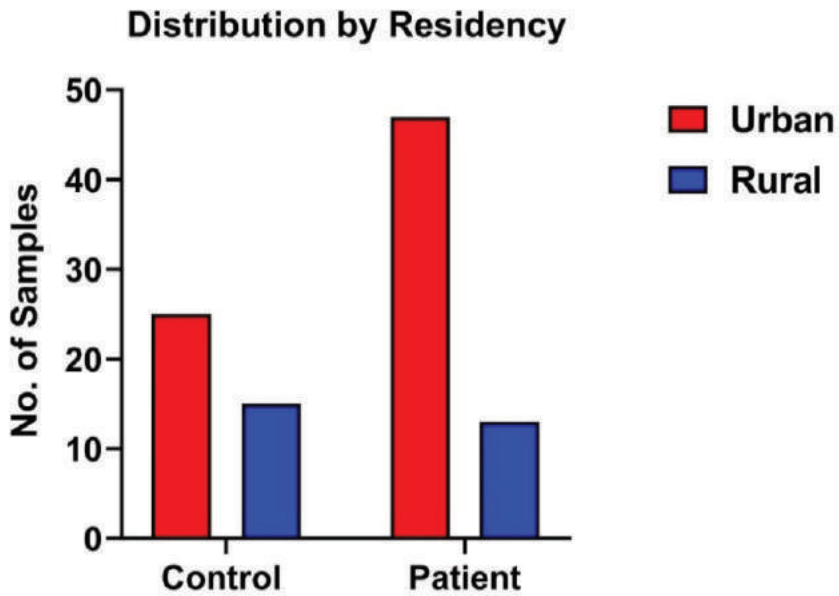



**Figure (2): Distribution of Study Population According to Residency**

The present study showed the prevalence of IBD in cities more than villages.

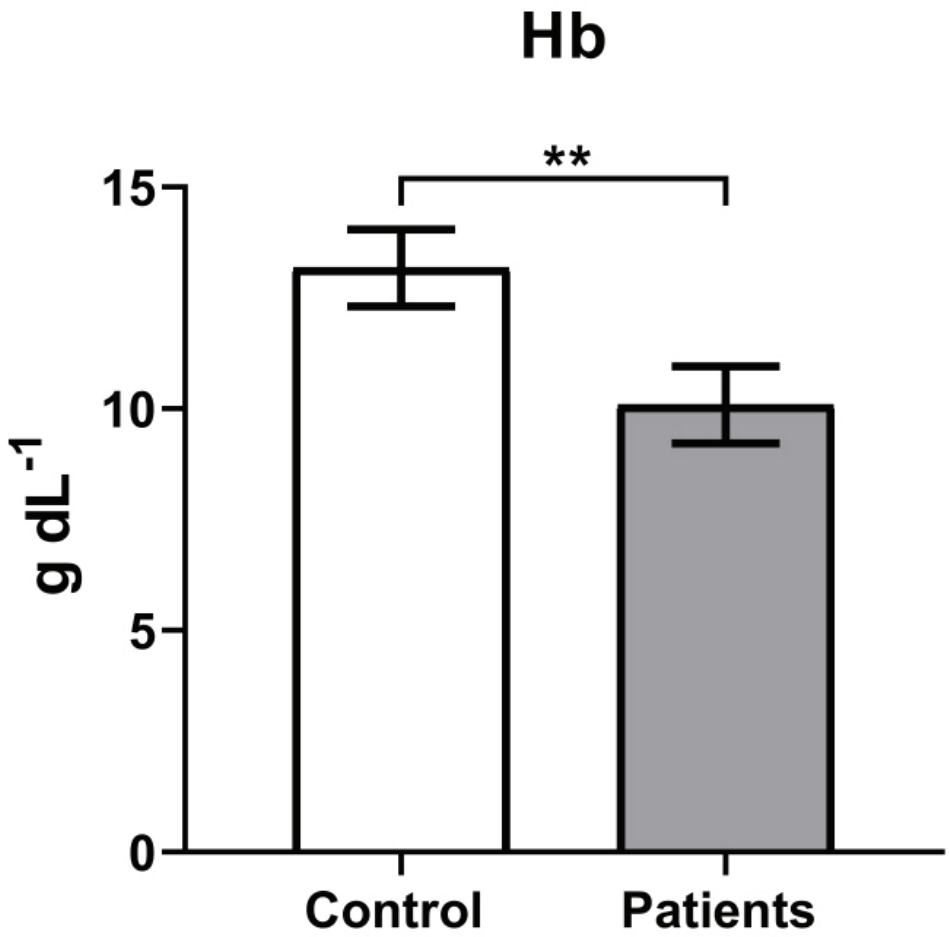
The present study is concordant with the study of Openshaw, [11] which showed a lower incidence of rural IBD compared to urban residence.

The urban diet may also influence the clinical course of IBD, as well as compared with its rural population, the urban diet contains significantly higher quantities of inert inorganic non-nutrient microparticles such as natural contaminants [soil and dust] and food additives, which may combine with intestinal luminal components such as bacterial cell wall lipopolysaccharides to form antigenic particles**.**

**Hematological Data Analysis** :

**Concentration of Hemoglobin (Hb) and Total Number of RBC :**

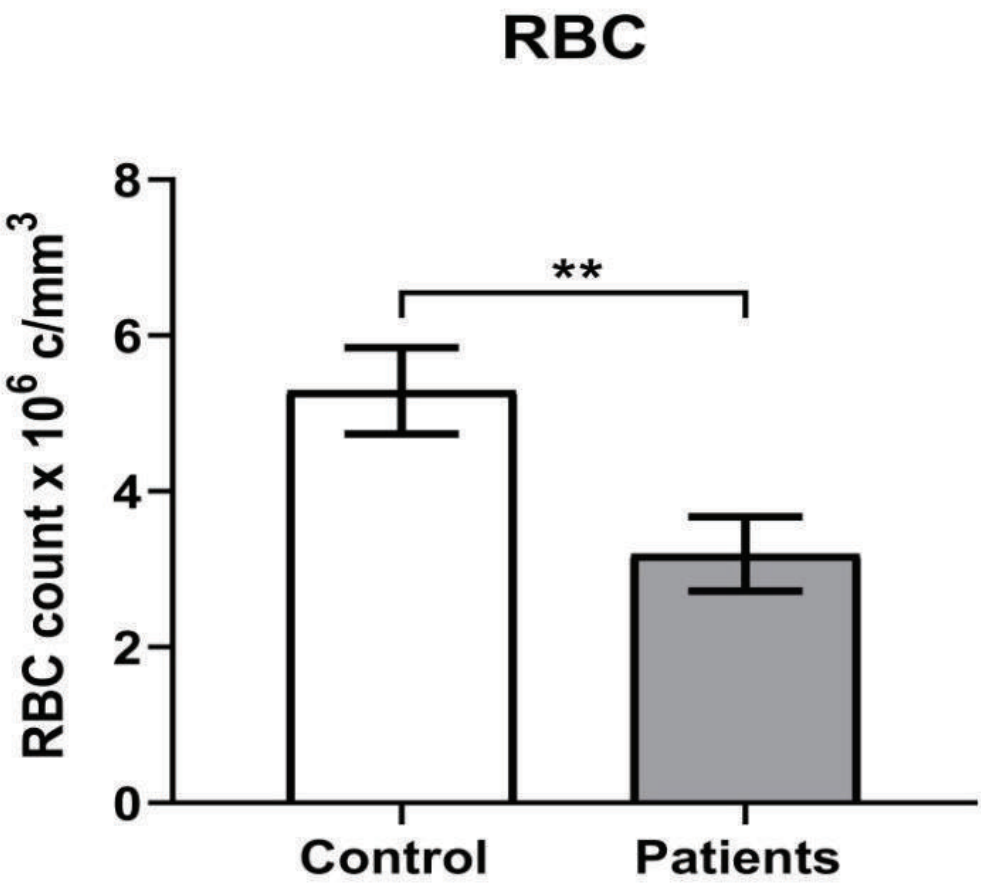
The present study showed significant difference in Hb concentration between cases and control with p-value(<0.05), the means of Hb for two groups respectively were (10.09 g/dl), (13.18 g/dl) , as shown in figure (3):




Std. Deviation: Control= 0.8658, patient = 0.8695

**Figure (3): Mean levels of Hb in Patients and Control.**

The present study showed significantly decrease in the Number of RBC in cases and control with p-value was (<0.05). the means of RBC for two groups respectively were ( 3.194 X $10^6$ c/ $mm^3$ ), (5.291X $10^6$ c/ $mm^3$) shown in figure (4).




Std. Deviation: Control= 0.5541, patient = 0.4767

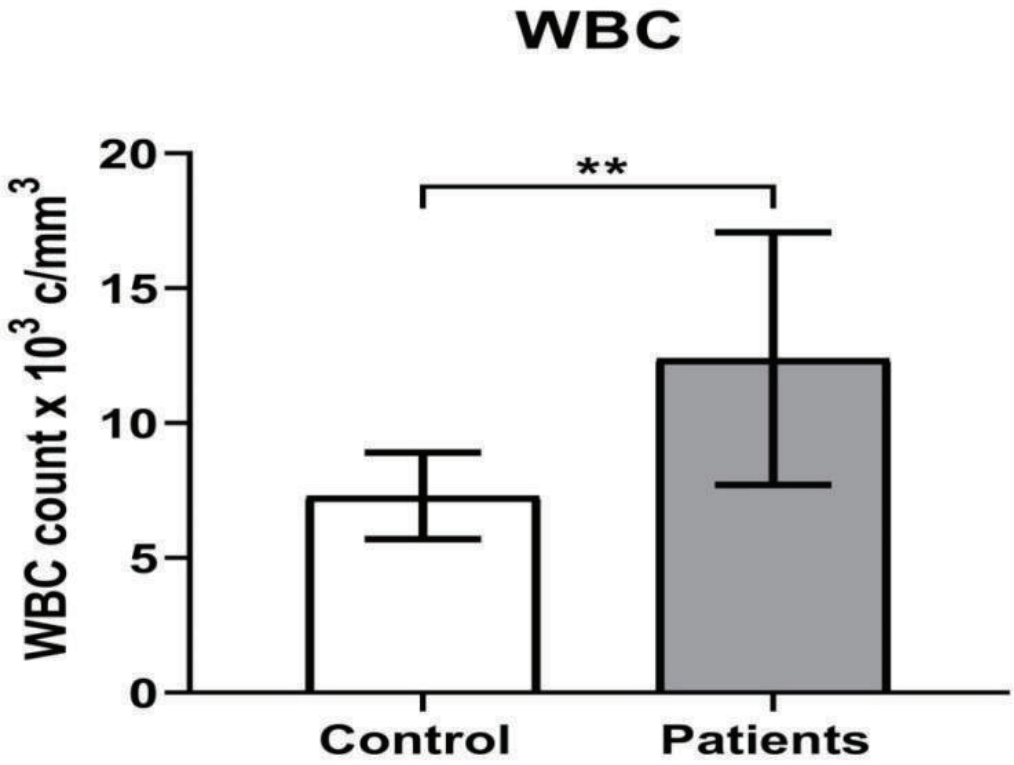
**Figure (4): Mean levels of RBC(c/$mm^3$) in Patients and Control.**

A major finding of the present study was the strong association between anemia and disease activity in IBD patients, Moderate disease activity was an independent factor that increased 3.5-fold the risk of anemia, patients with moderate activity have a higher prevalence of anemia than those in remission or presenting with mild activity [12]. The present study is concordant with the study of Veli *et al*., [13] which showed The frequency of anemia was higher in patients with IBD than in control The incidence rate of anemia for the entire IBD was Hemoglobin 8.6 g/ Dl. The present study is also consistent with the study of Bergamaschi *et al*., [14] which showed prevalence of anemia found in IBD patients with activity disease. Anemia in IBD seems to be multifactorial [15] ; As well as in conditions of B12 and folate deprivation, while it is known that the degree and severity of anemia in both Crohn's disease (CD) and ulcerative colitis (UC) are linked to disease activity [16]. Moreover, adequate supplementation of food was decreased in IBD patients due to the associated anorexia Additionally,

the immunosuppressive drugs given for IBD patients may result in iron malabsorption [16]. Moreover active IBD with inflammation activates the inflammatory cytokine e.g., interleukins-6 and TNF-alpha, among others) , which causes elevated hepcidin levels, To one side from blocking the intestinal iron absorption, hepcidin also causes reduced release of iron from iron storing cells, leading to functional iron deficiency leading to anemia ; also Higher oxidative stress may decrease erythrocyte life span [17].

Total Number of WBC and Platelet:

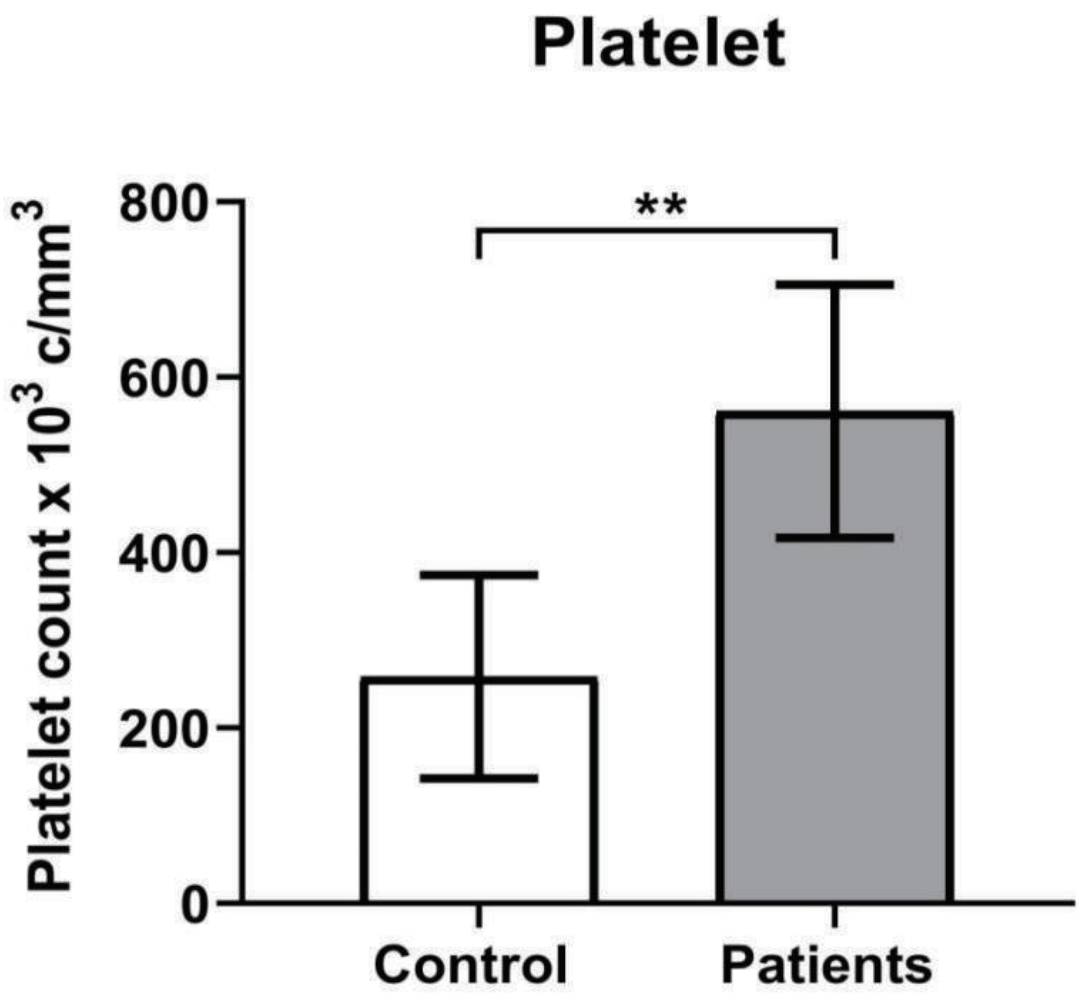
The present study showed significantly increase in the Number of WBC in cases and control with p-value was (<0.05). the means of WBC for two groups respectively were (12.39X $10^3$ c/ $mm^3$) , (7.300X $10^3$ c/ $mm^3$), as shown in figure (5).




Std. Deviation: Control= 1.600, patient = 4.684

**Figure (5): Mean levels of WBC(c/$mm^3$) in Patients and Control.**

The present study showed significantly increase in the Number of PLTs in cases and control with p-value was (<0.05). the means of PLTs for two groups respectively were (561.2X $10^3$ c/ $mm^3$) , (258.4X $10^3$ c/ $mm^3$), as shown in figure (6).




Std. Deviation: Control= 116.1, patient = 144.4

**Figure (6): Mean levels of platelet (c/mm$^3$ ) in patients and control .**

The leukocyte count has emerged as a marker of inflammation. the number of WBC proliferations significantly during infections, and useful information about the health status at the organismal and population level [18]. For example autoimmune diseases, are accompanied by increased systemic inflammation and elevated leukocyte counts [19] . The present study is concordant with the study of Veli et al., [13] which showed the number of White blood cell was higher in patients with IBD than in control the incidence rate was 16.710 mm3. The present study is also concordant with the study of Liaz *et al*., [20] which showed the number of White blood cell was higher in patients with IBD than in control. An elevated WBC count is prevalence in patients with active IBD and high leukocyte count is also common in patients taking steroids due to drug-induced mobilization of marginated neutrophils [21].Elevations in WBCs and is associated with several chronic conditions [22] that does consistent with present study. The typical laboratory findings of IBD are elevated in leukocytes number [23] that does consistent with present study. An increase in the circulating white blood-cell count It may reveal a primary disorder of bone marrow

production response to a disease process, drug, or toxin [21].The number of white blood cells increases during the acute phase response and is also influenced by the drugs utilized in IBD, as glucocorticoids [22]. Platelets play an important role in pathogenesis of IBD, platelets are involved in the pathogenesis of chronic inflammations such as IBD [24] . The present study is concordant with the study of Veli *et al*., [13] which showed the number of platelets was higher in patients with IBD than in control the incidence rate o was 434,000 $mm^3$. The present study is concordant with the study of Liaz *et al*., [20] which showed The number of platelets was higher in patients with IBD the mean with p-value 0.006 The PLT value may be increased if inflammation occurs, Both CD and UC are associated with abnormalities in the number and function of platelets [24] that does consistent with present study. The present study is concordant with the study of Frolkis *et al*., [25] which showed the obtained results indicate that the level is significantly higher in the IBD patients group. The present study is also concordant with the study of Tayyibe *et al*., [26] which showed The number of PLT is significantly higher in the IBD patients which was 316.66 group comparison with control which was 265.68 .

Injury site molecules such as subendothelial collagen, activated leukocyte cytokines and endothelial cells, increased local adenosine diphosphate (ADP) concentration due to reduced capillary blood flow, substances released from neighboring cells, arachidonic acid, activating factor PLT, and thrombin production increase the accumulation and activation of PLT in the intestinal microvasculature [27].

Thrombocyte activation seen in the active period of disease not only regulates coagulation also improves mucosal inflammation, Platelets initiate and support inflammatory processes by secretion of numerous biologically active substances like platelet activation factor, platelet-derived growth factor, platelets factor 4, IL-1, beta-tromboglobulin .The increased concentration of circulating PLT activation markers in the systemic circulation of patients was confirmed in several studies [28].

PLT misses its typical discoid shape during activation, obtains projecting forms called pseudopodia, releases an increased number of microparticles and increases in size and density, several metabolic reactions occur within their cytoplasm, where different inflammatory mediators are produced [24] .

## Conclusions

1-prevalence of Inflammatory Bowel Disease I(BD) in elderly persons, and in cities more than villages .

2- There was significant difference in hematological parameters levels (Hb ,WBC ,RBC ,PLT) between patients with IBD and control.

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# Coronavirus (COVID-19) and Online Studying Cas's Study Alasala University KAS Law School

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## Abstract

The first quarter of 2020 is one of the most difficult times for the region and whole world. Where the epidemic (COVID-19) invaded the world and thus affected many aspects of human aspects: in terms of low industrial production, the global economy stopping work, and educational aspects closed to students. All educational institutions modified their academic assessments, and then educational programs were modified during this period. As stakeholders and management of higher education institutions do not have another option is to take advantage of Internet and new technologies, and therefore go to online learning to continue academic activities at all levels of education around the region and the whole world.

The study aims at assess whether students in KSA, especially Alasala University in general, and in particular the College of Law, if they are satisfied with the "group" online learning experience in higher education institutions, therefore, the study used an online survey to investigate the level of satisfaction with online learning and how students adapt to these "new initiatives".

The study undertakes a case study of a law school within a leading university such as Alasala, which has implemented a quality system. The study reviews the development of the quality system and examines the concept of service quality in law education. The aim of this study is to address the paucity of service quality research in law education in this region. Empirical research is used to determine the factors that influence student evaluation of service quality. With data collected from 664 students, the study identifies six factors that influence students' evaluations of service quality. Research implications of the study are then discussed. The study results indicate that the implementation of online learning programs was a very impressive idea as the majority of students included in the sample supported the initiative.

***Keywords:*** *Distance education; student satisfaction, online Learning, COVID-19 pandemic, higher educational institutions, Alasala, KAS.*

## Introduction

''Never before have we witnessed educational disruption on such a large scale'' said UNESCO Director General Audrey Azoulay (2020). The global academic calendar has been thrown into a state of disarray by the Coronavirus outbreak. Most schools from basic to universities have shut down their doors and students have returned home to their parents and together self-quarantined (UNESCO, 2020). Convocations and graduations have been cancelled, and some classes have been cancelled, some examinations have been cancelled; university research programs have been postponed. Leaders around the world are feared and at the same time facing a major dilemma. It is imperative that education leaders make the decision to end the spring semester in most cases. But these sudden and decisive decisions related to

coronavirus (COVID-19) will change the history of the entire word on all levels, so it must be thoughtful and accurate.([1])

In March 2020, Distance Education had a major revolution in the in the Middle East and they are continuing to progress into a more widespread form of educational technology. However, to what extent can these work and what are the motives for applying this new technology to the KSA? It is important and necessary to recognize that online education is the critical solution in times of crisis, especially to avoid direct communication. Where many universities began to transfer their programs gradually over the Internet and to reduce the presence of huge numbers of students in the university edifices and to alleviate administrative and financial burdens alike (Paw, 2020). Where the best universities in the world have done this like Tsinghua, Peking University, Harvard, MIT.([2])

Pao (2020), Philius et al. (2019) they argue that a full online transition requires effort, massive budgets, bigger planning, and investments from all sectors. So, fundamental changes to the laws recognize the study certificates online. We cannot compare the first world country with countries in the third world, especially since there is a different boom in terms of laws and economic and political systems.([3]) Many universities have not taken into consideration the training of students and teachers through online teaching, and they may not have sufficient resources, including both registration platforms. The campus, and also through the work of the faculty from her home to record the work and submit it in a way that can be accessed by students, then the plan ends online here.

There have been increased levels of sophistication and effectiveness in a number of schools that have embraced digital learning (Murphy, 2020). According to UNESCO (2020), over 1.5 billion learners in 165 countries are attracted by COVID-19 school closure([4]). This translates to 87% of the world's student population. According to another survey conducted by an education think-tank the 'Times Higher Education'', on the prospects of higher learning from the perspectives of leaders of major universities in the world in 2018, about 200 respondents drawn from 45 countries across the 6 continents pointed to a specific fact; online education cannot match the normal teaching method. However, 63% predicted that by 2030 most prestigious universities will be offering their full courses online. However, only 24% agreed that electronic learning would be more popular than traditional methods of learning. Technology will reshape the universities by 2030. Though the online system of education is viewed as relatively new, according to research, in the future, it will just be as effective as school-based methods (Murphy, 2020; UNESCO, 2020).

The Alasala University is becoming increasingly competitive and consolidated, which means that local universities are facing shareholder pressure to focus on value rather than revenue growth. The ease and convenience offered by technologies such as mobile phones and the internet have help KSA higher education insituations serve the domestic education and their students. Education are now facing strong and intense competition. Universities in Saud Arabia are formulating various strategies to retain the quality services, the key to which is increasing service quality. KSA universities need a system to foster student satisfactions.([5])

## Literature Review

Educators and schoolers have long sought to define quality in several forms of education. Distance and open education and online education spreading with the support of the Internet and new technologies, the tasks required to evaluate the quality of online programs become more difficult. To help teachers and institutions search for quality assurance methods for the continuous improvement of their distance learning programs, the Sloan-C Framework (Moore, 2002), with five pillars identified for quality - effective learning, Access, student satisfaction, faculty satisfaction, and

cost-effectiveness of online programs. Based on the relevant literature review, this article explores the reasons behind online program quality assurance, key standards recommended by major accrediting agencies and some of the best practices currently used to ensure online program quality standards. It serves as a starting point for education officials and teachers to formulate program goals and evaluation policies regarding their online programs.

It is important to note that such problems can only be faced due to the high speed that universities in Saudi Arabia go through and they have been forced to transform the teaching method online to preserve the health of their students due to the coronary virus outbreak (COVID-19) that greatly paralyzed the region and the whole world. However, there are many complaints among students on social media platforms about their frustration with online education. There are cases when students are forced to rush to the kitchen to answer the calls of their teachers or to stop the video channels because the family plays and screams in the background. Other major issues of great concern to students participating in the Internet are the effectiveness and credibility of the content of the online learning course.

In KSA, several distance education students have positive perceptions and attitudes towards open and distance learning while many people who are outside the system are sceptical about the quality of evaluation and mode of service delivery in distance education. We observed that students in particular have mixed feelings about the inadequate coverage of the syllabus in distance learning. Hence, we assert that many students criticize distance education because of high rate of malpractices in home work, assignments, tests and examinations, which invariably lower the quality of education in the system. That many students still have wrong impression about distance learning in KSA, stressing that despite the popularity of the system in CORONA time, it is still facing credibility problem.

**Theoretical Framework**

The survey assesses the associations among the system quality, information quality, service quality on student satisfaction and use of systems in virtual learning environments .The survey was carried out by means of a distance education program offered to 646 students from law school. Confirmatory Factor Analysis and Structural Equation Modelling were used for data analysis in order to understand the student satisfaction process in virtual learning system.

The survey guided by a well-structured questionnaire had been administered across a valuable sample of 646 students of law school. The findings generally indicate that the majority of students are satisfied with the facilities provided by law school. Such finding law school make butter strategic plan as to enhance student's satisfaction in particular and its service quality were corrected with student satisfaction.

The survey was carried out through an online program presented to 646 students Law Faculty of Al-Alasala University. Analysis and structural equation modelling were used to analyse data for understand the student satisfaction process in a virtual learning system. Results show differences in system quality, information quality, and quality of service affect using the system, building user satisfaction was 75% of the variance previously explained quality of information and quality of service.

**Research Aims and Questions**

This study aims to identify the views of students in relation to the quality of distance education. The objective is to determine what could be done to improve the programmes, and the institutional framework in which they exist? In this sense, the following research questions were formulated to guide the study:

- Explore KSA students' perceptions of the importance of quality standards in distance education.
- Explore KSA students' perceptions of the

current implementation of quality standards in their current distance education.

· Investigate KSA students' perceptions of the strengths and weaknesses of their distance education courses.

· Investigate KSA students' perceptions of the barriers facing distance e-learning.

Hence, to achieve our research goals, the study seeks to answer these questions:

RQ 1 What are the KSA students' perceptions of the importance of quality standards in a distance study?

RQ 2 What is the expected challenges that students are likely to encounter during the online teaching and learning?

RQ 3 What is KSA students' perceptions of strengths and weaknesses through distance learning applications?

RQ 4 How much satisfied is the students with the "Learning Resources" available?

These study questions will determine the structure of the current study. We have made student perceptions are the basis for an investigation of both quality and strengths and weaknesses the obstacles students face, along with their suggestions for changes that may be reinforced their experience.

**Statement of the Problem**

Linking quality education with student satisfaction the relationship between quality education and student satisfaction is much debated. One view is that, if the provision of high quality education is a "service", then it is closely related to levels of student satisfaction and that quality also results from a comparison of expectations with perceptions of performance.([6]) ([7])

In Saudi Arabia's density populated countries have a good opportunity to benefit significantly from the gains of education via distance learning as a means to reach large numbers of students scattered in the outskirts of the Kingdom in order to achieve education for all in a reasonable period of time and to break the barrier of fear and the unknown. Perhaps, many Saudi students do not have the desire to study remotely, but the crises have forced them to do so, but they lack the skills, but they are quickly adaptable, as the questionnaire at the College of Law showed the great desire of his law request to study remotely by a percentage. Also, there is a problem of poor teaching materials, as if they are not programmed for distance study and poor methods of remote teaching, which creates for us a problem called dropout from study.

**Model of Research and Hypotheses:**

System Quality is characterized by factors related to the software managing the distance course, such as ease of use, stability, and visual resources.([8]) Information Quality refers to characteristics about the course content, and Service Quality comprises actions of the subjects who are part of the teaching-learning process, such as teachers, tutors, and technicians. Jointly, both these dimensions, information and services, promote higher satisfaction and greater intention to use the resources delivered in the virtual environment.([9])

The use construct refers to the frequency with which the user accesses the resources offered by the virtual learning environment, and User Satisfaction is the positive feeling derived from participating in the course([10]) .Identified a relationship among multidimensions in which system quality, information quality, and service quality are independent variables that influence students' use of and satisfaction with virtual learning environments([11]).

Lee-Post (2009) reinforced the causality of the independent dimensions indicated in this study and termed them Satisfaction, Benefits Perceived, and Intention of Use ([12]). In research on the antecedents of continuity of use in distance learning, disclosed that "information quality, technology quality, perceived usefulness, and satisfaction with the system influence

the intention to use.

### Importance of the study

By the study, the instructions will be able to know the necessity of students and they will try the best to the student's satisfaction. The study also will help to find the problems in distance education. The serviced of quality of the new college is questionable due to due to lack of fulltime faculty members, up dated curriculum intrastation facilities and libraries teaching aid session jam, students-teachers polices and proper monitoring.

**Distance e-Learning**: Merisotis (1999) stated, "It is important to understand what is meant by 'distance learning.' Because the technology is evolving, the definition of what distance learning is continues to change."([13]) In the same report, they illustrated the fact that much of the research in distance learning since 1990 has serious, methodological flaws and there is "a relative paucity of true, original research dedicated to explaining or predicting phenomena related to distance learning." ([14]). This is a definition that does not distinguish formal and informal learning or different types of distance (temporal and physical). Newby, Stepich, Lehman and Russell (2000) define distance learning as "an organized instructional program in which teacher and learners are physically separated."([15]) ([16]) Moore and Kearsley 2012*([17])*.

**Student's satisfaction**: what student thinks about their university experiences, quality of education facilities teachers, students support services and other important aspect related to life on the university.

### Concepts of Quality

Quality in education has been defined by many researchers. A brief summary is presented by Sahney, Banwet, and Karunes (2003) in various words, "excellence in education and academics ([18]), value addition process in educational output fitness for purpose fitness of educational outcomes to planned goals and objectives, specifications and requirements in education. Avoidance of defects in the educational process and meeting or exceeding students' expectation from education ([19]).

## Methodology

The questionnaire was adapted according to online-learning requirements and is around four dimensions namely: effectiveness and credibility of online learning, availability of learning resources, students' challenges with the issues of the e-learning as well as questions on students' knowledge on COVID19. A total of 646 questionnaires were distributed, which consist of 35 Likert scale survey items, sorted into four dimensions/factors.

Out of 700 survey questionnaires administered, 646 survey questionnaires were responded to; nevertheless, 646 were applicable since 52 questionnaires remained unfinished or mistakenly filled out. This translates to a response rate of 90%. Cited that any study with a response rate of 50% and above is appropriate for analysis, hence our response rate of 95% was very good to continue with the analysis . Survey Monkey software was used to get data for the research. A self-structured questionnaire called Perception Profile of Distance Education (PPDE) was structured in Likert format using SA for Strongly Agree, A for Agree, D for Disagree, and SD for Strongly Disagree, which were weighed 4, 3, 2, and 1 respectively for data collection. The results were recorded on a 5 point Likert scale. The range was from strongly disagree to agree strongly. A mean score was used to evaluate students' responses to each dimension the reading for the Mean is 1.1.8-1.81-2.61-3,41-5.0.

### Data Collection

The questionnaires were given to the students of the Alasala law school at KSA University used for the study to administer among literate students in the areas. Out of 700 copies of the questionnaire given out, a total of 646 copies were returned and used for data analysis.

## Data Analysis

The data collected were analysed using the Raw-Data- ENG-xIsx platform. The research questions were answered using descriptive statistics (mean and standard deviation) while the hypotheses were tested at 0.05 level using inferential statistics (t-test and ANOVA). For the interpretation of the results in Tables 1, a mean of 2.5 and above is an indication that an item was accepted while a mean score of less than 2.5 is an indication that an item was rejected by the respondents. Results The results of the study are as presented in Tables 1-6 below.

## Results

The results of the research were discussed in detail based on the questions that were provided. The purpose of the study was to investigate Coronavirus (COVID-19) and quality of Online Learning in Alasala law school in the analysis, data from questionnaires were coded and analysed by the use of version 20 of SSPS software. The software was used to excellent reliability among multiple measures of variables of the study. During the study, 700 questionnaires were given out to the respondents, out of which 646 were valid.

### Instrumentation

A self-structured questionnaire called Perception Profile of Distance Education (PPDE) was structured in Likert format using SA for Strongly Agree, A for Agree, D for Disagree, and SD for strongly disagree, which were weighed 4, 3, 2, and 1 respectively for data collection. Through face validity, the instrument was validated by an expert in guidance and counselling. Out of 24 items proposed in the draft copy of the instrument, fifteen of them were used for final construction after careful and painstaking scrutiny by the expert. Below the instrument was an open-ended question on the general impression of distance education in Alasala law school. The final version of the instrument was subjected to Cronbach alpha analysis using people working in Alasala University who were not part of the study. A result of Cronbach's alpha 0.870 was obtained to ascertain the reliability of the instrument. And the split-half coefficient was 0.891.

**Sex of Respondents, Female** Frequency 430 ,the present 66.56, valid present 66.56 and cumulative 66.56. the man Frequency 216,the percent 33.44,valid present 33.44 and cumulative 100.0.

**Sex of Respondents** of the study describes the whole number of respondents along with the frequency of two options, first is 'Female' 430 with the percentage of 66.56 the second is 'Male' 216 with a percentage of 33.44.

Educational Qualification of Participants of the study presents the total number of respondents which is 646. Also has the frequency of each level such as level 1 (12.69 %) next level 2 (14.71%) level 3 (7.12 %)level (21.83 )4 level(21.83 %) 5 level (6.97)6 (13.47) level 7(8.36 ) level 8 (10.53)then and 'level 9( 4.33) .

The study presents a numerical summary of the 646 usable responses obtained from students who participated in the questionnaire survey. The average ratings on these questions are mostly between 3.0 (indication a neutral stances) and 4.0 (indication a favorable stance). For example, in response to question If the Ministry of Education's decision to continue distance education during the first semester of next year continues, how many hours do you expect to enrol in that semester?, the average rating is 3.4suggestion that students were generally confident and comfortable with using the relevant technology required foe online-learning. Similarly. The mean response to question, Do you prefer recorded lectures or live lectures during the distance learning experience? indicating that students had a moderate amount of trust in the information that was presents through online-learning. The mean response to question, Do you complete lectures, tests and tasks through distance learning? quite high (4.3), suggestion considerable enthusiasm for recorded

lectures or live lectures during the distance learning experience, and pointing to an area where online-learning technology clearly has the potential to make a positive contribution. The mean rating for question. Do you think that the educational materials provided for the courses is also favorable (3.6) confirmation that students found it useful to be able to download and/or or pint learning materials for ready access.

It is interesting to compare the moderately favorable responses (mean =3.6) to If Distance Learning continues, how many hours will you enrol for in Semester 1? which asks about the If Distance Learning continues, how many hours will you enrol for in Semester 1? Which mean students accepted the use of online-learning instead of face to face classes? With the rating of only (3.7) obtained for If the Ministry of Education's decision to continue distance education during the first semester of next year continues, how many hours do you expect to enrol in that semester. Taken together, these responses suggest that although students with access to face-to-face classes did not particularly wish see greater use of online-learning technology they did recognise the essential role of online -learning in the current KSA context to meet the requirements of a rapidly growing education system and to address the needs of female students as well as students in remote areas.

The question about has educational content during the distance learning experience helped you understand the course and prepare for evaluations? The most general, asking students to provide on overall rating on the current use of online-learning. The answer is generally negative mean rating of only 1.7, the lowest of all mean ratings. Similarly, response to the What kind of educational content do you prefer in distance learning? about, what kind of educational content do you prefer in distance learning? The answer generally negative (mean=1.9) to extent that dissatisfaction which current systems could be reduced via changes in information technology systems.

Validity and reliability of the questionnaire

Honestly meant the internal validity and consistency of each paragraph of the questionnaire with the domain that this paragraph belong to, and the researcher calculates the internal consistency of the questionnaire through the expense of correlation coefficients between each paragraph of the areas of the questionnaire and the total score of the field itself.

**Reliability of the questionnaire:**

The steadfastly questionnaire means to give this questionnaire the same result if the questionnaire re-distributed more than once under the same circumstances and conditions, or in other words, the stability of the questionnaire means stability in the results of the questionnaire and not to change significantly as if it were re-distributed to individuals several times during certain periods of time .

In 5 & 6, as discussed already, reliability entails the level upon which an instrument used to make the measurements have variable errors that vary every time the use of the instrument successfully measures a unit. Also contains the mean of each instrument and the cumulative variance of each instrument. The questionnaire consisted of 04 subscales and used 5 points Likert Scale, following is showing a score of each point of the Likert scale. In the first question, what are the expected challenges that students are likely to encounter during the online teaching and learning?.

In addition the discussion of Student's Perception of the Effectiveness and Credibility of Course Content for Online Learning What are the Saudi students' perceptions of the importance of quality standards in a distance study? Learning refers to the use of texts, software, videos together with some other forms of materials recommended by the teacher to help a student meet the required expectations of learning. A mean of 3.6 shows that students are satisfied with the learning resources available Self-motivation is a prerequisite for e-learning; however, many online

learners lack this, which surprised them. Students need to find the motivation to follow new educational trends as well as properly equip themselves to face future challenges in their education and careers. Only a positive attitude will help them overcome challenges in e-learning; although this is difficult to do, students need to understand that it is necessary to reap the benefits of e-learning in the future

**Discussions Reliability of the questionnaire**

Student's Perception of the Effectiveness and Credibility of Course Content for Online Learning What are the Saudi students' perceptions of the importance of quality standards in a distance study?

Learning refers to the use of texts, software, videos together with some other forms of materials recommended by the teacher to help a student meet the required expectations of learning. A mean of 3.74 shows that students are satisfied with the learning resources available. What are the expected challenges that students are likely to encounter during the online teaching and learning?

Many students are not provided with the high bandwidth or strong internet connection required for online study, and thus fail to catch up with their virtual classmates: their weak screens make it difficult to follow the study management system and their learning experience becomes a problem. Moreover, some of them do not have modern computers or electronic devices. The only solution to this problem is knowing exactly what kind of technology support they will need for a particular course before enrolling in it, as well as preparing themselves properly to successfully complete the course.

Although students are generally technologically intelligent and thus able to manage computers well, the lack of computer literacy is a major problem among students today. Many of them cannot run basic programs like Microsoft Word and PowerPoint and therefore cannot handle their files. Moreover, many students find that fixing basic computer problems is troublesome, as they know nothing in this field. However, technological competence is a must for online courses, as it enables students to manage their assignments and curricula in an organized manner without difficulty. Basic computer literacy courses enhance students' knowledge in this field. Having a basic knowledge of computers will help them participate in classes online without interruption or hindrance.

What are students' perceptions of strengths and weaknesses through distance learning applications?

In the course of the study, one major challenge of online learning is meant to impart a sense of togetherness in a community in an online environment. Other issues Students identified the high cost of internet data for students . Self-motivation is a prerequisite for e-learning; However, many online learners lack this, which surprised them. Students need to find the motivation to follow new educational trends as well as properly equip themselves to face future challenges in their education and careers. Only a positive attitude will help them overcome challenges in e-learning; Although this is difficult to do, students need to understand that it is necessary to reap the benefits of e-learning in the future. How much Satisfied are the Students with "Learning Resources" Available?

Learning refers to the use of texts, software, live study with some other forms of materials recommended by the teacher to help a student meet the required expectations of learning. A mean of 3.7 shows that students are satisfied with the learning resources available.

## Conclusion

The results of the study indicate that the KSA students' experience in a distance study is insufficient and immature, as they have not previously experienced it or providing adequate training for students compared to what was happening in traditional education. Where as, the method of presenting the lecture is weak and traditional and does not correspond to the era of rapid

development, and this indicates weakness of quality and service.

The interaction of KSA students is weak and sometimes non-existent in distance education; online services are not enough for the student to help him with educational materials to provide good service and high-quality education. The study also found that the quality assurance mechanism does not exist. More criticism as discovered in this study is that distance education from where the supervision of the lecturer does not exist, which makes the system weak.

A total of 646 participated in the study, 66.56% were females, and 33.4% males. The reliability of all subscales and the overall scale was found higher, which substantiated the reliability of the instrument. The survey found out that the learners were contented with online learning education provided by Institutions of Higher Education in Alasala, KSA. Student's Perception of the effectiveness and credibility of the online learning program yielded the highest mean score (3.77), and challenges that students encounter during the online Teaching and Learning received lowest mean score (3.51); on a 05 point Likert scale these scores are appreciative. Based on the findings, it is clear students are satisfied with the online teaching and learning instituted by Alasala law school, despite the few challenges identified. The results of the study indicate that the students' experience in a distance study is insufficient and immature, as they have not previously experienced it or providing adequate training for students compared to what was happening in traditional education. Where as, the method of presenting the lecture is weak and traditional and does not correspond to the era of rapid development, and this indicates weakness of quality and service the system.

The results indicated the importance of raising the level of interaction and social cooperation. Therefore, we saw the importance of partnership between teachers and students together to formulate modern methods of interaction and cooperation to create an effective and effective community online.

**Declarations**

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# Human Papillomavirus DNA in a Sample of Iraqi Women with Positive Visual Inspection by Acetic Acid

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## Abstract

**Background**: This study has conducted in Baghdad Teaching Hospital, Department of Obstetrics and Gyanecology, Al-Habebyia H. outpatient,Ibn-Albalady H. outpatient and 6 Primary Health Care Centers in Baghdad between June 2019 to March 2020.A Prospective and multicentric study.

**Aim of the Study:** To asses the prevalence of HPV in a sample of Iraqi women.

**Patients and methods:** The study include 268 married women between the age of 30 years old and more, who were attending the outpatient department. Both screening tests; naked eye visual inspection with acetic acid &HPV test were performed.

**Results:** The study included 268 women.The positivity rate of HPV for all women survived was 8.9% ,19 % of women with positive VIA ,81% with negative VIA.All the positive VIA cases(19 %)and some ofnegative VIA cases(18.6%) were randomly selected(because of their symptoms) underwentHPV test. After comparing the results of HPV test and VIA results, the sensitivity of VIA 95.8 %The specificity of VIAwas 63.6%. Positive predictive values was 45.1%. Negative predictive values was 98%.Accuracy rate 71.3%.

**Conclusions:**

1. The prevalence of HPV infection among a sample of Iraqis women was very low.

2. Estimation of HPV infection would provide valuable data particularly to introduce the strategies for the screening of cervical cancer and HPV vaccination in Iraq.

3.VIA with HPV DNA test may be a suitable substitute to Pap. smear as a screening tests for premalignant and malignant disease of the cervix.

***Keywords:*** *HPV, Iraqi women, DNA,acetic acid.*

## Introduction

Routine cytological screening and treatment of women has resulted in a dramatic decline in cervical cancer deaths over the past four decades in wealthier countries. In developing countries cervical cancer is preventable by screeningasymptomatic women for precancerous cervical lesions and treating the lesions before they progress to invasive disease. In other words, those deaths are largely preventable. The cure

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rate for invasive cervical cancer is closely related to the stage of disease at diagnosis and the availability of treatment,ifleft untreated cervical cancer is almost fatal.(1)

Human papillomavirus plays a major role in the development of cervical cancers. Also increasing evidence suggests that HPV oncoproteins may be a critical component of continued cancer cell proliferation. Unlike low-risk serotypes, oncogenic HPV serotypes can integrate into the human genome. As a result, with infection, oncogenic HPV's early replication proteins E1 and E2 enable the virus to replicate within cervical cells. These proteins are expressed in high levels early inHPV infection. They can lead to cytologic changes detected as low-grade squamous intraepithelial (LSIL) cytologic findings on Pap smears(2).

**Cancer subtypes**

Though squamous cell carcinoma is the cervical cancer with the most incidence, the incidence of adenocarcinoma of the cervix has been increasing in recent decades(3).

**Human papillomavirus**

Detection of HPV is associated with a 250 fold increase risk of high grade CIN, while persistent high-risk HPV infection increase the risk of developing CIN 300 fold (4) In fact, most of the behavioral and sexual risk factors for cervicalneoplasia become statistically insignificant as independent variable s after adjusting for HPV infection(5). persisting HPV type after treatment have anincreased risk of residual CIN(2,6). pre-cancerouslesion become more severe, the koilocytes disappear, the HPV copy numbers decrease, and the capsid antigen disappears, indicating that the virusisnotcapable of reproducinginlessdifferentiatedcells.Instead, portions of the HPV DNA become integrated into the hostcell. Integration of the transcriptionally active DNA into the host cell appears tobeessential to malignant growth(4).There have been remarkable improvements in both the sensitivity and specificity of HPV in the last three decades(7).

Direct detection of HPV DNA can be done histologically by either:

**a**-nucleic acid amplification via polymerase chain reaction (PCR)(8)

**Prevention of Ca. Cervix**

Since the most common form of cervical cancer starts with pre-cancerous changes, there are two types of prevention:

**1.Primary prevention by vaccination:**

Although cervical cancer is a preventable disease and is completely curable if detected at an early stage, cytological screening alone will not lead to the, The tetravalent HPV vaccine is most effective if given before any sexual exposure, but sexually active women can receive and benefit from vaccination. Tetravalent vaccine is not recommended

The second one (Cervarix) is a bivalent HPV vaccine of HPV types 16/18, used at 0,1 and 6 months as a 0.5 ml intramuscular injection.The efficacy of these vaccines in preventing persistent HPV infection has been found to range between 90% and 100%, and immunity provided has been shown to last for in excess of six years. (9).

**2.Secondary prevention by early diagnosis of precancerous lesions(screening):**

Cervical cytology screening programs in the developed countriesof Europe and North America have been followed by substantialreduction in disease burden. However, screening programs do notexist in most developing countries.The success of prevention programs based on cytology screeningdepends upon availability of adequate technical personnel, goodquality cytology smears, adequate laboratory services with internaland external quality control, a good organisation to ensure highcoverage of the target population with screening and

diagnosis,treatment and follow-up of screen-positive women. In many developingcountries, technical, manpower and financial resources are inadequate to provide the necessary infrastructure. The difficulties in introducing cervical cytology screening in low-resource settings have prompted the evaluation of simple and inexpensive non cytological methods of detecting precursor lesions, such as visual inspection of the cervix after application of 3–5% acetic acid (VIA) or after the application of Lugol's iodine (VILI)[10]

**Types of visual detection:**

**1- Naked eye inspection after application of 3-5% acetic acid (VIA):**

The acetic acid coagulates protein of cytoplasm and nuclei and since abnormal epithelium is of a high nuclear density, this prevents light from passing through the epithelium, which thus appears white[5], the provider can recommend further treatment as needed.

**2-Visual inspection after the application of Lugol's iodine (VILI):**

If iodine is applied to the cervix, precancerous and cancerous lesions appear well-defined, thick, and mustard or saffron-yellow in colour, while squamous epithelium stains brown or black, and columnar epithelium retains its normal pink colour.[1]

**Aim of the study**

To asses the prevalence of HPV in a sample of Iraqi women.

## Materials & Methods

**Study Design:**

This is a Prospective study which has conducted in Baghdad Teaching Hospital, Department of Obstetrics and Gynaecology, Al-Habebyia H. outpatient,Ibn-Albalady H. outpatient and 6 Primary Health Care Centers in Baghdad between June 2019 to March 2021.

268 women were enrolled in the study, some of them were complaining from different gynaecological problems and others had no complaint.

**Inclusion criteria**:

268 married women ages equal or more than 30 years. Written informed consent was obtained from all participants. Information on demographics and risk factors was obtained by a self-administered questionnaire.All patients examinedby VIA and HPV DNA test done for all positive VIA patients &for some patients with negative VIA & had complaint.

**Exclusion criteria**:

Women who were lacked a cervix (hysterectomies), were pregnant, had a history of cervical cancer, or were unable to provide consent were excluded.

**Materials:**

vaginal speculum (Cusco's speculum), a sterile rubber gloves, adequate light source about 100 watt(flash light), cotton swabs,labelled positive charge slides, Ayres spatula, Cuplan`s jar contain 95% ethyl alcohol. Freshly prepared 5% acetic acid solution (5ml of glacial acetic acid with 95 ml of distilled water).

The woman is asked to lie in a modified lithiotomy position onto the examination table after she has emptied her bladder.Inspection of the external genitalia was done for the presence of lesions. Thenfull pelvic examination was done in the usual way. A sterile Cusco's speculumwas carefully inserted in the vagina, and avoid use antiseptic solution forsterilization of genetilia. Inspection of the cervix was done for cervicitis,ectropian, nabothian cyst, cervical ulcer or erosion, polyp, outgrowth, andbleeding for which the women is giving treatment and appointment for reassesment. The four vaginal fornices then examined to make sure that they arefree from any growth. VIA was done for all patients and it involve gentle application of 5% acetic acid. The woman is informed

that she might feel a slight stingingsensation. After 1□2 minutes a naked eye evaluation was performed under 100-watt illumination. The transformation zone is carefully checked for any dense non movable acetowhite areas in the mucosa. If acetowhite areas are identified on the cervix after 1-2 minute, the test is positive then HPV smear was done for those positive VIApatients&those who had complaint on the same day using a conventional disposable wooden Ayre□s spatula, scrape the cervix around theentire transformation zone and smearing the cells onto a labeled glass positive charge slides, 2 slides for each patient. The smear is fixed with 95% ethyl alcohol for 20- 30 minutes. these slides were sent to laboratory to be examined.

**Criteria for categorization**

VIA findings were categorised as negative when any of the following findings were observed:

- No acetowhite lesions, or faint, ill-defined, bluish white or doubtful lesions.
- Acetowhitening on cervical polyps or on nabothian cysts
- Dot- or streak-like acetowhitening on the cervix.
- White line-like prominent squamocolumnarjunction (SCJ) after application of acetic acid.
- Angular or geographic acetowhite lesions far away from the SCJ or the external os, if SCJ was not visible (satellite lesions) .

VIA was categorized as positive when any of the following were observed:

- Well-defined, opaque, acetowhite lesions touching the SCJ or the external os, if SCJ was not visible.
- A large circumferential acetowhite lesion surrounding the external os.
- Pre-existing wart or leukoplakia turning intensely white after application of acetic acid.
- Ulceroproliferative growth turning densely acetowhite after application of acetic acid[10].

The woman after completition of the tests can go back to her normal activities.

When the results of HPV test appear, follow up the patients was done.

**In Situ Hybridization for the Detection of Hpv-16, HPV-18**

**Principle of the Test:** In situ hybridization is a method of localizing and detecting specific DNA or RNA sequences in morphologically preserved tissue sections. Briefly, the method involved deproteinization of fixed tissue sections mounted on slides hybridization of a biotinylated probe to the target sequence, the hybridized probe was then detected by addition of a streptavidin – alkalinephosphatase (streptavidin-AP) conjugate (DNA probe hybridization/Detection system in situ kit,Dako biotech, USA).

Upon addition of the single component BCIP/ NBT solution (substrate) which is 5-brom-4 chloro-3 indolyl phosphate/Nitro blue tetrazolium, an intense blue signal appeared at the specific site of the hybridized probe. This streptavidin-AP conjugate directly linked to the biotinylated probe provides a rapid and highly sensitive detection method.

**Statistical analysis**

Analysis of data was carried out using the available statistical package of SPSS-20 (Statistical Packages for Social Sciences- version 20).

Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different percentages (qualitative data) were tested using chi-square test ($c^2$-test) with application of Yet's correction or Fisher Exact test whenever applicable.

Statistical significance was considered whenever the P value was equal or less than 0.05.

The sensitivity, specificity, positive predictive value, negative predictivevalue and accuracy rate were calculated.

Sensitivity= True positive / (True positive+False negative).

Specificity= True negative/ (False Positive+True negative

## Results

Women under study. The total number of women included in this study was 268they had an agespectrum between 30-54 years old. The number of women in the reproductiveage was 264(98.5%)while the number of post menopause was 4 (1.5%), the mean±SD for age was 36.4±5.2. the mean±SD for age of first sexual intercourse (years) was 19.9±4.1, grand multiparous 134the mean±SD was 3.8±2.0. Themajority of patients were non-smoker 266 (99.3%), and most of them 232(86.6%) were not use oral contraceptive pills.

Table 1 shows VIA results for all 268 women under study. 51(19%) women hadpositive VIA test, the remaining 217(81%) had negative VIA test.

Table 2 show that 24 (23.8%) had positive HPV test, the remaining 77(76.2%) had negative HPV test.

Table 3 shows that 23 women with positive VIAhad HPV positive test&28 women with positive VIA hadHPV negative test, onewoman with negative VIA had HPV positive testand 49 women with negative VIAhad HPV negative test. the sensitivity of VIA was 95.8%, the specificity 63.6%,the positive predictive value 45.1%, the negative predictive value 98%. Figure 1.

**Table 1: VIA results**

| VIA | No. | % |
|---|---|---|
| Positive | 51 | 19% |
| Negative | 217 | 81% |
| Total | 268 | 100% |

**Table 2: HPV results**

| HPV | No. | % |
|---|---|---|
| Positive | 24 | 23.8 |
| Negative | 77 | 76.2 |
| Total | 101 | 100% |

**Table 3: Comparism between VIA test outcome and HPV resultsHPV**

| | | Positive | Negative | P value |
|---|---|---|---|---|
| VIA | Positive | 23 | 28 | PPV 45.1 |
| | Negative | 1 | 49 | NPPV 98.0 |
| | | Sensitivity 95.8 | Specificity 63.6 | |

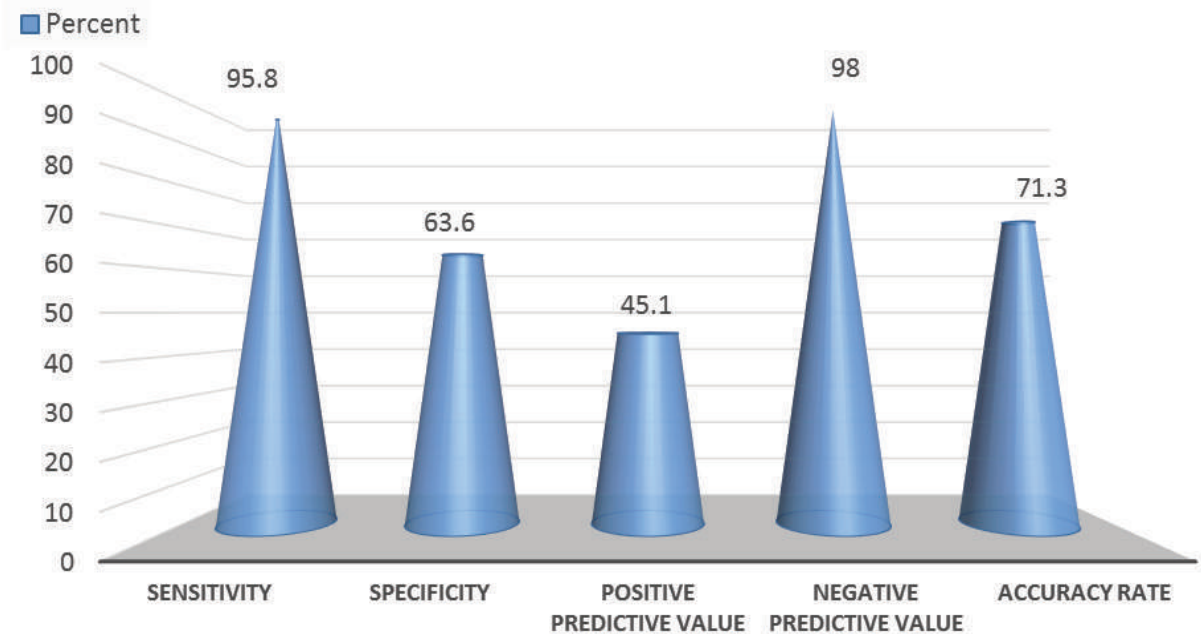


**Figure 1: This figure show VIA statistical results**

## Discussion

Infection with oncogenic HPV has been identified as the underlying cause of cervical cancer therefore there is interest in the use of HPV DNA testing as a primary screening test for cervical cancer. The overall prevalence of HPV among cervical cancers in a large international study was more than 99%, the highest attributable fraction ever identified for a specific cause of cancer [12].

In our study the positivity rate ofHPV infection in all survied women was 8.9%, all viral types wereofthe high risk genotype (type16&18), other types of HPV not included in our study because of difficulty in availability of the kits& probs of HPV tests, so we chose the most oncogenic types.

Inameta-analysissummarizingthegloballiteraturef orwomen with negative cytology results, **de Sanjosé et al** in July 2007, the worldwide prevalence of women harboring HPV DNA was 10.4% [13]. In other studies the rates range from 7.6% to 27%, depending on the region [14,15] while in June2010arandomized trial in Turkey by**Eren et al** [16], found that the prevalence of HPV amongTurkishwomenwas16.5%, multiplegenotypes were found in 35.8% of the infected women, and 75% of the typeswerehigh risk. While in Japan study done by **Inoue** in 2010[17], the prevalence was14.5%.Another randomized trial done by **Fernandes et al** in Brazil in 2009, shows that the overall HPV prevalence in Brazil was 48%[18].

A similar study in Iran was done by **Khodakarami, Net al.** in 2012shows that the prevalence of HPV was 7.8%[19].

In our study the results showed the highest incidence of HPV infection among patient aged between 40-44 years old, this results also found in Turkish study [16].

AlthoughagecurvesforHPVinfectiondiffer notably acrossregions, HPV prevalence is strongly associated with ageworldwide.

Inallworld regions, HPV prevalence was highest in women younger than 35 years of age, decreasing in women of older age [13, 14].

Oral contraception has been proclaimed as a risk factor of cervical cancer on prolonged use by high-risk HPV positive women.In our study OCCPS was used

by 36 women(13.4%) ,only 1 of them hadpositiveHPV test ( which is statisticallynot significant).

**Syrjanen K et al** 2006 suggest that the use of OCCPS is not an independentrisk factor for cervical cancer or its precursors but sexual behavior is different among OCCPS users andnonusers ofcontraception, these factors predispose women to HR-HPV, high grade CIN and determine the outcome of their cervical disease / HR-HPV infection[20]

0.7% of women in our study were smoker, 99.3% of women were not smoker,all of themhad disease negative, which not reach statistically significant level. **Bosch FX et al** 2007 study state that smoking is a co-factor that modify the risk among HPV DNA positive women include the use of occps for five or more years[21].

**Syrjanen K et al** 2007 in Finland study conclude that cigarette smoking is not an independent risk factor of HSIL, but the increased risk ascribed to smoking is mediated by acquisition of high risk \HPV,of which current smoking was an independent predictor in multivariate model[22].

], the sensitivity of VIA and Pap smear were 74.3% and 37.1% respectively, and also by **Cohn *et al***the sensitivity of VIA was 76.7% which is higher than the sensitivity of Pap.smear 44.3%. Also, by **Rana *et al***2010 [23] reported that there was no statistically significant difference between the specificity ofVIA and cervical cytology.

## Conclusion

1.The prevalence of HPV infection among a sample of Iraqi women was very low.

2. Estimation of HPV infection would provide valuable data particularly to introduce the strategies for the screening of cervical cancer and HPV vaccination in Iraq.

3.VIA with HPV DNA test may be a suitable substitute to Pap. smear as a screening tests for premalignant and malignant disease of the cervix.

**Declarations Conflict of Interes**t the authors declare that the reareno potential conflicts of interestrelatedtothestudy.

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# Soluble Cluster of Differentiation 25 (sCD25) as a Predictor of Mortality of COVID-19 Patients in Surabaya, Indonesia

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## Abstract

The purpose of this study was to analyze and determine the cut-off level of sCD25 as a predictor of mortality in COVID-19 patients. In an observational analytic study with a prospective cohort design, the study population was COVID-19 patients who were hospitalized at RIK RSUD Dr. Soetomo Surabaya for the period July 2020-December 2020. Sampling was taken by consecutive sampling, divided into two groups, mild-moderate and severe-critical groups. The examination of sCD25 levels in both groups was carried out on day-0 and day-6 of hospitalization using the sandwich ELISA method. The paired group statistical analysis used the Wilcoxon range test, the unpaired group used the Mann Withney U test. ROC curve analysis to determine the cut off level of sCD25 as a predictor of mortality. There were a total of 83 study patients consisting of 36 patients in the mild-moderate group, 47 patients in the severe-critical group. There was a difference in sCD25 levels between mild-moderate COVID-19 patients who were treated on day-6 compared to day-0, whereas in the severe-critical group there was no difference in sCD25 levels. There was a difference in sCD25 levels in COVID-19 patients between the mild-moderate group by severe-critical. The level of sCD25 with a cut off of 3.14 ng/mL (AUC 0.719, p = 0.001) can be used as a predictor of mortality in COVID-19 patients with a sensitivity of 96.2%, a specificity of 47.4%. Levels of sCD25 >3.14 ng/mL can be used as a predictor of mortality in COVID-19 patients

***Keywords:*** *sCD25, COVID-19, severe-critical, mild-moderate, mortality*

## Introduction

COVID-19 is an infectious disease caused by SARS-CoV-2. SARS-CoV-2 is a new type of Coronavirus that has never been previously identified in humans. This disease was identified starting with the emergence of a pneumonia case of unknown etiology in Wuhan, China at the end of December 2019[1,2,3]. COVID-19 spreads to various countries in a short time so that on March 11, 2020, the WHO determined this incident to be a Pandemic[2,4,5]. Indonesia reported its first case on 2 March 2020 then reported 70,736 confirmed COVID-19 cases with 3,417 deaths (CFR 4.8%) as of 9 July 2020[3].

COVID-19 gives varying symptoms, about 80% of sufferers have mild or asymptomatic infections, 15% have severe infections and need oxygen, and 5% are in critical condition and need a ventilator[6]. It is estimated that 10-15% of mild cases progress to severe, and 15-20% of severe cases become critical. The CFR among critical cases was 49% and patients with older age and comorbids are factors that can increase the mortality of COVID-19 patients[7]. The severity of the disease in COVID-19 patients generally occurs 1 week after the appearance of symptoms[8,9].

Hyperinflammation or cytokine storms are known to play an important role in the severity process of the disease[10]. Clinicians must be aware of this potential for deterioration so that it can reduce the mortality rate for COVID-19 patients.

Soluble Cluster of Differentiation 25 (sCD25) or also known as Soluble Interleukin-2 receptor α (sIL-2Rα) is a marker of inflammation and a diagnostic marker of immune system activation[11]. COVID-19 patients develop a hyperinflammatory syndrome that has similarities with other hyperinflammatory disorders[12], so it should be expected that the sCD25 marker can assess the inflammatory process and can be used as a predictor of mortality in COVID-19 patients. Soluble CD25 (sCD25) results from cleaving the CD25 receptor when T cells, B cells and dendritic cells are activated. The CD25 receptor is cut from the surface of the cell membrane by a protease enzyme, namely matrix metalloproteinase-9 (MMP-9) produced by T cells, macrophages, and dendritic cells induced by inflammatory conditions. The cutting of CD25 to form soluble CD25 (sCD25) can also reflect IL-2 activity attached to its receptors as well as the proliferation and activation of T lymphocytes[13,14].

Based on the above explanation, the researchers wanted to examine whether sCD25 levels could be used as a predictor of mortality in COVID-19 patients associated with immune cell activation and inflammatory processes that occur in COVID-19 patients, so that they can be used to predict mortality in COVID-19 patients.

## Materials and Methods

This type of study was an observational analytic study with a prospective cohort design. The study population was COVID-19 patients who were hospitalization in Ruang Isolasi Khusus (RIK) Dr. Soetomo Surabaya for the period July–December 2020. Study patients were taken from the study population who met the inclusion and exclusion criteria using consecutive sampling technique. Based on the severity level, it is divided into two groups, namely the mild-moderate and severe-critical groups. The examination of sCD25 levels was carried out on day-0 and day-6 of hospitalization. Serum samples were stored at -80ºC pending examination. Examination of sCD25 levels used Bioassay Technology Laboratory reagent with the sandwich ELISA method. Paired group statistical analysis used the Wilcoxon sign rank test method, the unpaired group used method mann withney u test. ROC curve analysis was used to determine the cut-off level of sCD25 on day-0 of hospitalization as a predictor of mortality within 30 days of COVID-19 patients.

## Results and Discussion

There was a total of 83 study patients who met the inclusion and exclusion criteria consisting of 28 female patients (33.7%) and 55 male patients (63.3%). The mild-moderate group consisted of 36 patients and the severe-critical group of 47 patients. Based on gender, men were more in the severe-critical group, while women were more in the mild-moderate group (Table 1). COVID-19 shows different case fatality between men (2.8%) and women (1.7%), this is associated with ACE2 expression. ACE2 activity in men is lower than women because estrogen triggers an increase in ACE2 expression more than androgens[15].

In the severe-critical group, it was found that the median age of patients was older than the mild-moderate group and comorbids were more common in the severe-critical group (Table 1). Age >65 years is a factor associated with the severity and mortality of COVID-19 patients[15,16,17]. This is related to the function of the immune system and organ function. The function of T cells and B cells decreases with age[18,19,20]. The presence of comorbidities is known to increase the case fatality rate (CFR) and provide a poor prognosis in COVID-19 patients[21,22]. In this study, there were no significant differences based on comorbid (Table 1).

**Table 1. Characteristics of research subjects based on gender, age and co-morbidity in the mild-moderate and severe-critical groups.**

| Variable | Mild – Moderate (N = 36) n (%) | Severe – Critical (N = 47) n (%) | P value |
|---|---|---|---|
| **Gender** | | | |
| Male n (%) | 17 (31.5%) | 37 (68.5%) | 0.005 |
| Female n (%) | 19 (65.5%) | 10 (34.5%) | |
| Age | 51 (24-68)* | 53 (25-72)* | 0.076 |
| **Obesity** | | | |
| N n (%) | 27 (40.9%) | 39 (59.1%) | 0.419 |
| Y n (%) | 9 (52.9%) | 8 (47.1%) | |
| **Asthma** | | | |
| N n (%) | 34 (42.5%) | 46 (57.5%) | 0.576 |
| Y n (%) | 2 (66.7%) | 1 (33.3%) | |
| **Hypertension** | | | |
| N n (%) | 30 (47.6%) | 33 (52.4%) | 0.202 |
| Y n (%) | 6 (30.0%) | 14 (70.0%) | |
| **Diabetes Mellitus** | | | |
| N n (%) | 27 (49.1%) | 28 (50.9%) | 0.165 |
| Y n (%) | 9 (32.1%) | 19 (67.9%) | |

*Median (Min-Max). Description: Y: Yes, N = No,

In this study, the levels of sCD25 were not significantly different between the mild-moderate and severe-critical groups based on sex, age and comorbid obesity, asthma, hypertension. Significant differences in sCD25 levels were only found in patients with comorbid diabetes mellitus in the two groups (p = 0.005; p = 0.018) (Table 2). Increased levels of sCD25 may be related to the chronic inflammatory process that occurs in diabetes mellitus called chronic low-grade inflammation, which is characterized by the production of cytokines, chemokines, and adipokines[19].

**Table 2. Levels of sCD25 by age, sex and comorbidity in the mild-moderate and severe-critical groups.**

| Characteristics of sCD25 | Group | | | |
|---|---|---|---|---|
| | Mild - Moderate | | Severe - Critical | |
| | Median (Min-Max) | p Value | Median (Min-Max) | p Value |
| Age | | | | |
| ≤ 50 | 3.1 (1.7-9.9) | 0.129 | 3.5 (0.4-24.1) | 0.121 |
| > 50 | 2.7 (0.9-9.9) | | 5.3 (0.6-18.4) | |
| Gender | | | | |
| Male | 3.1 (0.9-7.4) | 0.925 | 4.8 (0.4 - 24.1) | 0.404 |
| Female | 2.7 (1.1-9.9) | | 4.7 (3.5 - 13.3) | |
| Obesity | | | | |
| N | 2.9 (0.9-9.9) | 0.886 | 3.8 (0.4 - 24.1) | 0.132 |
| Y | 2.7 (2.2-8.6) | | 6.0 (3.5 - 17.1) | |
| Asthma | | | | |
| N | 2.8 (0.9-9.9) | 0.133 | 4.6 (0.4-24.1) | 1.000 |
| Y | 6.1 (4.8-7.4) | | - | |
| Hypertension | | | | |
| N | 3.1 (0.9-9.9) | 0.123 | 5.0 (0.4-24.1) | 0.383 |
| Y | 2,6 (1.2-4.3) | | 3.7 (2.2-13.3) | |
| Diabetes Mellitus | | | | |
| N | 3.1 (1.7-9.9) | 0.005 | 3.5 (0.4-24.1) | 0.018 |
| Y | 2.5 (0.9-9.9) | | 6.3 (3.0-18.4) | |

Information: Y= Yes, N= No

In the mild-moderate groups, there was a significant difference in sCD25 levels between day-6 and day-0 of hospitalization (p = 0.016) (Table 3). Of a total of 36 patients in the mild-moderate group, 23 patients (64%) had decreased levels of sCD25 and 13 patients (36%) had an increase in sCD25. The level of sCD25 decreased from a median of 2.92 ng/mL on day-0 to 2.80 ng/mL on day-6 of hospitalization (Table 3). This can indicate that in patients there has been a decrease in the activation of immune cells, especially cells that express interleukin-2 receptor (IL-2R), so that the level of sCD25 in the blood decreases. In the severe-critical group, there was no significant difference in sCD25 levels between day-6 and day-0 of hospitalization (Table 3). This is because of a total of 47 patients in the severe-critical group, 27 patients (57%) experienced a decrease in sCD25 levels and 20 patients (43%) experienced an increase in sCD25 levels so that their sCD25 levels were not significantly different.

There were significant differences in sCD25 levels on day-0 and day-6 between the mild-moderate group and the severe-critical group (p = 0.001; 0.004). The median level of sCD25 for the critical severe group tended to be higher when compared to the median for the mild-moderate group both on day-0 and day-6 of hospitalization (Table 3). This is consistent with a number of studies where sCD25 levels are positively correlated with severity[10,20,21,22]. In severe degrees of COVID-19, the increase in sCD25 levels is thought to be due to inflammation which increases the action of a proteolytic enzyme, namely MMP-9 which cuts sCD25 on the cell surface[23].

Based on the patient's outcome of the severe-critical groups, 21 patients recovered and 26 patients died. There was no significant difference in sCD25 levels between 6 and 0 days of hospitalization in the critically severe group with outcomes of recovery or death (Table 3). In patients who recovered, the median sCD25 decreased, while in patients who died, the median sCD25 increased although it was not statistically significant. A longitudinal analytical study identified innate and adaptive immune response patterns severe COVID-19. In severe patients there is a delay in the immune response or the course of the immune response appears to be protracted and irregular. In the severe COVID-19 cohort, changes in immune response occurred less dynamically and varied for 15 days after hospitalization. This may explain why in the severe-critical group there was no significant change in sCD25 levels on the 6$^{th}$ day of hospitalization[23]. It takes a longer observation time to get a significant change in sCD25 levels.

**Table 3. sCD25 levels in COVID-19 patients by group on day-0 and day-6 of hospitalization.**

| Variable | Group | Days to- | Median (Min-Max) | p-value |
|---|---|---|---|---|
| sCD25 (ng/mL) | Mild - Moderate | 0 | 2.92 (0.94-9.91) | 0.016 |
| | | 6 | 2.80 (0.81-8.84) | |
| | Severe - Critical | 0 | 4.78 (0.24-24.07) | 0.167 |
| | | 6 | 4.09 (1.03-23.55) | |
| | Mild - Moderate | 0 | 2.92 (0.94-9.91) | 0.001 |
| | Severe - Critical | 0 | 4.78 (0.40-24.07) | |
| | Mild - Moderate | 6 | 2.80 (0.81-8.84) | 0.004 |
| | Severe - Critical | 6 | 4.09 (1.03-23.55) | |
| | Severe - Critical (Outcome Recovery) | 0 | 3.58 (0.4-24.07) | 0.198 |
| | | 6 | 3.17 (1.03-23.55) | |
| | Severe - Critical (Outcome Deaths) | 0 | 5.30 (2.17-18.36) | 0.477 |
| | | 6 | 5.46 (1.06-23.55) | |

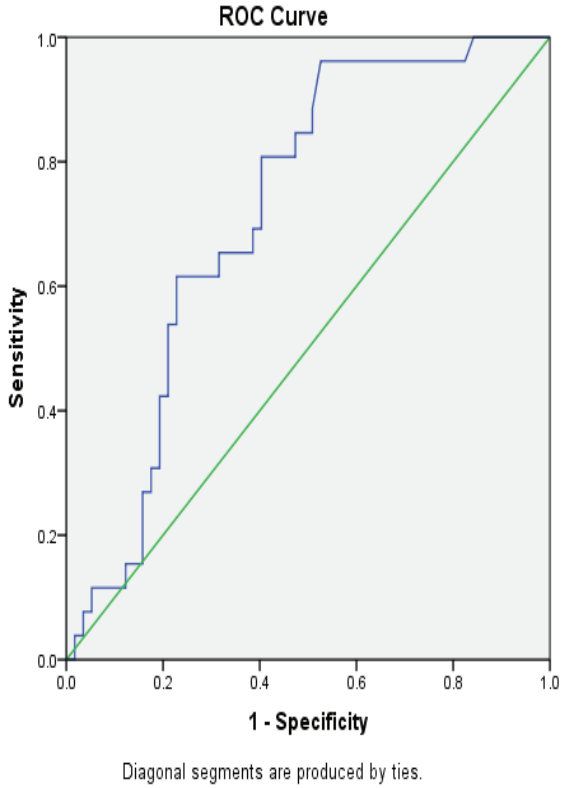


**Figure 1. Receiver Operating Characteristics (ROC) curve to determine the cut-off level of sCD25 as a predictor of mortality in COVID-19 patients.**

ROC analysis was performed to determine the cut off of sCD25 levels on day-0 to predict mortality within 30 days of COVID-19 patients. The mortality analysis was carried out on all patients (mild-moderate, severe-critical) as many as 83 people with a death outcome of 26 patients and 57 patients recovered. The results of the ROC analysis showed that the cut off level of sCD25 was 3.14 ng/mL (Area Under Curve 0.719, p = 0.001) with a sensitivity value of 96.2% and a specificity of 47.4% (Figure 1). With this cut off, clinicians are expected to be more aware of the potential worsening of COVID-19 patients so that they can reduce the mortality rate for COVID-19 patients. Low specificity can indicate that sCD25 can be said to be not a specific marker in predicting mortality in COVID-19 patients, combination with other markers is needed to increase its specificity.

This study has limitations, that there is no complete data regarding when the patient was infected so that the onset of symptoms cannot be ascertained and may can affect the level of sCD25 at the time of examination. Observation and measurement of sCD25 levels in the severe-critical group were carried out more than 6$^{th}$ day of hospitalization, a longer treatment time was needed in order to get a picture of the changes in sCD25 after the 6$^{th}$ day of hospitalization.

## Conclusion

In summary, there was a difference in sCD25 levels between the mild-moderate group of COVID-19 patients who were hospitalized on day-6 and day-0, whereas in the severe-critical group there was no difference in sCD25 levels. There was a difference in the sCD25 of COVID-19 patients between the mild-

moderate and severe-critical groups. The sCD25 level at day-0 with a cut off >3.14 ng/mL can be used as a predictor of mortality within 30 days of COVID-19 patients with a sensitivity of 96.2%, a specificity of 47.4%.

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# Post Traumatic Tuberculous Tenosynovitis in a Patient that Manifests as Soft Tissue Tumor: A Case Report

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## Abstract

Mycobacterium tuberculosis infection is one of the oldest diseases of the human race. This bacteria can attack any organ in the human body. Extra Pulmonary Tuberculosis (EPTB) infection diagnosis are rarely straightforward, and in many cases, delayed due to various reasons. Tuberculous tenosynovitis (inflammation of the tendon and its capsule because of Mycobacterium tuberculosis complex infection) is a rare form of EPTB. Tuberculosis infection at sites of previous trauma have been reported consistently though rarely. We present the case of a 48 years old male with complains of lumps on his lower left arm (with prior history of blunt trauma on the location) and progressive inability to flex the fingers of his left hand. Early examinations suggest the diagnosis to be a soft tissue tumour. However, tissue biopsy later showed that the patient was actually suffering from tuberculosis infection. The patient later showed satisfying response to tuberculosis medication on subsequent follow ups.

***Key Words:*** *Mycobacterium Tuberculosis, Tuberculous Tenosynovitis, Extra Pulmonary Tuberculosis*

## Introduction

Tuberculosis (TB) is one of the oldest diseases of the human race, thought to first appear around 70,000 years ago in Africa. This Mycobacterium complex infection primarily affects the lung parenchyma, but other organs and tissues can also be infected[1].

TB is one of the diseases with the heaviest global burden. More than a third of the human race is infected. In 2016, the WHO reported 1.7 million deaths caused by the disease, with 10.4 million of new cases of TB. Seven countries hosted 64 % of all new cases: India, Indonesia, China, Pakistan, Nigeria, and South Africa[2].

Extra Pulmonary Tuberculosis (EPTB) are defined as Mycobacterium tuberculosis infection of any tissue apart from lung tissue. EPTB represents 20-25 % of all tuberculosis. This number increases significantly in Human Immunodeficiency Virus patients[1,3].

The diagnosis of EPTB is rarely straightforward. In many cases, it took a considerable amount of time before the diagnosis is established. This phenomenon most often is caused by the delayed addition of EPTB into the differential diagnosis due to various reasons[4].

Tuberculous tenosynovitis (inflammation of the tendon and its capsule because of Mycobacterium tuberculosis complex infection) is a rare form of EPTB. Among tuberculosis patients, 1-3 %

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have musculoskeletal infection, and tuberculous tenosynovitis accounts for 5 % of this group, comprising of around 0.05 % of all TB cases[5]

EPTB developing at sites of previous trauma have been reported consistently, though rarely, in the literature of the past century. The mechanism that can explain how these cases emerge has been rarely researched. The study into the pathogenesis of post-traumatic tuberculosis might offer new understandings of tuberculosis pathogenesis[6].

## Case Report

A 48 years old male came to the hospital with complaint of progressive inability to flex the fingers on his left hand. The patient has been having difficulty in moving his left hand since 6 months ago. The disturbance worsened until he was not able to hold a glass filled with water. No other part of the body was affected. The patient also complained about two bumps on his left lower arm. The first bump appeared in 8 months ago on the lateral side just below his elbow. About one month before the appearance of the first bump, his lower left arm was hit by a wood while working at the same location. The skin was red, with mild pain for a few days after the trauma, but no visible wound was observed on the skin. The first bump gradually increases in size, with local paresthesia and light pain when pressed. About one month after the first bump appears, a second bump emerges close to his wrist, also gradually increasing in size. The skin on both bumps appears normal during the course of the disease. Five months after the trauma, the increasing difficulty experienced by the patient in moving his fingers brought him to the local hospital, which referred him to our hospital. The patient's appetite is normal, no complaints of night sweats or decrease of body weight. No history of past illness or family history of cancer. The patient is a farmer from a rural area.

The patient is fully alert and in good general condition. Height 155 cm. Weight 55.2 kg. BMI 23 kg/m2. Blood pressure 120/70 mmHg. Pulse 82 bpm. Breathing 17x/min. Temperature 36.3 degree celsius. On head and neck examination, we found no anemia, jaundice, cyanosis, or dyspnea. Lymph nodes were not enlarged. On thoracic examination, chest wall appears symmetrical, no intercostal retractions. Heart sound and breath sound normal. On abdominal examination, the surface appears normal, bowel movement normal, no ascites, and no organ enlargement. On extremity examination, the patient's perfusion was normal. Two bumps were observed on the patient's left antebrachium, the first was on the lateral side, just below the elbow, with the size of 5 x 3 x 1 cm. The second bump was on the medial side, just above the wrist, with the size of 4 x 2 x 1 cm. Both bumps have a rubbery consistency on palpation and were immobile.

Lab result showed: Hb 14,5 g/dL, RBC 5,65 jt/µl, HCT 43,9%, MCV 77,7 fL, MCH 25,5 pg, MCHC 33,0 g/dL, PLT 356.000/ µl, WBC 8.420/µl (Eos 3,4 %, Baso 1,1 %, Neut 63,9 %, Lymph 22,8 %, Mono 8,8 %) SGOT 29 U/L, SGPT 14 U/L, Serum creatinine 0,65 mg/dL, BUN 9 mg/dL, Random Blood Glucose 86 mg/dL, C-Reactive Protein 4 mg/dL. Antebracium x-ray of the patient's left arm within normal limit. US of both masses showed hypoechoic, well defined mass that attaches to the surrounding tendons and muscles, possibly a soft tissue tumor. Fine Needle Aspiration Biopsy showed proximal mass: hypocellular smear showing macrophage and lymphocyte distribution with purplish mucoid matrix. Distal mass: adequate cell smear showing distribution of round-nucleated cells with smooth chromatin, spacious cytoplasm. Small number of round-spindle nucleated cells with smooth chromatin (conclusion: Proximal mass: no signs of malignancy; tenosynovitis suspected. Distal mass: probably fibrohystiocytic tumor). MRI result: Synovial proliferation with enhancements that obliterates the tendon of the flexor pollicis longus and flexor digitorum profundus muscles along distal of the radius until the palm region, as high as the left metacarpal head. Hypointens lesion observed on T1M1 and T2M1, strict demarcation, regularly shaped with a size of 3.2 x 2.3 x 1.9 cm, underneath the extensor

carpi radialis longus muscle and anterolateral of the left radial bone. Normal bone trabeculation. Joint gap and surface appeared normal. Conclusion: Sclerosing tenosynovitis along distal of the radius until palmar region as high as the left metacarpal head; Soft tissue mass with strict demarcation, regular edge, in the size of 3.2 x 2.33 x 1.9 cm profundus of the long extensor carpi radial muscle and anterolateral from the left radial bone. Allegedly a deep myxoid tumor.

The patient then underwent surgical removal of the tumors. Biopsy result from the tumors showed granulomatic inflammation that's consistent with tuberculosis.

The patient received category I treatment for extra pulmonary tuberculosis for 6 months using the Fixed Dose Combination (FDC) regiment: Rifampicin 600 mg, Isoniazid 300 mg, Pyrazinamide 1600 mg, and Etambuthol 1100 mg daily for 2 months, followed by Rifampicin 600 mg and Isoniazid 600 mg three times a week for 4 months.

At the follow op 2 months after EPTB medication started, the range of movement of the patient's left hand fingers has improved, although not yet at the previous normal level. The patient is now able to hold a cup filled with water without any problem. Light activity which involves the hand, such as doing laundry, gardening, or lifting water from a water dipper can be done with relatively little problem. Pain and paresthesia are gone, and surgical wound was healing well.

## Discussion

From the physical examination, two bumps were observed on the patient's left antebrachium, the first was on the lateral side, just below the elbow, with the size of 5 x 3 x 1 cm. The second bump was on the medial side, just above the wrist, with the size of 4 x 2 x 1 cm. Both bumps have a rubbery consistency on palpation, immobile, with very mild pain on pressure. No other signs of infection or inflammation.

Frank pain, pathological fracture, and bone abnormality on x-ray was not found in the patient, excluding bone tumor from the differential diagnosis. Signs of infection such as tenderness, erythema, swelling, and warmth was not found. Pain was minimun on pressure of the lesion. The size of the lesions, their increase in size, and immobility points towards a possibility of a soft tissue tumor.

USG was planned to confirm the existence of soft tissue tumor and revealed hypoechoic, well defined mass that attaches to the surrounding tendons and muscles.

The immobility of the tumors and their adherence to the surrounding muscles and tendons, augmented by the fact that they kept getting bigger raised the suspicion of malignancy. FNAB was chosen as the earlier examination to minimize morbidity and risk of tumor spread.

FNAB examination revealed no signs of malignancy on proximal mass with tenosynovitis suspected. Distal mass analysis revealed probable fibrohistiocytic tumor. Tenosynovitis can be caused by fibrohistiocytic tumor that infiltrates tendon capsule[7,8]. MRI was planned as the next step for evaluation of the soft tissue lesion. The result of the MRI will help with planning the biopsy and tumor excision, if necessary.

MRI showed sclerosing tenosynovitis along distal of the radius to palmar region as high as the left metacarpal head; Soft tissue mass with strict demarcation, regular edge, in the size of 3.2 x 2.33 x 1.9 cm profundus of the long extensor carpi radial muscle and anterolateral from the left radial bone. Allegedly a deep myxoid tumor. Open biopsy and tumor excision if possible were our next planned course of action.

Biopsy result from the left elbow and wrist: both preparations showed similar picture. Tissue samples consist of groups of epitheloid hystiocytes, surrounded by lymphocytic inflammatory cells that

forms a granuloma. Also visible are distributions of datia langhans cells and necrosis. Muscle and fat tissue visible. No sign of malignancy. Conclusion: granulomatic inflammation, consistent with tuberculosis is the cause of the tenosynovitis.

Tuberculous tenosynovitis (inflammation of the tendon and its capsule because of Mycobacterium tuberculosis complex infection) is a rare form of EPTB, comprising of around 0.05 % of all TB cases[5]. A number of predisposing factors exist for tuberculous tenosynovtis: trauma, joint overuse, old age, low socioeconomic status, malnutrition, alcohol consumption, and immunosuppression. By far, the most affected location is flexor tendons of the hand and wrist. Involvement of other location is very rare. The dominant extremity is affected more (possibly because of the higher probability of trauma and overuse). Men are affected more than women. Infection results from direct inoculation from a wound or adjacent bone or joint, or the spread of tuberculosis from pre-existing lesions in the body[9].

The clinical manifestation of tuberculous tenosynovitis is slow-growing mass along the inflamed tendon without (or with minimum) pain. The patient may experience carpal tunnel syndrome, decreased range of motion, even tendon rupture when treatment is delayed. The slow progresivity of this disease often causes late diagnosis, when extensive damage has been done[9].

In tuberculous tenosynovitis, laboratory examination is generally within normal limits except for increasing erythrocyte sedimentation rate. MRI can provide a good evaluation of tendon and its capsule. Nonspecific tenosynovitis with serous exudate is the most frequent MRI result in higromatous phase. In serofibrous phase, we can usually find thickening of the synovium, thinning of the tendon, tendon damage or adhesion. Extensive spread of granuloma forming a soft tissue tumor is often observed in the fungoid phase[10].

According to the WHO, the diagnosis of EPTB must be based on one of the three criteria below, followed by administration of antituberculosis medications by the clinician[11]:

1. A culture that's positive for Mycobacterium tuberculosis complex.

2. Acid fast bacilli in histological examination.

3. A sound clinical evidence of an active EPTB (radiology, pathology, response to treatment).

Diagnosing EPTB is rarely an easy task. Wide spectrum of manifestation, affected by location, agresivity of infection, and patient's immune response add to the complexity of diagnosis effort. In many cases, it took a considerable amount of time before the diagnosis is established. This phenomenon most often is caused by delayed addition of EPTB into the differential diagnosis due to various reasons. Not often, empiric antibiotics have been given when TB is suspected. EPTB lesions are often pauci-bacilli, making histological diagnosis more difficult. Hard-to reach lesions complicates efforts to obtain samples for microscopy, histology, culture, or molecular examination[4].

The conformity we found in the patient's socioeconomic status, clinical manifestation, radiological, and pathological examination with clinical picture of serofibrous phase of tuberculous tenosynovitis convinced us to conclude that it is the right diagnosis.

Tuberculosis infection developing at sites of previous trauma has been reported consistently (albeit rarely) in the literature in the last century. The publication of these cases is divided into three cathegories: Tuberculosis in the location of previous open or stab wounds; Tuberculosis at sites of previous fracture fixation or prosthetic joint insertion; Tuberculosis at sites of previous blunt trauma (excluding direct inoculation of Mycobacterium)[6].

Barr et al. studied published cases of post traumatic tuberculosis in the last 50 years. Among 26 patients reported during that period, 25 of them was in or emigrated from tuberculosis endemic areas (Indonesia is, unfortunately still an endemic area for TB). Time span between trauma and onset of symptom ranged from 1 to 42 weeks with a median of 8 weeks[6] (4 weeks in our patient).

A number of studies on animals showed tuberculosis infection developing at sites of previous injury, not long after systemic injection of Mycobacterium Tuberculosis. In latent TB patients, granuloma formed surrounding focus of Mycobacterium infection to contain it. Recent data from animal models showed that mycobacterium-infected monocytes and dendritic cells can travel freely in and out of granulomas. Meanwhile, a continuous influx of monocytes and macrophages keep moving into granulomas, phagocyting dead or dying infected macrophages. Granuloma that once thought to be limiting infection, appears to be playing a role in disease spreading[6,12].

Sterile tissue injury provokes the release of several chemoattractant. Macrophage Chemoattractant Protein-1 (MCP-1) pulls monocytes from the circulation into inflamed locations. Hypothesis for the mechanism of post-traumatic tuberculosis is that in latent TB patient, infected monocytes can move out of granulomas to follow chemoattractant signals from inflamed tissues caused by trauma. This inflamed tissue might provide an environment that allows TB reactivation inside the infected monocytes, rapidly infecting numerous monocytes and macrophages gathered there, establishing a new Mycobacterium infection focus[6,13].

Reports of infection by Salmonella enteritidis at sites of previous trauma is an interesting information that might support the above hypothesis. Salmonella enteritidis is an intracellular facultative bacteria, just like Mycobacterium tuberculosis[6].

## Conclusion

In regions where tuberculosis is endemic, it is important to have a high suspicion index for cases of EPTB. The multitude of signs and symptoms it can exhibit can be very problematic in determining its true cause. This increased awareness can help hasten diagnosis, avoiding worse outcome as a result of a delayed treatment.

Signs of chronic infection or inflammation at sites of previous trauma in TB endemic regions, should alert clinicians of a potential EPTB. Post-traumatic TB can probably be explained by transportation of mycobacterium by monocytes and macrophages in the circulation, contrary to previous understandings that in latent TB patients, the infection is contained and inactive inside granulomas. Further pursuit to understand this mechanism is important to expand current knowledge of TB pathogenesis.

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# COVID-19 Outbreak and Health literacy of Health Institutions: The Role of Strategic Theory

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## Abstract

The local outbreak of COVID-19 and its development into a global pandemic in a short time have made health literacy more critical than ever in facing these global health threats. The main objective of the research is to know the reflection of the strategic theory in health literacy and the relationship and impact statement, and then it is possible to know the determinants of the health institution's success in managing health literacy efforts as the Department of Health of Najaf was the organization in question. The researcher surveyed the opinions of a number of its employees, as the research community was (248) employees, and the research sample reached (155) responses on which the analysis can be performed. A set of specialized descriptive and inferential tests and methods were conducted on the data obtained by using (SPSS v.26) program. The results show that the health institution's interest in strategic theory will contribute to achieving health literacy by demonstrating the relationship and impact between the two research variables. The research extracts a set of recommendations, the most important of which is to increase the interest of the departments in the Najaf Health Department in the concept of strategic theory and make health literacy programs a priority that must be implemented even in light of the scarcity of financial resources.

***Keywords:*** *Strategic theory, health literacy, and the COVID-19 pandemic.*

## Introduction

The theory means looking, seeing, observing. Many of the theories that have been used and developed in strategic management research, as in other established disciplines, implicitly or explicitly reflect the understanding of social activities and behavior that has occurred in the contemporary business environment. There are clear opportunities to develop new theoretical concepts that reflect assumptions, ideas, and relationships that differ from traditional settings either as entirely new or fundamental differences [(1)]. Strategic theory refers to models that describe or explain how strategies are developed, implemented, and changed. Models of strategic processes identify patterns in decisions or actions over time and address the mechanisms and pathways that shape and govern strategies. Research in strategic management is often categorized as related to either the content of plan or the strategy process [(2)].

Good theories provide valuable and relevant conceptual frameworks to understand the general requirements of a strategy and the general logic associated with its effective employment. This theoretical and conceptual knowledge is crucial for policymaking [(3)], and strategic theory may be the theory of interconnected decision-making under conditions of uncertainty [(4)]. It is able to resolve conflicts in the attempt to assess social activity designed to achieve

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the goals of arbitrary moral assessments. In this way, strategic theory facilitates clarity of understanding. Hence, strategic theory opens the mind and liberates it intellectually [5].

A disciplined strategic theory allows a professional to evaluate the merits of a particular strategy. The framework provided by this theory can be a systematic basis for the disciplined thinking process to aid the strategist in developing strategy and act as a guide for others to follow in understanding, evaluating, and criticizing the merits of a particular approach [6]. The strategic theory is a theory of action. From this standpoint, Colin Gray developed the idea of a "strategic theory" that helps educate the strategist so that he can visualize, plan and implement strategy through his leadership performance [7]. A brief review of the main developments in strategy theory indicates. The conventional wisdom suggests that the increasing polarization and fragmentation of strategy theory has gradually limited its applicability to actual strategic management practice [8].

"Making appropriate health decisions" is a consequence, as the means are usually language, communication, knowledge, mobility in the health system, an understanding of mathematical concepts of risk, an understanding of basic biology, disease processes, and health issues [9]. Another definition most often used is "interpersonal, cognitive and social skills that determine the ability of individuals to access, understand and use information to promote and maintain good health." People with limited health knowledge often have low educational levels, are elderly, and immigrants who depend on various forms of Public Transfer Payments [10].

Over the past decade, health literacy has become a significant topic in health research, policy, and surveys that test literacy skills in health care settings [8]. Health literacy, as a construct, was introduced in public health research nearly 45 years ago, and since that time, it has become an issue of increasing relevance to global public health [11]. Simonds was the first person to use the term health literacy in 1974. However, the concept of health literacy differed from the current concept.

## Background

### Dimensions of Strategic Theory

Given the evolving and diverse nature of strategy research, it is challenging to construct a comprehensive theory of the organization, and it is too early to choose one idea to the exclusion of every other idea. The construction of view in the field of strategic management often relies on an inductive approach based on empirical evidence rather than using deductive reasoning [12].

### Resource-Based View

The resource-based theory (RBV) is essentially a theory of the conditions necessary for competitive advantage, the main objective of which is to assess whether the current resources have the potential to earn differential rents (profits). Hence, if the resources meet the conditions (jointly necessary) for being of value, Rare and expensive to imitate and replace, it may result in a sustainable competitive advantage [13]. The resource-based view (RBV), which is firmly based on ideas developed by economists, has become the dominant strategic management viewpoint.

### Dynamic Capabilities Theory

In theory, any organization can create a unique set of resources for the current market situation and gain an advantage over its competitors. But in the long run, this feature will be lost for two reasons: competitors will try to bypass this feature, and they will sooner or later; Ultimately, technology and demand change and the market will then demand a different kinds of products or services. One of the results that an organization's economic theory can derive from the concept of dynamic capabilities Conclusions is that the organization's most valuable resource is its first manager because it represents the higher-order dynamic capabilities necessary to reconfigure all

remaining resources [14]. The dynamic capabilities view has evolved as an imminent criticism of the lack of dynamics in resource-based theory.

**Knowledge-Based Theory**

Kogut and Zander 1992 emphasized the strategic importance of knowledge as a source of benefits and laid the foundation for organization theory. They have assumed that what organizations do best in markets is creating and transferring knowledge within the organization. In their view, knowledge is possessed by individuals, yet it is also an integral part of the organizational principles under which the individuals working voluntarily cooperate in an organizational context. Since the creation of new knowledge depends on existing capabilities and corporate regulations, the organization's knowledge develops in a path-dependent manner through the iteration and recombination of existing knowledge. In contrast, it can form the basis of strategic theory [15].

**The Real Option Theory**

Organizations are among the other investment alternatives [16]. Kogut and Kulatilaka 2001 indicate that fundamental options capabilities act as dynamic remedies against organizational inertia. The role of knowledge and managerial competencies has been emphasized as a critical precondition for flexible development strategies. Patterns of market entry, forms of governance, and innovation investments [17].

**Dimensions of Health Literacy**

There are four conceptually distinct dimensions of health literacy: the ability to find health information, the ability to evaluate health information, the ability to understand health information well enough to know what to do, and the ability to actively manage one's health. According to Sorensen et al., The health literacy dimensions of health promotion include:

(1) The ability to regularly update oneself, (2) understanding information, (3) interpretation and critical thinking, (4) the ability to make informed decisions about health determinants in the social and physical environment [18]. When a society is healthy learning, it refers to its ability to gather information about (the social determinants of health), to mobilize collective resources to act on them, to efficiently advocate for structural changes to improve the daily living conditions of its members [18].

The authors developed a single conceptual model based on the four dimensions of health literacy, as shown in Table (1). The dimensions are: (1) obtaining, (2) processing, and (3) understanding basic health information based on the traditional definition of health literacy. In addition, a fourth dimension has been added, which is the information application [19].

**Table 1. dimensions of health literacy**

| | Access to health information | Understand health information | Evaluating practical health information | Use of health information |
|---|---|---|---|---|
| **Health care** | Access to information on medical or clinical matters | The ability to understand medical information and derive meaning | The ability to interpret and evaluate medical information | The ability to make informed decisions on medical issues |
| **Prevention of diseases** | The ability to access information on health risk factors | The ability to understand information about risk factors and derive meaning | The ability to interpret and evaluate information on health risk factors | The ability to make informed decisions about health risk factors |
| **Promoting health** | The ability to update itself on health determinants in the social and physical environment | The ability to understand information about health determinants in the social and material environment and derive meaning | The ability to interpret and evaluate information on health determinants in the social and material environment | The ability to make informed decisions about health determinants in the social and physical environment |

## Findings

Table 2 shows the results of the correlation analysis between strategic theory and health literacy at the level of the health institution under study. It is evident that there is a positive and significant correlation (overall indicator) between the strategic theory combined with health literacy and its dimensions, as the general correlation coefficient reached (495 **). It is a positive (positive) correlation relationship, and it was marked with a sign (**) indicating that it is a statistically significant level (0.01). With the dimensions, there were two important relationships, which is the relationship between (strategic theory, obtaining health information and evaluating health information) ).

Table 2 results of correlation relationships between study variables.

**Table 2. Results of correlation relationships between study variables**

| Variables approved Variables Independent | Health literacy | | | | | |
|---|---|---|---|---|---|---|
| | Access to health information DSN | Understanding health information YU | Evaluating practical health information Eat | Use of health information YU | Total index St | Statistical significance with the overall indicator |
| Strategic Theory | .407** | 0.129 | .494** | 0.149 | .495** | 0.00 |

Table 2 summarizes the findings of the effect relationship test between strategic theory and health literacy, assuming that there is a substantial link between the real value of strategic theory (X) and health literacy (Y), as indicated by the following equation: (H.LITRACY) = $B_o$ + ı * (STR.THE.)

H.LITRACY = Health Literacy.

STR.THE. = Strategic theory.

Bı = slope of the equation (the amount of change in y that occurs as a result of change of x units).

$B_o$ = constant term.

This equation shows that health literacy is a function of the actual theoretical and strategic value, that the estimates of this equation and its statistical indicators were calculated at the level of the research sample of (155), which showed a simple linear regression model of the effect of strategic theory on health literacy.

As it appears from the results of Table (31) that the value of the constant ($B_o$) coefficient is (2.213), which means that there is an existence of health literacy of (2.213) when the value of the strategic theory is equal to (zero).

**Table 3. Correlation Coefficients Between Strategic Theory and Health Literacy**

| prototype | | Non-standard transactions | | Standard transactions | T Calculated | Level of morale |
|---|---|---|---|---|---|---|
| | | coefficient $B_o$ | Standard error | bI | | |
| 1 | Hard | 2.213 | 0.206 | | 10.719 | .000 |
| | Strategic Theory | 0.245 | 0.060 | 0.495 | 7.043 | .000 |

According to Table (6), the value of (R) has reached (.4950). This demonstrates that the strategic theory explains for a component of the change in health literacy (0.245) and that the remaining interpretation explains for a portion of the change in health literacy (0.755). It is for other factors that we propose to study in future studies.

The calculated value of (F) was high, as it amounted to (49.603) compared to its tabular value, which amounted to (3.915). On this basis, the first central hypothesis is accepted.

**Table 4. Contrast Analysis (ANOVA) of the relationship between strategic theory and health literacy**

| prototype | Link R | R2 Rate | Standard error | Statistical change | | | |
|---|---|---|---|---|---|---|---|
| | | | | R2 | F | df | Level of morale |
| Strategic Theory | .4950 | 0.240 | 0.418 | 0.245 | 49.603 | 155 | .000 |

## Conclusions

There is a clear and positive difference for the research sample in the Najaf Health Department in the availability of strategic theory variables in it, but not at the required level. The reason for this is attributed to the fact that the Najaf Health Department is subject to the Iraqi Ministry of Health in its central decisions. The results of the research show that the strategic theoretical variable has a good effect on health literacy in the health institution, and then it is possible to know the determinants of the health institution's success in managing health literacy efforts. Strategic theory helps to open the mind to all possibilities and influencing forces, and that it may serve to explain or predict multiple events of interest. It prompts us to consider the costs and risks of our decisions and weigh the outcomes of those decisions. Strategic theory provides strategy-makers with tools to help guide and shape their strategies, and establish a systematic basis for the disciplined thinking process in strategy development. The strategic approach assists in overcoming uncertainties and uncertainties. Public organizations are most needed when planning to manage health literacy efforts and facing risks, disasters, and crises, as is the case in the spread of epidemics, by developing emergency strategies using scenarios, for example. The process of health literacy in the community is of the utmost necessity, as this

concept has great importance in ensuring the health of its members. In return, it works to reduce individuals' review of health institutions and then reduce them and save money for health care. The health institution should take advantage of the dimensions of strategic theory and its hypotheses in developing programs and plans, such as caring for resources, harnessing capabilities, investing knowledge, and creating multiple options to face emergencies and continuous changes. It should also be urged to conduct constant field surveys necessary for collecting data and information required on health phenomena and forecasting them and developing appropriate strategies to serve the directions of the health institution.

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# Qualitative Analysis of *Cinnamomum burmannii* Content using GCMS (Gas Chromatography Mass Spectometry) Method

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## Abstract

To identify the bioactive compounds in *Cinnamomum burmannii* and their biological activity. Cinnamon bark originating from Batu Malang, East Java, which was located 700-1300 meters above sea level, was processed into dry extract by maceration method with 96% ethanol solvent. Furthermore, cinnamon ethanol extract was analyzed using the GCMS method to look at the content of the bioactive component for further testing biological activity with the server Way2Drug PASS. GCMS results showed 40 active compounds such as trans-cinnamaldehyde, trans-anethole, cinnamyl acetate, calacorene, cadina-1, 4-diene, delta-cadinene. Furthermore, of the 40 compounds, the biological activity potential was tested for 29 bioactive compounds based on PA (probable to be active) values predicted by the Way2Drug PASS server. *Cinnamomum* was tested against the potential as anti-fungi, anti-bacterial, anti-oxidant, anti-inflammatory, anti-diabetic, anti-neoplastic. Trans-cinnamaldehyde showed PA 0,583 as anti inflammatory, L-limonene PA0,818 as anti neoplastic, Tans-anethole PA 0,614 as anti neoplastic, Cinnamyl acetate PA 0,669 as anti inflammatory, calacorene PA 0,698 as anti inflammatory, Delta-cadinene PA 0,651 as anti neoplastic, , Cathechin PA 0,828 as anti oxidant, alpha.-Cubebene PA 0,888 as anti inflammatory and PA 0,837 as anti neoplastic, melilotin PA 0,929 as anti neoplastic, Caryophyllene PA0,915 as anti neoplastic. *Cinnamomum burmannii* had biological potency based on potential activity (PA) 0,432 (+0,117) as antifungi, PA 0,335 (+0,090) as antibacterial, PA 0,304 (+0,199) as antioxidan, PA 0,561 (+0,190) as anti inflammatory, PA 0,373 (+0,170) as antidiabetic, PA 0,584 (+0,234) as antineoplastic.

***Keywords:*** *Cinnamomum burmannii, antifungal, antibacterial, antioxidant, anti-inflammatory, antineoplastic.*

## Introduction

The natural wealth of plants in Indonesia includes 30,000 species of plants from a total of 40,000 species of plants in the world, 940 of which are medicinal plants. This amount is 90% of medicinal plants in Asia[1,2,3,4,5,6,7]. One of the medicinal plants that has been used as a traditional treatment product is cinnamon[8].

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Twelve species of cinnamon out of 54 cinnamon species (*Cinnamomum* spp) in the world are found in Indonesia and the most widely planted are *C. burmanii*, *C. zeylanicum* and *C. cassia* in addition to those that grow wild in forests such as *C. massoi* and *C. culilawan* . The five types of cinnamon can produce essential oils, especially from the skin and leaves. Cinnamon plants contain many phytochemical compounds from the phenylproponoids class in the form of cinnamic acid, which function as antioxidants. Cinnamon bark extract contains trans sinamaldehide as an antioxidant compound that can act as radical scavenger and can prevent free radical and be able to repair oxidative damage.[9,10]

Cinnamon is a native plant of South Asia, Southeast Asia and mainland China, Indonesia included. *Cinnamomum burmannii* is a native plant of Indonesia. This plant is generally cultivated by the people and the main producing areas are West Sumatra, Jambi, North Sumatra. Until now, Indonesia is a major producer and exporter of cassia bark which is exported to 44 countries, with the main aim are the United States and a number of European and Asian countries. This is different from Sri Lanka and China which have been able to utilize essential oils from *C. zaelanicum* and *C. cassia* as export commodities.[11]

The leaves containing essential oils, saponins and flavonoids. Besides that, the bark also contains tannin, the leaves contain alkaloids and polyphenols. The chemical content of cinnamon is essential oils, eugenol, safrole, cynnamaldehyde, tannin, calcium oxalate, resin and tanning agent. The part that is used for medicine is bark.[12]

*Cinnamomum burmannii* is a medicinal plant that is often found in the territory of Indonesia. Cinnamon has been acted as anti-bacterial in *Baccillus aereus*, *Listeria monocytogenes*, *Staphylococcus aereus*, *Helicobacter pylori*, *Salmonella typimurium*, *Salmonella anatum*, and *Eschericia coli*, in addition to acting as an anti-inflammatory, anti-fungal, anti-oxidant, anti-diabetic insecticides and nematicides.[13]

## Materials and Methods

### Determination test (certification test) of cinnamon

The certification test conducted in Indonesian Institute of Sciences (LIPI) Plant Conservation Center for the Botanical Gardens of Purwodadi, East Java was identified/deteminated based on herbarium and garden collections and scientific references, with the following results:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genus: *Cinnamomum*

Species: *Cinnamomum burmannii* Ness ex BI

### Making cinnamon ethanol extract

The instrument used in making cinnamon ethanol extract is a set of distillation equipment consisting of a distilled kettle, cooler (condenser) and a condensation container and separator funnel. The tools used in making cinnamon ethanol extract are a flouring machine, 30 and 50 mesh sifter, three neck flask, hot plate, turning cooler, rotary evaporator vacuum, pipette, funnel, beaker glass, and filter paper. Cinnamon bark that was tested came from Batu Malang, East Java. Cinnamon sticks are cleaned and scraped off the outer skin and then dried. The dried skin is then mashed up to 1 kg of flour. The 1 kg powder was then soaked with 1.5 liters of 96% ethanol for 24 hours and then filtered and followed by the second and third soaking using 1 lite of 96% ethanol. The yield is collected as much as 300 grams and distilled using a Buchi rotary evaporator to remove residual ethanol. Furthermore, freeze drying is done to make the yield into a dry extract.

The bark of the cinnamon is cleaned and the outer skin is scraped and then dried. The dry skin is crushed into 1 kg of flour. The powder is immersed in 1.5 liters of 96% ethanol for the first 6 hours while stirring occasionally, then left to stand for 18 hours before filtered, filtering is carried out 3 times with the same amount and type of solvent. After the viscous extract was obtained, it was distilled using a Buchi rotary evaporator to remove ethanol residue, then freeze-dried to make a dry extract yield. The collected yield was 300 grams.

**GCMS test method**

The tools used in this study were 10 mL volumetric flask, micro pipette, volume pipette, 100 mL beaker, Gas Chromatography (HP 6890 GC model number Agilent 19091S-433), column HP-5MS% 30 m long; 250 μm diameter, 0.25 μm film thickness and 1.0 mL/minute flow rate, with Phenyl Methyl Siloxane stationary phase, MSD detector, helium carrier gas (He). 200 mg of cinnamon viscous extract was dissolved with 2 ml ad ethanol and then filtered using 0.45 μm 13mm millipore wathman (Whatman-R nuclepore tract-etched membranes) until a clear solution was obtained, then the solution was pipetted as much as 1.00 μL then injected into a gas chromatography injector. Before measuring the sample, optimization and validation of the gas chromatography conditions were carried out. The analysis conditions used were the injector temperature 3000C, the detector temperature 2300C, with a split ratio of 20.1; 1 The initial temperature of the 1000 C column is held for two minutes at this temperature, gradually increasing by 100C/minute until the temperature reaches a maximum of 1500C with a hold of 21 minutes, a total rate of 28 minutes; The flow rate of the selected column is 1.0 ml/minute. Helium carrier gas with an average velocity of 37 cm/s with a pressure of 10.46 psi. Fraction identification is done by comparing the fragmentation pattern of the mass spectrum with the fragmentation pattern of reference compounds from the NIST02.L and Wiley 275.L databases.[14]

***In silico* test prediction of the bioactive potential of cinnamon**

Based on predictions from PASS Way2Drug, it is proven that cinnamon has a variety of bioactive potential based on the value of potential activity (PA).[15]

## Results and Discussion

Based on the average test value of cinnamon bioactive potential is as below:

1. Antineoplastic 0.584285714
2. Anti-inflammatory 0.570818182
3. Antifungal 0.432448276
4. Antidiabetic 0.373333
5. Antibacterial 0,33537931
6. Antioxidant 0.304

Based on the GCMS test results found that in cinnamon obtained the highest content based on the lowest and highest ppm.

1. Trans-cinnamaldehyde 2450-54000 (SD 1)
2. cinnamaldehyde 6000-30000 (SD 1)
3. EO 3500-40000 (SD 2.5284410)
4. Eugenol 140-16800 (SD 1.4140787)

**Table 1. Bioactive potential based on the value of potential activity (PA) on the highest content of cinnamon.**

| Compounds | Anti-Fungal | Anti Bacteia | Anti Oxidant | Anti Inflamatory | Anti Diabetic | Anti Neoplastic |
|---|---|---|---|---|---|---|
| Trans-Cinnamaldehyde | 0.485 | 0.287 | 0.175 | 0.583 | - | 0.37 |
| Catechin | 0.583 | 0.35 | 0.828 | 0.597 | 0.396 | 0.681 |
| Alpha.-Cubebene | 0.298 | 0.278 | - | 0.888 | - | 0.837 |
| Trans-Anethole | 0.444 | 0.323 | 0.323 | 0.525 | - | 0.614 |
| Melilotin | 0.788 | 0.553 | 0.482 | 0.83 | 0.531 | 0.929 |
| Caryophyllene | 0.582 | 0.437 | 0.174 | 0.745 | - | 0.915 |
| Cinnamyl Acetate | 0.424 | 0.345 | 0.283 | 0.669 | 0.193 | 0.501 |
| Alpha-Caryophyllene | 0.339 | 0.431 | - | 0.877 | - | 0.827 |
| Valencene | 0.379 | 0.307 | 0.142 | 0.653 | - | 0.68 |
| Calacorene | 0.338 | 0.209 | - | 0.698 | - | - |
| Delta-Cadinene | 0.482 | 0.385 | 0.147 | 0.492 | - | 0.651 |
| Stdev | 0.11751 | 0.09033 | 0.199945 | 0.190325 | 0.170136 | 0.234521 |

Cinnamon has long been used as a spice, food preservative and food flavoring. Based on the experience of traditional communities cinnamon bark can be efficacious as a medicine for lozenges, mouth ulcers, anti-rheumatism, anti-diarrhea and cough medicines, gout medicines, high blood pressure, stomach ulcers, headaches, flatulence, vomiting, difficulty urinating large, asthma, pain relief and diabetes mellitus.[16]

Kayu manis is a plant native to South Asia, Southeast Asia and mainland China. This plant belongs to the family Lauraceae which has economic value and is an annual plant that requires a long time to be taken. The main products of cinnamon are the bark and branches, while the byproducts are twigs and leaves. This commodity is often used as a spice while its processed products such as essential oils and oleoresin are widely used in the pharmaceutical, cosmetic, food, beverage, cigarette and other industries.[17]

Along with the motto “back to nature” people’s interest in using natural materials is increasing.

This is proven by the existence of small and large industries that use plants as medicinal ingredients. One such medicinal plant is cinnamon plant. Cinnamon processed products can be made in the form of powder, essential oils and oleoresin. Oleorisin and essential oils can be used in the food, beverage, pharmaceutical, flavor (tobacco/cigarette), fragrance, coloring and other industries.[18]

Based on the value of Probability to Be Active, the results of *Cinnamomum burmanii in silico* turned out to have biological potential as anti-neoplastic, anti-inflammatory, anti-oxidant, anti-diabetic, anti-fungal, anti-bacteria predicted PA (probable to be active) value using the Way2Drug PASS server. The Pa (Probability To Be Active) value is a value that describes the potential of a compound being tested. Active compounds that have values that vary between 0.3-0.7, which means low to moderate if tested on a laboratory scale. If the Pa value is more than 0.7, it indicates that the compound is predicted to have high potential in computational and laboratory tests. Meanwhile, if the Pa value is more than 0.3 but less than 0.7, the compound has computationally ability in the activity being tested, but in laboratory tests it has not been proven or its potential is small. If it is less than 0.3, the compound is computationally and laboratory tested has little potential.[15,19,20]

## Conclusion

Based on GCMS test the active compounds contained in *Cinnamomum burmannii* which are dominant based on the lowest and highest values are Trans-cinnamaldehyde 2450-54000 (+1), cinnamaldehyde 6000-30000 (+1), EO 3500-40000 (+2.5284410), Eugenol 140-16800 (+1.4140787). So, it can be concluded that based on the results of Way2Drug PASS server *in silico* with probability to be active (PA) values between 0.3-0.7. *Cinnamomum burmannii* has the ability as anti-inflammatory, anti-neoplastic, anti-oxidant, anti-diabetic, anti-bacteria and anti-fungal computationally but in laboratory tests it has not been proven or has little potential.

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# Niclosamide as a Prospective Therapeutic in L-Arginine Induced Acute Pancreatitis in Rats; Concerning Autophagic p62/ NF-*k*B signaling pathway

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## Abstract

Autophagic flux impairment is recently reported as a cardinal factor in acute pancreatitis (AP) pathogenesis. Niclosamide, an anthelmintic drug, has been lately proved to be a potent autophagy enhancer**.** The diminution of the various inflammatory factors unrestrained release via autophagy improvement may be helpful to improve the prognosis of AP. This study spots on investigating the potential ameliorative effect of niclosamide on autophagic flux and its consequent curative outcome on L-arginine-induced AP in rats. Thirty male wistar rats were divided into three groups. The first one is the control one, the second is L-arginine induced AP group, the third group is niclosamide treated L-arginine induced AP. Serum lipase, amylase, pancreatic tissue homogenate IL6, IL1β, TNFα, NF-kB, oxidative stress biomarkers; glutathione peroxidase activity, Hydroxy-2'-Deoxy-Guanosine and total antioxidant capacity levels were evaluated. Besides, the DNA-binding activity of nuclear erythroid related factor 2 (Nrf-2) was assessed using a pancreatic tissue nuclear extract. Both LC3-II subunit & P62 mRNA were quantified using PCR technique. Morphometric analysis of histopathological changes was done. The obtained data showed that niclosamide improved L-arginine induced AP as evidenced by significantly reduced serum lipase and amylase levels, which could be related to improvement of autophagy flux impairment as evidenced by decreased levels of LC3-II and p62 expression in pancreatic cells, in addition to anti-inflammatory effect as evidenced by decreased NF-kB and proinflammatory cytokines levels, along with improving the antioxidant capacity of the pancreatic tissue. As manifested by elevation of Nrf-2- DNA binding activity and normalization of oxidative stress biomarkers levels. These results could pave the way for niclosamide as a potential therapeutic role in acute pancreatitis.

***Key words:*** *Acute Pancreatitis; Niclosamide; autophagy; LC3-II; P62; Nrf-2.*

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## Introduction

Acute pancreatitis (AP) is a pancreatic inflammatory disorder, ranging from a mild self-limited disease to a severe necrotizing inflammation in nearly 15–20% of patients with up to 30% mortality rate. No specific drugs have been presented till now for the reducing the fore-mentioned risks as AP pathophysiology is

still poorly known [1]. Experimentally induced AP by L-arginine is considered a severe necrotizing model. Twenty-four hours after intraperitoneal (I.P) injection of L-arginine, characteristic tissue inflammatory changes are verified by histological and biochemical examinations. The high reproducibility, non-invasiveness and dose-dependent acinar necrosis make this model highly suitable for exploring the AP pathogenesis as well as novel therapeutic options [2].

Several recent studies revealed that impaired autophagy is a pancreatitis key provoking event and a converging step of multiple deranged pathways, leading to disease initiation and propagation of inflammatory response that defines the severity of AP [3].

Autophagy is a sequential process via which cells degrade long-lived proteins and cytoplasmic organelles, LC3 is the most extensively used indicator of autophagosomes. LC3 is an essential protein in the process of autophagy substrate selection and autophagosome formation, serving as a recognition site for LC3-binding chaperones such as p62 that deliver their cargo to autophagosomes. Once formed, autophagosomes travel along microtubules to reach lysosomes where they fuse to generate autophagolysosomes allowing degradation of their constituents by lysosomal acid hydrolases [4].

Dysfunctional autophagy influx results in activation of NF-kB with promoting macrophages infiltration to the pancreas, leading to up-regulation of cytokines and chemokines as well as pancreatic tissue infiltration by inflammatory cells [5]. The mechanisms of these processes involve rise of reactive species of oxygen (ROS) generation due to inadequate clearance of damaged mitochondria, and accumulation of p62-containing protein aggregates; P62 accumulation causes endoplasmic reticulum stress, which sequentially leads to NF-κB activation and triggers inflammatory protein transcription [6]. This generates unrestricted release of excess pro-inflammatory factors like tumor necrosis factor- α (TNF-α), interleukin-6 (IL-6), IL-1β and IL-10, which are the cornerstone triggering components of AP. The abovementioned factors stimulate both necrotic and apoptotic pathways of pancreatic acinar cells and ultimately intensify the pancreatic tissue damage. Therefore, the diminution of the unrestrained release of several inflammatory factors via restoration of normal autophagy flux may be valuable to improve the prognosis of AP [7].

Noteworthy, the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a mainstay factor in the antioxidant pancreatic defense mechanism through initiating the transcription of cytoprotective genes, and its modulation may boost the prognosis of AP. Interestingly, NF-kB competes with CH1-KIX domain of CBP (cAMP-response- element-binding protein-binding protein), so it decreases the availability of CBP which is a transcriptional co-activator of Nrf2. In the meantime, it additionally promotes phosphorylation of p65 at Ser276 which in turn hinders CBP binding to Nrf2 [8].

Niclosamide is an approved anthelmintic drug with a good safety profile. Recently, niclosamide has been documented as an effective autophagy enhancer and inducer of mitochondrial fission [9]. NF-κB, ROS as well as rapamycin complex 1 (mTORC-1) are additional mechanistic targets of niclosamide [10], which suggests that this drug may be an encouraging agent for the treatment of numerous conditions including AP. So, this study was designed to discover the potential mitigating niclosamide effect on dysfunctional autophagic flux and its possible therapeutic outcome on L-arginine induced AP in rats.

## Material and Methods

### Animals

Thirty male wistar rats aged eight weeks old with an average weight (150-200 g) were obtained from Tanta University Animal House. All rats were housed in wire mesh cages (20 ± 2 °C, 65 ± 10% relative humidity, 12-hour light/dark cycle, standard

laboratory diet and water ad libitum) and allowed to adapt for one week before starting the experiment. All experiments carried out following the guidelines for the care and use of experimental animals in Faculty of Medicine, Tanta University, Egypt, with an approval (#48661/11/22) of the Tanta Faculty of Medicine's Animal Experiment Ethics Committee.

**Experimental design**

Rats were randomly equally divided into three groups (10 per each). Group 1 served as normal control group, received an intraperitoneal injection (I.P) of Dimethyl sulfoxide (DMSO) as a vehicle daily for 3 successive days. Group 2 (AP) received two I.P injections of 3.2g/kg L-arginine (Sigma, St., Louis, MO, USA), dissolved in distilled water at interval of 1 hour followed immediately by I.P injection of DMSO, the latter was administered daily for 3 days [11]. Group 3 (AP + Niclosamide) the rats were treated with L-arginine by the same regimen as group 2 followed immediately by I.P injection of 25 mg/kg/day niclosamide (Sigma, St. Louis, MO, USA) dissolved in DMSO (at a concentration of 5mg/ml), the latter was administered daily for 3 days [12].

**Blood and Tissue sampling**:

72 hours after the last L-arginine injection, animals were sacrificed under chloroform anesthesia. Blood was rapidly gathered in a well sterile dry centrifugation tube, standed for 30 min at room temperature to clot, followed by 20 min centrifugation (1000 x g at 4ºC). Sera were collected and kept at -80ºC for further biochemical analysis.

Pancreatic tissues were rapidly excised, washed at its place with ice-cold NaCl solution (0.9% w/v), allowed to dry on a filter paper, to be sectioned into four parts. The first one was stored in buffered paraformaldehyde (10%) for histo-pathological examination. The other three parts were stored at -80ºC till being used for tissue homogenate, nuclear extracts and RNA extraction.

**Preparation of pancreatic tissue homogenate**:

A piece of each stored pancreatic specimen was allowed to thaw then weighed and homogenized in 10 volumes of 50 mM, 7.4pH ice-cold phosphate-buffered saline (PBS) by a Potter–Elvenhjem tissue homogenizer. The previous homogenates were centrifuged (7700 x g for 30 min at 4ºC). The ultimate supernatant was collected and stored frozen at -80ºC for biochemical assay. The total protein content was determined according to the method illustrated by Lowry et al. [13]

Preparation of pancreatic nuclear extracts.

nuclear extracts of pancreatic tissue were prepared using Nuclear/Cytosol Fractionation Kit (Cat #K266-25, BioVision, Inc., CA, USA) following the manufacturer protocol.

**Biochemical analysis**

**Serum was used for colorimetric assay of both serum lipase and amylase** by using commercially available kits (Biodiagnostic., Egypt).

**Nuclear factor erythroid 2-related factor 2 (Nrf-2) DNA-binding activity** was estimated in pancreatic tissue nuclear extract by an enzyme-linked immunosorbent assay (ELISA) (Nrf2 Transcription FactorAssay Kit, Abcam, USA, Cat #ab207223) obeying to the manufacturer's instructions.

**Pancreatic tissue homogenate IL6, IL1β, TNFα and NF-kB** were evaluated using ELISA with available commercial kits of MyBiosource, Inc. Southern California, San Diego (USA) Catalog No: MBS175908, MBS355232, MBS175904 and MBS453975 respectively.

**Quantitative measurement of LC3-II subunit and P62 mRNA by quantitative real-time reverse transcription PCR (rt-PCR):**

***RNA extraction:*** According to the context of the protocol from the manufacturer. Total RNA was extracted from rat pancreatic tissue using the Gene

JET RNA Purification Kit (Thermo Scientific, #K0731 USA). By a NanoDrop spectrophotometer (NanoDrop Technologies, Inc. Wilmington, USA), The total RNA concentration and purity were measured at the OD260 and OD260/280 ratios, respectively, and the RNA was then preserved at-80 ° C.

***cDNA synthesis:*** RevertAid H Minus Reverse Transcriptase (Thermo Scientific, # EP0451,

USA) was utilized to revers transcribe the total RNA samples (5µg), producing cDNA that was stored at -20 ° C to be used for PCR.

***Real-time quantitative PCR:*** The cDNA was used as a template for determining the relative expression of the**LC3-II and p62** genes by usage of StepOnePlus real-time PCR system (Applied Biosystem, USA). By Primer 5.0 software, The above mentioned genes primers were designed and their sequences were as follow: **LC3-II subunit (NCBI GenBank Nucleotide accession # NM_022867.2)** F: 5' -CATGCCGTCCGAGAAGACCT -3', and R: 5'-GATGAGCCGGACATCTTCCACT -3'. **P62 (NCBI GenBankNucleotideaccession#NM_175843.4)**F:5'--TCCTGCAGACCAAGAACTATGACATCG -3', andR:5'-TCTACGCAAGCTTAACACAACTATGAGACA -3'. The housekeeping gene **GAPDH** with primer sequences **(NCBI GenBank Nucleotide accession # NM_017008.4)** F: 5'-ATGTTCCAGTATGACTCCACTCACG-3' and R: 5' GAAGACACCAGTAGACTCCACGACA-3' was used as a reference for fold change in target gene expression calculation. A final volume of 25-µL PCR mix was prepared by adding 12.5 µL of 2X Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, # K0221, USA), 2 µL of cDNA template, 1 µL forward primer, 1 µL reverse primer, and 8.5 µL of nuclease free water. Conditions of thermal cycling were started with initial denaturation at 95˚C for 10 minutes, followed by 40 cycles with denaturation (95˚C for 15 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec). At the end of the last cycle, the temperature was increased from 60 to 95 °C for melting curve analysis. The relative expression level of genes was normalized to GAPDH and analyzed using the threshold cycle (Ct) $2^{-\Delta\Delta Ct}$ method [14].

**Estimation of pancreatic tissue homogenates Oxidative stress Biomarkers:**

**Glutathione peroxidase GPx** (EC.1.11.1.6) activity was assessed spectrophotometrically according to Paglia and Valentine (1967) method [15]. **8-Hydroxy-2'-Deoxy-Guanosine levels (8-OHdG)** were determined by ELISA kit (Cat# ADI-EKS-350, ENZO LIFE SCIENCES INT'L,PA 19462-1202, USA) obeying manufacturer's protocol. However **total antioxidant capacity** was assessed according to the method of Koracevic et al. [16]

**Morphometric & statistical analysis for cell counting of inflammatory cells:**

The software (Image J) (National Institute of Health, Bethesda, Maryland, USA) was used to measure cell counting of inflammatory cells in all groups of the present research. By which, 10 LM images at a magnification X 400 from each group were used.

## Statistical Analysis

Data analysis was achieved using the GraphPad Instat software (Version 2.0 Philadelphia, 1993). Data were expressed as mean ± SD. Comparisons were done using one-way ANOVA followed by Tukey-Kramer as post ANOVA test. Criterion for significance was chosen to be at $p \leq 0.05$.

## Results

**Niclosamide administration improved L-arginine induced acute pancreatitis in rats**

I.P injection of L-arginine resulted in significant increase diagnostic markers of AP, which are serum lipase and amylase levels, compared to control group. On the other hand, Niclosamide treatment markedly reduced the serum lipase and amylase levels. Moreover,

histological investigation of L-arginine treated group **(Figure 2.B)** confirmed the development of severe necrotizing pancreatitis with focal loss of normal architecture of pancreatic acinar cells, separation of lobules, dilated blood capillaries, extravasation of red blood cells between the pancreatic acini and marked mononuclear cellular infiltration when compared to that of control group **(Figure 2.A)** (H&E, Mic. Mag. x 400). As assessed by the image analyzer, AP group showed statistically significant increase in the number of inflammatory cells when compared with control group. Data were illustrated in the histogram **(Figure 3).** Pancreatic tissue of niclosamide treated group showed regression of some of the microscopic lesions, most of pancreatic acini resembled the normal structure denoting improvement of histopathological changes **(Figure 2.C)**. Statistical results for number of inflammatory cells of niclosamide treated group showed statistically highly significant decrease of the number of inflammatory cells when compared with L-arginine induced AP group. Data were illustrated in the histogram **(Figure 3)**.

**Niclosamide administration improved dysfunctional autophagic flux in of L-arginine induced acute pancreatitis in rats (Figure 1.A & B):**

Intraperitoneal (I.P) injection of L-arginine resulted in elevated pancreatic LC3-II and p62 mRNA levels compared to control group (P< 0.001). However, Niclosamide treatment remarkably reduced pancreatic LC3-II and p62 mRNA levels (P< 0.001) compared to L-arginine induced AP group signifying amelioration of impaired autophagic flux in pancreatic tissues.

**Niclosamide administration ameliorated pancreatic inflammation and oxidative stress status of L-arginine induced acute pancreatitis in rats (Table 1&2):**

I.P injection of L-arginine resulted in significant increase in pancreatic tissue NF-kB and proinflammatory cytokines IL6, IL1β, TNF a (P< 0.001) levels compared to control group, in addition to a significant increase in hydroxy-2'-deoxyguanosine and significant decrease in GPx, total antioxidant capacity and Nrf-2 DNA binding activity compared to control group. On the other hand, Niclosamide treatment markedly reduced pancreatic tissue NF-kB and proinflammatory cytokines IL6, IL1β, TNF a (P< 0.001) levels compared to L-arginine induced AP group denoting improved pancreatic inflammation along with a significant decrease in hydroxy-2'-deoxyguanosine and significant increase in GPx, total antioxidant capacity and Nrf-2 DNA binding activity compared to L-arginine induced AP group (P< 0.001) suggesting efficient mitigation of pancreatic oxidative stress.

**Fig. 1.A: Pancreatic LC3/GADPH relative expression Fold change**

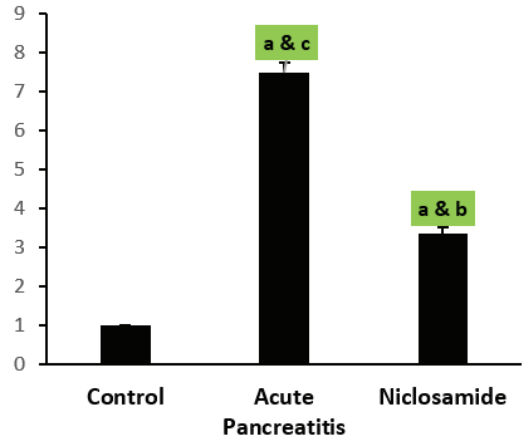


**Fig. 1.B: Pancreatic P62/GADPH relative expression Fold change**

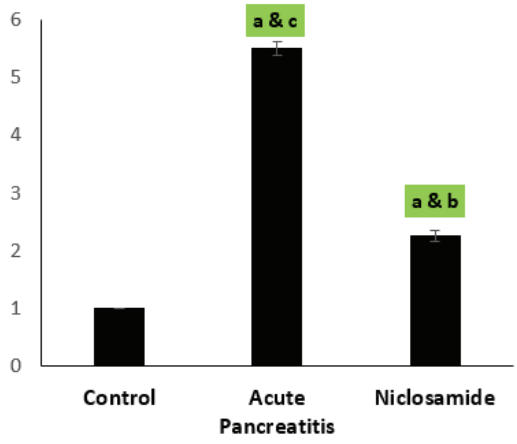


Figure (1.A&B): Quantitative real-time PCR

analysis of the mRNA expression of LC3-II subunit and P62 gene (in different groups, values expressed by fold changes ± SD. Figure 1.A shows a relative fold change of LC3-II subunit mRNA expression (7.47 ± 0.25 & 3.36 ± 0.115) for acute pancreatitis group and Niclosamide treated group respectively. However, Figure 1.B shows a relative fold change of P62 gene mRNA expression (5.50 ± 0.118 & 2.25 ± 0.095) for acute pancreatitis group and Niclosamide treated group respectively. a: $p < 0.01$ significant increase relative to control; b: $p < 0.01$ significant decrease relative to acute pancreatitis group; c: $p < 0.01$ significant increase relative to Niclosamide treated group.

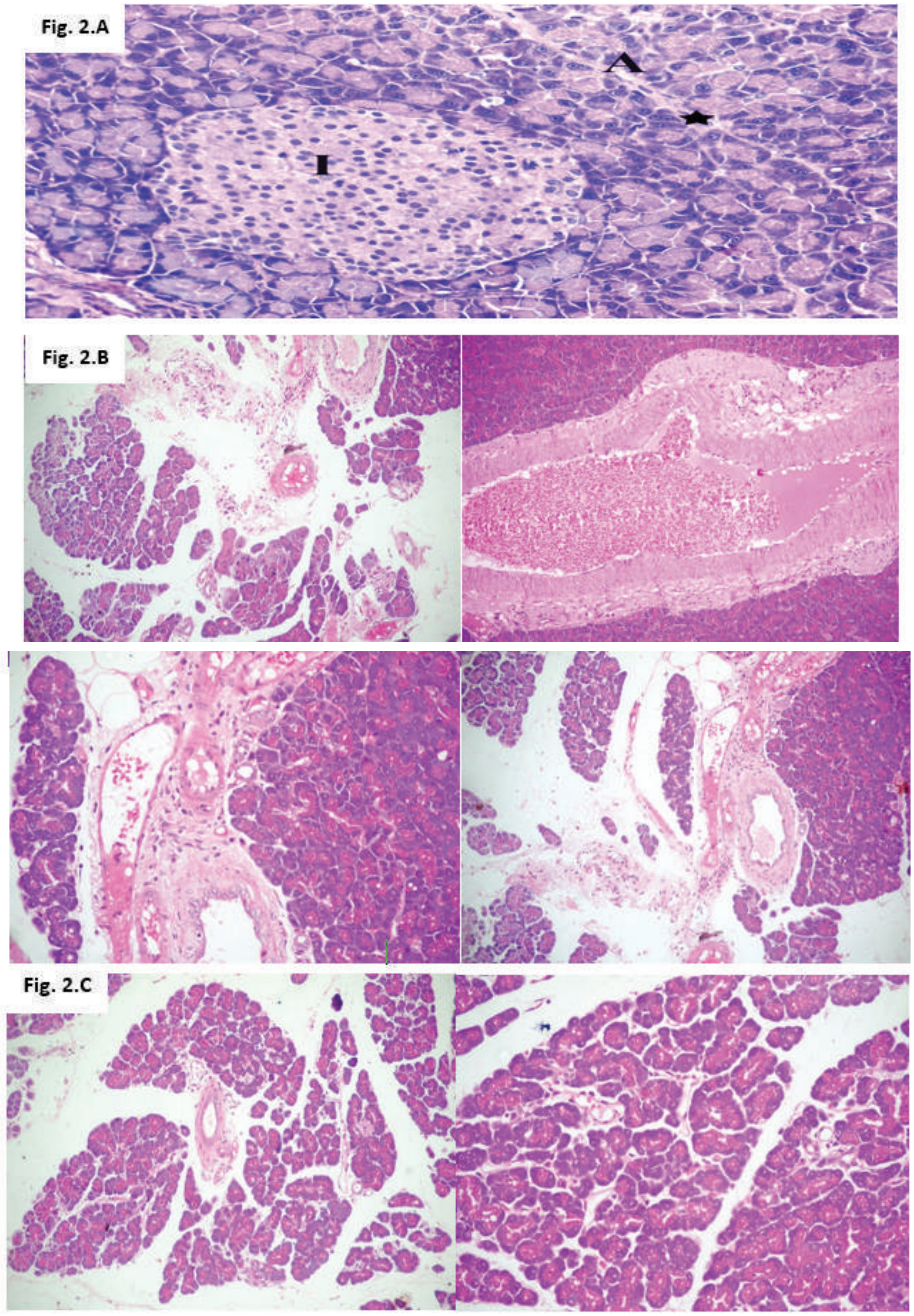

Figure 2: A photomicrograph of a pancreatic section from different studied groups (H&E, Mic. Mag. x 400). Fig. 1.A showing part of pancreatic lobules of control group, with closely packed exocrine acini (A) and a pale stained islet of Langerhans (I) is seen in-between the acini. Notice the thin connective tissue septae (asterisks) in-between the pancreatic lobules. An intralobular duct lined with cubical cells is seen in-between the acini. Notice the normal blood vessel. Fig. 2.B: pancreatic sections from acute pancreatitis group, showing focal loss of normal architecture of pancreatic acinar cells and separation of lobules, dilated blood capillaries with extravasation of red blood cells between the pancreatic acini and marked mononuclear cellular infiltration. Fig. 2.C: pancreatic sections from niclosamide treated group, regression of some of the microscopic lesions, most of pancreatic acini resembled the normal structure. (H&E, Mic. Mag. x 400).

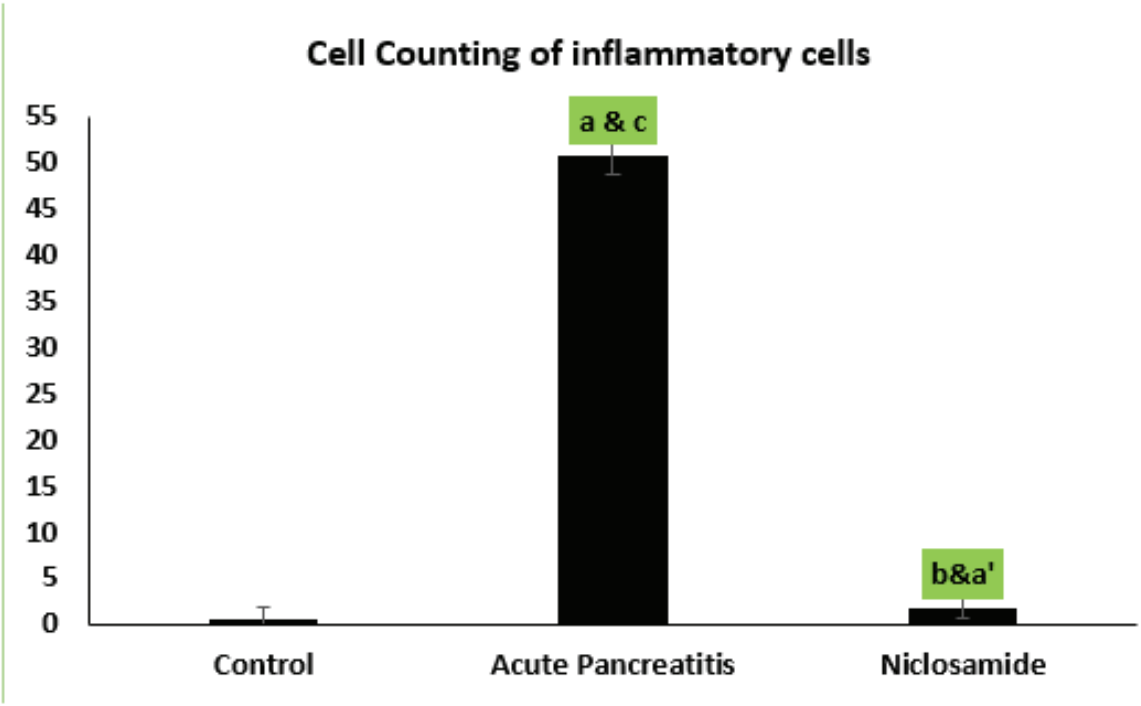


**Figure 3: The number of inflammatory cells in different studied groups expressed as mean ± SD. Figure 3 shows a cell counting (0.600 ± 0.843, 50.800 ± 2.044 & 1.700 ± 1.160) for control group, acute pancreatitis group and Niclosamide treated group respectively.**

a: $p < 0.01$ highly significant increase relative to control; a': $p > 0.05$ non-significant relative to control; b: $p < 0.05$ significant decrease relative to acute pancreatitis diseased group. c: $p < 0.01$ significant increase relative to Niclosamide treated group.

**Table 1: A comparison of serum amylase, lipase and pancreatic tissue homogenate inflammatory and oxidative stress biomarkers among the studied groups using ANOVA test.**

| | Parameters | Control n=10 | Acute pancreatitis n=10 | Nilosamide treated n=10 | ANOVA test | |
|---|---|---|---|---|---|---|
| | | Mean ± SD | Mean ± SD | Mean ± SD | F value | P value |
| Direct indicators of AP | Serum Amylase U/L | 876 ± 135b | 1908 ± 255 a, c | 1090 ± 171 b | 79.36 | <0.001** |
| | Serum Lipase U/L | 946 ± 112 b | 2701 ± 293 a, c | 1166 ± 155 b | 223.50 | <0.001** |
| Inflammatory markers | IL6 pg/mg.tissue ptn | 421 ± 87 b, c | 861 ± 94 a, c | 525 ± 96 a, b | 61.70 | <0.001** |
| | IL1β pg/mg.tissue ptn | 332 ± 101 b, c | 1064 ± 122 a, c | 445 ± 72.50 a, b | 153.80 | <0.001** |
| | TNF α pg/mg.tissue ptn | 26.90 ± 9.98 b | 193 ± 52.70 a, c | 35.10 ± 10.90 b | 88.31 | <0.001** |
| | NFKB ng/mg.tissue ptn | 2.17 ± 0.44 b | 4.77 ± 0.94 a, c | 2.72 ± 0.38 b | 45.85 | <0.001** |
| Oxidative stress markers | Nfr-2 DNA binding activity | 1.97 ±0.37 b | 0.66 ±0.57 a, c | 1.53 ±0.23 b | 26.19 | <0.001** |
| | GPx U/mg.tissue ptn | 6.82 ± 2.10 b | 1.24 ± 0.31 a, c | 5.24 ± 1.63 b | 34.70 | <0.001** |
| | 8-hydroxy-2'-deoxyguanosine ng/ mg.tissue ptn | 4.85 ± 0.96 b | 9.08 ± 0.71 a, c | 5.82 ± 1.10 b | 56.27 | <0.001** |
| | Total AntiOxidant Capacity nmol/ mg.tissue ptn | 2.84 ± 0.26 b | 1.36 ± 0.16 a, c | 2.55 ± 0.31 b | 96.46 | <0.001** |

a= significant with control, b= significant with Acute pancreatitis, c= significant with niclosamide treated group, *= significant p< 0.05

**Table 2: Pearson's Correlation between serum amylase, lipase and pancreatic tissue homogenate inflammatory and oxidative stress biomarkers in niclosamide treated group**

| | Serum Amylase U/L | Serum Lipase U/L | IL6 pg/ mg.tissue ptn | IL1 β pg/ mg.tissue ptn | TNF α pg/ mg.tissue ptn | NFKB ng/ mg.tissue ptn | Nfr-2 DNA binding activity | GPx U/ mg.tissue ptn | 8-hydroxy-2'-deoxyguanosine ng/mg.tissue ptn |
|---|---|---|---|---|---|---|---|---|---|
| Serum Lipase U/L | 0.790* | | | | | | | | |
| IL6 pg/mg.tissue ptn | 0.870* | 0.733* | | | | | | | |
| IL1 β pg/mg.tissue ptn | 0.637* | 0.770* | 0.530 | | | | | | |
| TNF α pg/ mg.tissue ptn | 0.774* | 0.815* | 0.703* | 0.786* | | | | | |
| NFKB ng/ mg.tissue ptn | 0.679* | 0.520* | 0.729* | 0.515 | 0.613* | | | | |
| Nfr-2 DNA binding activity | -0.883* | -0.804* | -0.753* | -0.664* | -0.823* | -0.563* | | | |
| GPx U/mg.tissue ptn | -0.900* | -0.795* | -0.956* | -0.564* | -0.814* | -0.752* | 0.823* | | |
| 8-hydroxy-2'-deoxyguanosine ng/mg.tissue ptn | 0.920* | 0.769* | 0.947* | 0.464 | 0.664* | 0.716* | -0.831* | -0.931* | |
| Total Anti-Oxidant Capacity nmol/ mg.tissue ptn | -0.914* | -0.814* | -0.928* | -0.649* | -0.702* | -0.728* | 0.883* | 0.903* | -0.954* |

***= significant p< 0.05**

## Discussion

A potentially fatal inflammatory disease of the pancreas is acute pancreatitis (AP). Its pathogenesis remains mysterious, and it has not established clear therapies [17]. Exocrine pancreas cells are one of the highest protein synthesis and trafficking rates that make pancreatic acinar cells more in need of removing defective cytoplasmic organelles. Manifest acute pancreatitis occurs when autophagy deficiency overwhelms the unfolded protein response ability to remove protein aggregates [18]. AP is documented to have remarked early large vacuoles accumulation of in acinar cells with intra-acinar cell trypsinogen activation; the majority of these vacuoles are autophagic with reduced rates of long-lived proteins degradation along with LC3 as well as p62 accumulation that suggest impaired autophagic flux [19].

An expanding role of autophagy in controlling NF-kB signaling pathway has been lately explicated;

autophagy evidently inhibits expression of inflammatory cytokines, in particular by eradicating p62, the adaptor protein that usually triggers activation of NF-kB [20, 21]. Oppositely, failure of autophagic flux affects cellular clearance, causing pancreatic acinar cells cytokine release via activating the p62- Tumor necrosis factor receptor-associated factor 6 (TRAF6)-NF-kB pathway that sparks the inflammatory response through up-regulation of expression of inflammatory cytokines [22, 23], this finding comes in agreement with our results herein as L-arginine induced AP group displayed significant increase in pancreatic levels of LC3 and p62 signifying impaired autophagy flux in addition to upregulation of the level of the key transcription factor NF-kB with consequent increase in proinflammatory cytokines, TNF-α, IL-6 and IL1β levels.

A recently recognized interplay between autophagy, oxidative stress and inflammation plays a critical role in AP pathogenesis; Piplani et al., 2019 demonstrated that impaired autophagic flux resulted in downregulation of lysosomal-associated membrane protein-1 (LAMP-1) with consequent accumulation of dysfunctional mitochondria that excessively generate ROS, resulting in tissue damage [24].

Mounting proofs have demonstrated that Niclosamide amends diverse biochemical intracellular signaling pathways and biological processes, including uncoupling of oxidative phosphorylation, autophagy, and the Wnt/βcatenin signaling pathway [25], designating that beyond utilization in parasitic infection treatment, it can also be applicated in other diseases. The remarkable effect of niclosamide as an enhancer of autophagic flux, has been consistently reported in recent studies [26, 27] and attributed to its ability to inhibit the mammalian target of rapamycin complex 1 (mTORC1) via cytoplasmic acidification by releasing protons from lysosomes leading to prevention of ubiquitin- containing aggregates [28]. These findings are consistent with our results that showed that Niclosamide significantly decreased the expression of LC3-II, p62 indicating recovery of AP associated retarded autophagy flux process.

Notably, Zhang et al., 2017 demonstrated that Niclosamide treatment significantly increased levels of anti-inflammatory cytokines and decreased levels of pro-inflammatory cytokines, exerting a substantial protective role against renal ischemia perfusion injury in rats (12). Moreover, Jin et al. documented that niclosamide blocked (TNF-α)-induced IκBα phosphorylation, translocation of NF-κB p65 subunit, and expression of NF-κB-regulated genes in acute myelogenous leukemia cells. In addition, niclosamide prevented the DNA binding of NF-κB to the promoter of its target genes [29]. Furthermore, Cerles et al, 2016 reported that Niclosamide exerted a manifest neuroprotective effect in experimental oxaliplatin-induced neurotoxicity through downregulation of oxaliplatin-mediated H2O2 production in neuron-like cells, in addition to reducing levels of IL6, TNFα, and advanced oxidized protein products resulting in prevention of cell death and preserving the neuronal integrity [30], these results came in consonance with our findings which proved that niclosamide resulted in significant reduction of the level of the key transcription factor NF-kB with consequent declining of levels of proinflammatory cytokines, TNF-α, IL-6, IL-1β along with enhancing the antioxidant capacity of the pancreatic tissue as evidenced by upregulation of Nrf-2 DNA binding activity, Glutathione peroxidase activity as well as total antioxidant capacity in addition to decreasing Hydroxy-2'-Deoxy-Guanosine levels (8-OHdG). Interestingly, Liu et al., 2019 reported that Niclosamide could initiate non-canonical LC3 lipidation, indicating that Niclosamide may additionally induce Beclin 1-independent non-canonical autophagy [31].

**In conclusion**: based on our data herein, restoring normal autophagy flux process with reduced accumulation of p62 and consequent NF-kB downregulation, culminating into Nrf2 enhancement could be the underlying mechanism of Niclosamide-induced improvement of L-arginine induced AP. This finding paves the way for Niclosamide as potential

therapy of AP.

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# Relationship between Neutrophil-Lymphocyte Ratio and Disease Severity in COVID-19 Patients in Isolation Ward of Dr. Soetomo General Teaching Hospital

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## Abstract

This study was conducted to prove the relationship between the neutrophil-lymphocyte ratio and the severity of COVID-19. A retrospective cohort study using medical record data of inpatients from June to July 2020. Analysis of the NLR relationship and the degree of severity using the Mann Whitney test if the data had an abnormal distribution. Significant if $p<0.05$ and 95% confidence interval. If there were significant results, we try to measure the cut-off of NLR value to predict severe and non-severe clinical symptoms. Total study subjects were 110 patients, with a male as many as 65 (59.1%), the median age was 53.5 years (range 20-88). Most of the comorbidities were diabetes mellitus (35.5%), followed by hypertension (30%). The severity of clinical symptoms was 50% in the non-severe and severe groups, respectively. The NLR value was higher in the severe group. Mann-Whitney test showed significant differences in the value of NLR between the severe group and the not severe group with the p-value <0.001. Receiver operating characteristic (ROC) curve analysis area under the curve (AUC) of NLR on day-1 was 0.716 (CI 95%: 0.605 - 0.826), and the cut-off point of the prediction severity disease at day-7 was ≥ 6.14 with a sensitivity of 71% and a specificity of 69.1%. The neutrophil-lymphocyte ratio value with severe symptoms was higher than the neutrophil ratio value for lymphocytes with non-severe symptoms in patients with COVID-19.

***Keywords:*** *COVID-19, neutrophil lymphocytes ratio, risk-factors, clinical characteristics.*

## Introduction

COVID-19 is a contagious disease caused by SARS-CoV-2, a new type of coronavirus identified as the cause of a collection of pneumonia cases in the city of Wuhan, China, at the end of 2019. The virus is spreading rapidly, resulting in an epidemic across China, followed by an increase in the number of cases worldwide[1,2,3]. The number of fatalities due to COVID-19 continues to increase, and available evidence suggests the condition of some COVID-19 patients is rapidly deteriorating. Available therapy is supportive and may have limited effects with poor outcome[4,5,6]. World Health Organization (WHO) reported 11.874.226 confirmed cases with 545.481 deaths worldwide (CFR 4.6%) until July 9, 2020. Indonesia reported its first cases on March 2, 2020, and until July 9, 2020, there were 70.736 confirmed

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cases COVID-19 with 3.417 cases died (CFR 4.8%)[7]. The rapid spread and serious dangers of COVID-19 require rapid analysis in identifying laboratory results and clinical characteristics with good precision and explaining the risk factors associated with mortality[8,9].

The neutrophil-lymphocyte ratio is a biomarker that can represent inflammation and immune status so that it can be indicators of the inflammatory response that are useful for assessing the prognosis of COVID-19 patients[10]. The neutrophil-lymphocyte ratio can be rapidly calculated based on routine blood tests performed at admission so that clinicians can make a precise and accurate disease assessment at an early stage, identify severe patients, and take active treatment measures as soon as possible to reduce mortality. This study was conducted to analyze the relationship between the neutrophil-lymphocyte ratio with the severity of symptoms of COVID-19 patients.

## Materials and Methods

This research is an observational analytic study with a retrospective cohort study approach. Sampling was done by a consecutive sampling of the medical records of COVID-19 patients. The research sample was COVID-19 patients who came to the hospital emergency room Dr. Soetomo General Hospital from June-July 2020. The inclusion criteria were patients aged ≥18 years diagnosed with COVID-19 with positive PCR swab results. The exclusion criteria included: patients with aplastic anemia, myeloproliferative disease, blood malignancy or chemotherapy, systemic lupus erythematosus (SLE) disease, patients with end-stage renal disease (ESRD). The severity of COVID-19 consists of no symptoms (asymptomatic), mild illness, moderate illness, severe illness, and critical illness. Patients with severe and critical symptoms were classified as severe. Patients who did not meet these symptoms were classified as non-severe. All data were analyzed with the statistical program SPSS version 25.0 (SPSS Inc, Chicago, USA).

## Results and Discussion

There were 122 patients who met the inclusion criteria, and 12 patients were excluded so that 110 patients were eligible for the study. General characteristics of the subjects at admission to the hospital included age, gender, and comorbid (Table 1).

**Table 1. General Characteristics of Research Subjects.**

| General Characteristics | n | % |
|---|---|---|
| Male | 65 | 59.1 |
| Female | 45 | 40.9 |
| Comorbid | 76 | 69.1 |
| Type 2 diabetes mellitus | 39 | 35.5 |
| Hypertension | 33 | 30 |
| Chronic kidney disease | 17 | 15.5 |
| Heart disease | 12 | 10.9 |
| HIV | 4 | 3.6 |
| Obesity | 3 | 2.7 |
| Malignancy | 1 | 0.9 |
| No Comorbid | 34 | 30.9 |

Most of the COVID-19 hospitalized patients were male, with a total of 65 people (59.1%), while the female gender totaled 45 people (40.9%). 138 COVID-19 cases in 2020 in Wuhan showed 75 people (54.3%) were male and 63 (45.7%) female[7]. The Indonesian Health Ministry report until the end of June 2020 states that the percentage of COVID-19 in Indonesia in men is 51.5%, as well as a study conducted by the Chinese CDC which shows that most cases occur in men (51.4%). Most of the cases are found in the male sex[3,8,9,10].

In this study, T2DM was the most comorbid with 39 people (35,5%), followed by hypertension with 33 people (30%). Besides that, there were also 15,5% comorbid chronic kidney disease, 10,9% heart disease, and obesity 2,7%. 1482 cases in the United States as of the end of March 2020 showed some of the most common comorbidities such as hypertension (49,7%), obesity (48.3%), chronic lung disease (34,6%), diabetes (28,3%) and cardiovascular disease (27,8%)[11]. 5700 patients in New York in 2020 showed that the main comorbidities were hypertension (56,6%), obesity (41,7%), and diabetes (33,8%)[12]. This could be due to the likely increase in ACE-2 receptor expression in hypertension, diabetes mellitus, and smokers [8]. Patients with these comorbidities also had poorer immune function[13].

Respiratory symptoms become the most common symptoms because the transmission of SARS-CoV-2 in a human occurs mainly through respiratory droplets and infect the respiratory tract lining the alveoli [8]. Clinical symptoms such as fever (78,2 %), shortness of breath (76,4 %), and cough (75,5 %) were the most common symptoms in this study[14,15]. Clinical and laboratory characteristics can be seen in Table 2 and Table 3.

**Table 2 Clinical and Laboratory Characteristics.**

| Clinical and Laboratory Characteristics | N | % |
|---|---|---|
| Respiration symptoms | | |
| Cough | 83 | 75.5 |
| Cold | 31 | 28.2 |
| Shortness of breath | 84 | 76.4 |
| Symptoms | | |
| Fever | 86 | 78.2 |
| Anosmia | 22 | 20 |
| Gastrointestinal Symptoms | | |
| Diarrhea | 27 | 24.5 |
| Nauseous vomit | 43 | 39.1 |
| Abdominal pain | 23 | 20.9 |
| Rapid Test | | |
| Reactive | 55 | 50 |
| Non-reactive | 55 | 50 |
| CXR | | |

***Cont...*** **Table 2 Clinical and Laboratory Characteristics.**

| | | |
|---|---|---|
| No abnormalities | 13 | 11.8 |
| Basal peripheral bilateral intercourse | 53 | 48.2 |
| Bilateral diffuse intercourse | 25 | 22.7 |
| Unilateral intercourse | 11 | 10 |
| Central bilateral intercourse | 8 | 7.3 |
| Thoracic CT scan | | |
| Bilateral ground glass opacity | 9 | 8.2 |
| Unilateral ground glass opacity | 4 | 3.6 |
| No abnormalities | 2 | 1.8 |
| Not available | 95 | 86.4 |

**Table 3. Clinical and Laboratory Characteristics.**

| **Clinical and Laboratory Characteristics** | **Result** |
|---|---|
| Age (years), median (min - max) | 53.5 (20 - 88) |
| Duration of symptoms before admission (days) | 5.5 ( 1 - 14) |
| Median (min - max) | |
| Length of stay (days), median (min - max) | 18 (1 - 58) |
| GCS | 15 (8 - 15) |
| Vital signs, median (min - max) | |
| Systolic blood pressure (mmHg) | 130 (90 - 170) |
| Diastolic blood pressure (mmHg) | 80 (58 - 100) |
| Pulse (beat / minute) | 103 (64 - 126) |
| Respiration rate (x / minute) | 24 (16 - 36) |
| Temperature ( o C) | 37 (36.1 – 38.9) |
| SpO2 (%) | 98 (96 - 99) |
| Laboratory, median (min - max) | |
| Hemoglobin ( g / dl) | 13.2 (3.9-17.2) |

***Cont...*** **Table 3. Clinical and Laboratory Characteristics.**

| | |
|---|---|
| Leucocytes (/ mm 3 ) | 8475 (1720 - 31010) |
| Platelets (/ mm 3 ) | 236500 (18000 - 955000) |
| CRP (mg / dl) | 9 (0.4 – 31.4) |
| Procalcitonin (ng / ml) | 0.2 (0.01 - 14) |
| D-dimer (ng / ml) | 490 (190 - 22730) |
| BUN (mg / dl) | 15 (3 - 224) |
| Creatinine (mg / dl) | 1 (0.1 – 18.5) |
| SGOT (U / L) | 59 (12 - 293) |
| SGPT (U / L) | 45 (12 - 263) |
| PO2 / FiO 2 | 382.5 (23 - 895) |

In this study, the median time to symptoms experienced by patients before admission to the hospital was 5.5 days. The median interval from symptom onset to hospital admission was seven days with a range of 3-9 days[11]. A retrospective study by Wang et al. also showed the median interval from onset of symptoms to admission to hospital was 7 days with a range of 4-8 days[7]. This occurs because the symptoms of the second attack (pulmonary phase) occur four to seven days after the initial symptoms appear, which is characterized by the patient still having fever, starting hard to breathe, and the lung lesions worsening[8,16].

The neutrophil-lymphocyte ratio on the first day had a median value of 5.6 (range 0.94-58.36). It was recorded that from 110 samples, the median neutrophils on the first day of examination was 6230 (range 1048-26110) with an increase in neutrophils in 70,9% of patients, while the median lymphocyte was 1070 (range 230-5360) with a decrease in lymphocytes in 82,7% of patients. Neutrophil levels, lymphocytes, and neutrophil-lymphocyte ratios on day-1 can be seen in Table 4.

**Table 4. Neutrophil, Lymphocyte, and Neutrophil-Lymphocyte (NLR) Levels.**

| **Absolute count of day 1** | **Result (µL)** |
|---|---|
| Neutrophils, m edian (min-max) | 6230 (1048-26110) |
| Lymphocytes, median (min-max) | 1070 (180-5360) |
| Neutrofil lymphocytes ratio, median (min-max) | 5.6 (0.94-58.36 ) |

In 452 patients who showed a median neutrophil count of 3900 (range 2500-5800) with elevated neutrophils in 63.3% of patients with severe case symptoms and a median lymphocyte count of 900 (range 600 -1200) which is lower than the normal value[17]. 99 patients showed a median neutrophil count of 5000 (range 3300-8100) with showed an increase in 38% of patients and a decrease in the number of lymphocytes in 35% of patients [14]. These results indicate a decrease in the absolute value of lymphocytes that corresponds to the second phase of attack (pulmonary phase) of the course of the disease. The second attack phase occurred at a median of 4-7 days after the onset of initial symptoms[8,16], whereas the median time to symptoms experienced by the patient on admission was 5.5 days; in addition, the inflammatory system that began to occur in the second phase of attack significantly suppressed cellular immunity thereby reducing the number of lymphocytes[4,16,18,19]. The severity level of clinical symptoms of COVID-19 was the same at admission, 55 cases (50%) in the non-severe (mild and moderate) group and in the severe (severe and critically ill) groups, respectively.

**Table 5. Severity of Clinical Symptoms of COVID-19 Patients.**

| Severity Day 1 | N | % |
|---|---|---|
| Non-severe | 55 | 50 |
| Mild | 9 | 8.2 |
| Moderate | 46 | 41.8 |
| Severe | 55 | 50 |
| Severe - Critically ill | | |

The comparison of the number of cases in the non-severe and severe groups was almost the same, namely from a total of 828 patients, 407 (49,15%) were in the severe group. This meta-analysis using the NLR and the ratio of lymphocyte-CRP (LCR) as a marker of inflammation which reflects systemic inflammation [20]. This could be because the data observed were data from patients who received treatment at the hospital. Generally, patients have entered the second phase of attack (pulmonary phase), where the hyper-inflammatory system response begins to occur in this phase.

The results of the Mann Whitney test showed that there is a significant difference in the value of the neutrophil-lymphocyte ratio on day 1 between the non-severe and severe groups on the 1st day ($p<0.001$) and 7th day ($p=0.001$) observations, however there was no significant difference in the value of the neutrophil-lymphocyte ratio on day 1 between the non-severe and severe groups at the end of observations ($p=0.054$). In this study, the median neutrophil-lymphocyte ratio on the first day of observation was 3.9 (range 0.95-58.36) in the non-severe group and 7.6 (range 0.94-48.06) in the severe group.

**Table 6. Difference of Neutrophil-Lymphocyte Ratio Day 1 Based on Time of Observation.**

| Severity | N | Neutrophil-Lymphocyte Ratio Day 1 | |
|---|---|---|---|
| | | Median (min - max) | P value |
| Day 1 | | | |
| Non-severe | 55 | 3.9 ( 0.95 – 58.36 ) | <0.001 |
| Severe | 55 | 7.6 (0.94 – 48.06) | |
| Day 7 | | | |
| Non-severe | 55 | 4 (0.95 – 58.36) | 0.001 |
| Severe | 31 | 8.2 (2.53 – 20.05) | |
| End | | | |
| Non-severe | 77 | 5 (0.95 – 58.36) | 0.054 |
| Severe | 33 | 6.8 (0.94 – 48.06 ) | |

A total of 443 cases. The median NLR score in the severe group was 4.75 (2.51-9.42) higher than the median NLR for the non-severe group of 2.38 (1.57-3.72) with a p-value <0.001. A retrospective study conducted by Yang et al. on 93 cases also found an association between NLR values and the severity of COVID-19. The mean NLR value for the severe group was 20.7±24.1 higher than the mean NLR value for the non-severe group was 4.8 ± 3.5 with p-value <0.001. Meta-analysis conducted by Lagunas Rangel showed that NLR was significantly increased in COVID-19 patients with severe disease (SMD: 2.404, 95% CI: 0.98-3.82)[20].

The results of the Mann-Whitney test in this study showed a significant difference in the value of the neutrophil-lymphocyte ratio on the 1st day between the non-severe and severe groups on the 7th day of observation (p=0.001). 115 patients, it was found that the neutrophil-lymphocyte ratio was higher in the severe group at admission than in the non-severe group (p = 0.0240 for cohort derivation and p <0.0001 for cohort validation) observed on day three and day seven. COVID-19 pneumonia is not severe in its early stages, but critically ill patients will worsen within 7-14 days of the disease and cause severe pneumonia and acute respiratory failure[14].

Analysis of the ROC curve ratio of neutrophil-lymphocyte first day on severity observation of the seventh day showed the area under the curve (AUC) 0.716 (CI 95%: 0.605-0.826) and the best cut-off point of neutrophils lymphocytes ratio for predicting severe disease on day 7 were ≥6.14 with a sensitivity of 71% and a specificity of 69.1% (Figure 1). Extensive neutrophil-lymphocyte ratio AUC 0.841 and optimal cut-off point NLR to predict the poor clinical outcome is ≥3.3 with a sensitivity of 88% and a specificity of 63.6% [4]. The AUC area of the neutrophil-lymphocyte ratio was 0.737, and the optimal NLR cut-off value for

predicting the level of severe disease was ≥4.28 with a sensitivity of 56.3% and a specificity of 83.7%[5].

## Conclusion

In summary, there is a relationship between the neutrophil-lymphocyte ratio and the clinical severity of COVID-19 patients. The neutrophil-lymphocyte ratio can evaluate the level of clinical symptom severity, where the neutrophil-lymphocyte ratio value with severe symptoms is higher than the neutrophil-lymphocyte ratio value with non-severe symptoms in hospitalized COVID-19 patients.

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# Effect of Work Stress on the Productivity of Shift Workers in Production Department at Pt.x Makassar City

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## Abstract

Productivity can be defined as worker's performance in terms of quantity, quality and timeliness. There are several factors that can affect productivity. This study aims to determine the effect of monotony and work stress on production department's shift workers at PT. X wirh age, sex, years of service and quality of sleep as a control variables.

This study was an analytic observational study with cross sectional design. The number of respondents was 49 people which were determined using purposive sampling technique. Data were obtained using online questionnaires. Chi square was used for bivariate data analysis and multiple logistic regression for multivariate data analysis.

From the results of bivariate data analysis, it was found that work stress had an effect on productivity with a value of p = 0.001 (<0.05). For the analysis of work stress based on age, it affected older workers with a value of p = 0.004, while based on gender, it affected the female workers with a value of p = 0.001, based on years service, it had effect on long service years with a value of p = 0.018 and based on sleep quality, it affected on poor sleep quality with a value of p = 0.003. The results of multivariate analysis showed that work stress affected the productivity with a value of p = 0.001 (p <0.05) with gender and sleep quality as variable controls. It was suggested for workers to maximize the rest time after serviced a night shift in order to restore their physical condition and for company leaders to establish good communication towards workers in order to find out critics or suggestion related to the job.

***Keywords :*** *productivity, work stress, age, sex, working period, sleep quality.*

## Background

Employees are the major part of a company or organization. Companies that have qualified employees would likely run optimally. Employee quality can be measured through work productivity. The concept of productivity can be seen from two dimensions, namely the individual dimension and the organizational dimension. Individual productivity is a mental attitude which perceive that today's life quality must be better than yesterday, and tomorrow must be better than today[1]. Meanwhile, according to Gaol[2], employee productivity is the actual behavior

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that is displayed by each person as work performance in accordance with their role in the organization or company.

The objects of this research were shift workers at PT. X in Makassar city. During the initial observation, it was found that the level of absenteeism at PT. X was quite high. This certainly affected employee productivity because not coming to work, means no productivity. Research conducted by Rina Fitiriana[3] suggested that attendance had an effect on employee productivity. Having employees absent from work will also increase the workload for other employees where it could lead to fatigue which would affect employee productivity. From secondary data, it was found that 20 employees of PT.X generally experienced work fatigue.

Work accidents might also affect employee productivity. Work accidents such as getting cuts by scissors and knives often occur in shift workers, where these would hinder the production process. One of the factor causing work accidents is fatigue which mainly due to excessive working time [4].

In meeting market demand, PT. Cahaya Anugerah Sentosa applies a shift work system. Extending working time more than the ability to work long hours is usually not accompanied by optimal efficiency, effectiveness and work productivity. Decrease in quality and work results and work for a long time creates a tendency to fatigue, health problems, illness and accidents as well as dissatisfaction[5].

Based on these preliminary observations, the researcher assumed that absenteeism, work fatigue and work accidents that occurred at PT. X which affected employee p roductivity might be caused by work stress experienced by shift workers.

Many factors can affect worker productivity. This study aims to see the effect of work stress on productivity with age, sex, years of service and sleep quality as control variables.

Work stress can affect employee productivity. Stress is a condition of tension that affects a person's emotions, thought processes, and conditions[6]. Work stress eventually might cause financial losses to organizations which are not small in number. Stress is an important problem because this situation may affect job satisfaction and productivity[7]. Research conducted by Zuhroh Fatimatuz, et all[8], also suggests that there was a significant correlation between work stress and work productivity.

Gender can affect a person's productivity level. In general, the productivity level of men is higher than women. This is influenced by factors possessed by women such as being physically weak, tend to use more feelings or biological factors such as having to leave when giving birth. Ukkas Imran[9] in his research stated that gender affects worker productivity.

Age and years of service can affect worker productivity. Amron and Taufik[10] stated that older workers have weak and limited physical energy, while young workers have strong physical abilities. A worker who has worked for a long time, had possessed experience in carrying out work and was able to increase his technical maturity which would support his productivity[11]. On the other hand, workers who have a long working period also have greater potential to be exposed to occupational diseases which, if not handled properly, will reduce worker productivity.

The sleep quality of workers with work shifts is different from workers who do not carry out work shifts. If the sleep adequacy of workers is disturbed, it will cause work fatigue. Sleep problems experienced by a person can interfere with daily activities, and can even be life-threatening either directly (such as insomnia) or indirectly (such as accidents due to sleep disorders)[12]. Park, Eunok, PhD, RN, et al[13], stated that poor sleep quality affects nurse productivity.

## Research Method

Type of research used in this thesis was quantitative with the research design used was analytic

observational with cross sectional study approach. Samples were taken by using exhaustive sampling method, where the population was designated as the research sample, amounting to 49 shift workers in production department at PT. X in Makassar city. Fishers's exact test was used for bivariate data analysis and multiple logistic regression for multivariate data analysis.

## Results and Discussions

### A. Univariate Analysis

Univariate analysis was carried out to determine the frequency distribution of respondents based on research variables which as seen in the following table :

Results of Univariate Test Analysis based On the Studied Varibales On Shift Workers At Production Department of PT. X Makassar City, 2021

| Variables | Frequency (n) | Percentage (%) |
|---|---|---|
| Work Stress | | |
| Moderate/Severe | 35 | 71.4 |
| Mild | 14 | 28.6 |
| Age | | |
| Old | 27 | 55.1 |
| Young | 22 | 44.9 |
| Sex | | |
| Male | 22 | 44.9 |
| Female | 27 | 55.1 |
| Working Periode | | |
| Long | 29 | 59.2 |
| Short (New) | 20 | 40.8 |
| Sleep Quality | | |
| Poor | 31 | 63.3 |
| Good | 18 | 36.7 |
| Productivity | | |
| Less | 35 | 71.4 |
| Good | 14 | 28.6 |

Based on the results of univariate analysis, stated that respondents who experienced moderate/ severe were 35 people (71.4%) and mild stress were 14 people (28.6%). Respondents who included into the old age category were 27 people (55.1%) and 22 people were in the young age category (44.9%) with 22 men (44.9%) and 27 women (55.1%). Respondents who included into the category of long working period were 29 people (59.2%) and 22 people were in the short working period category (40.8%). There were 31 people have a poor sleep quality (63.3%) and good sleep quality were18 people (36.7%). The worker who had less productivity were 35 people (71.4 %) and good productivity were 14 people (28.6%).

**B. Bivariate Analysis**

1. Correlation Between Work Stress and Productivity

The analysis results of work stress on productivity, it was found that the value of p = 0.001 (p <0.05). These results showed that work stress had a significant correlation to worker productivity. This was in line with research conducted by Jemilohun, V.G, et all[14] on 250 insurance employees who stated that stress had an effect on worker productivity.

2. Correlation Between Work Stress and Productivity with Base on Age.

The age of the employees is quite crucial in determining a good working outcome, both physical and non-physical. Generally, older workers were weaker and had limited physical energy, on the other hand, young workers tend to have stronger physical abilities.

In this study, the effect of work stress on productivity based on age had a significant correlation with the old workers group with a value of p = 0.004 (<0.05.). This was because the more we age, the more organ abilities degraded which would make us prone to experience fatigue where it would decrease the productivity. This was in line with research conducted by Rizki Aulia Dina Safira and Ela Nurdiawati[15] which stated that age affects the productivity of workers at PT. KHI Pipe Industries with p value = 0.000.

3. Correlation Between Work Stress and Productivity Based on Sex

Gender can affect a person's productivity level, where the productivity of men is higher than women. This is influenced by factors possessed by women such as being physically weak, and tend to use feelings at work[10]. The analysis results were not in accordance with the theory stated above. The indicated that there was an effect of work stress on productivity on female gender with a value of p = 0.001 (<0.05).

4. Correlation Between Work Stress and Productivity Based on Sex

Workers who had a longer working period had advantages in detecting, understanding and finding the causes of errors in work, so as to minimize errors in the production process. However, as the working period increases, the worker was at risk of being exposed to illness in the workplace.

The analysis results indicated that there was a correlation between work stress and productivity in workers who had long working periods with a value of p = 0.000 <0.05. This was due to the fact that the longer a person works in the same company, the more feeling of boredom piled up which, if not resolved immediately, could affect their productivity.

This research was in line with research conducted by Fajar Pasaribu [16] which stated that working period affected employee productivity. Working period was also related to the age of the worker. The longer the work period, the older the worker got where their physical abilities would tend to decrease.

5. **Correlation Between Work Stress and Productivity Based on Sleep Quality**

Good and regular sleep quality causes body activities and daily activities to run normally. People who have good quality sleep and healthy help maintain physical health, mental health and quality of life in general. Fatigue due to excessive activity or stress can interfere with sleep. A person's sleep quality is said to be good if he does not show signs of sleep deprivation and does not experience sleep problems.

The results of the analysis in this study stated that stress had a significant relationship with productivity in workers who had poor sleep quality with p value = 0.003 (<0.05). In line with research conducted by Yoshiki Ishibashi and Akiyoshi Shimura[17] with a cross sectional design, on 2897 respondents in Japan, stated that poor sleep quality has a significant relationship to worker productivity. Respondents who have poor

sleep quality will affect the level of attendance at work so that it can affect productivity.

Sleeping at night is one way to restore a person's physical condition to be fit to work the next day. Workers who experience sleep disturbances at night will feel tired the next day even if it lasts a long time can experience health problems which in turn can affect worker productivity.

### C. Multivariate Analysis

The results of multivariate analysis show that work stress affects the productivity of shift workers at PT.X, Makassar City with gender and sleep quality as confounding variables that have changes in OR values above 10%.

## Conclusion

Based on the research results conducted at PT. X shift workers at Makassar city can be concluded that ork stress variables had a significant effect on productivity among older workers, female gender, workers with long working period and poor sleep quality. It was suggested for workers to maximize the rest time after serviced a night shift in order to restore their physical condition and for company leaders to establish good communication towards workers in order to find out critics or suggestion related to the job and not to mention to give more attention about the workload of older workers and give awards to employees who excel so as to stimulate the enthusiasm of other workers to increase worker productivity.

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# Cardiac Tamponade in a Patient With Hypothyroidism: A Case Report

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## Abstract

Cardiac tamponade as a manifestation in newly diagnosed hypothyroidism is a rare condition because fluid accumulate slowly. We present a 38-year-old woman with massive pericardial effusion with cardiac tamponade findings in echocardiography without hemodynamic disturbance. Pericardiocentesis was done, but pericardial effusion was not improved. Other secondary causes of pericardial effusion including inflammation, infection, autoimmune disease, and malignancy were ruled out. The patient had a history of thyroidectomy 4 years prior and thyroid function test confirm the diagnosis of hypothyroidism. The patient was given levothyroxine with initial dose of 400 mcg and continued with 100 mcg daily. The pericardial effusion significantly improved in a week.

***Keywords:*** *cardiac tamponade, hypothyroidism, levothyroxine*

## Introduction

Cardiac tamponade is a life threatening heart compression caused by accumulation of fluid, pus, blood, blood clot, or gas resulting from inflammation, trauma, cardiac rupture, or aortic dissection. (1, 2)

In hypothyroidism induced cardiac tamponade, atypical symptoms such as syncope, fatigue, dyspnea, lower extremity edema, or even gastrointestinal manifestations are often found and tachycardia is absent. (1, 2)

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Incidence of pericardial effusion caused by hypothyroidism are around 3-37% and it mainly occurs on severe hypothyroidism but no data about cardiac tamponade. (3, 4)

Diagnosis of hypothyroidism as a cause of pericardial effusion is made if related to increased TSH and other secondary causes have been ruled out. Early recognition of thyroid disorder can prevent complication and mediate prompt treatment. Its management consists of hormone replacement therapy and pericardial drainage in tamponade cases. (3)

### Case Illustration

A 38-year-old female was admitted because of dispneu since a month. She also felt heaviness on her chest. She also complained extremity swelling. Patient had history of thyroidectomy in 2014 without routine follow-up.

Patient was alert, blood pressure was 110/70 mmHg with paradoxical pulse, pulse rate was 67 x/min, respiration rate 24 x/min, and temperature was 36.2ºC with pitting edema on all extremities.

Laboratory results show: haemoglobin 14.6 g/dl, haematocrit 44 %, leucocytes 6.090 /µL with neutrophil 72.9 %, lymphocytes 15.7 %, platelets 324.000/µL, AST 25 U/L, ALT 10 U/L, albumin 3.3 g/dL, BUN 8.28 mg/dL, creatinine serum 0,89 mg/dl, and blood glucose 104 mg/dl, ESR 28 mm/h, CRP 0.1, C3 116 mg/L, C4 32.9 mg/L, ANA test 8.94, FT4 0.12 µg/dL (Normal: 4.5-10.9 µg/dl) and TSH 106.726 uIU/mL (Normal: 0.55-4.78 uIU/mL)

Chest X-ray revealed cardiomegaly and "water bottle sign". Electrocardiography revealed regular sinus rhythm at 67 x/min and low voltage.

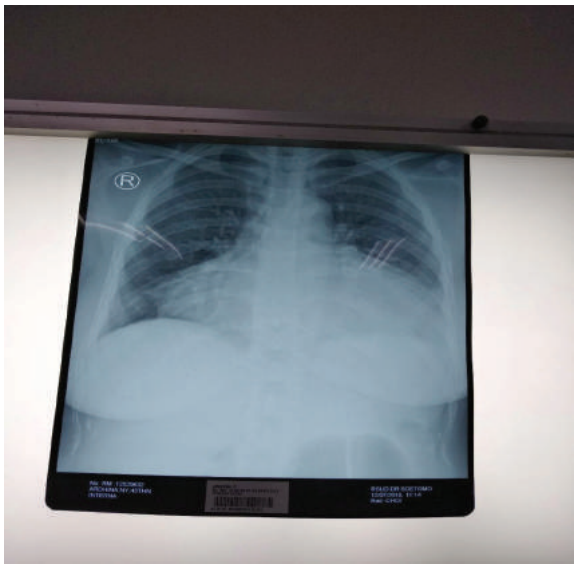

**Figure 1: Chest xray show water bottle sign**

Echocardiography show, concentric LVH, normal left ventricular systolic function with EF by Teich 63%, and diastolic function show abnormal relaxation. Massive pericardial effusion was present in anterior (2.1 cm), posterior (2.6 cm), left lateral (2.5 cm), and basal (2.3 cm) with right atrial collapse and right ventricle with mitral-tricuspid respirophasic 43%.



**Figure 2. Echocardiography show massive pericardial effusion in anterior, posterior, left lateral and basal**

The next day pericardiocentesis was done. Pericardial fluid cytology, analysis, and culture was done, the fluid was exudative and no malignant cells was found. ADA test was normal (7.9 U/L). The GeneXpert pericardial fluid was negative. 400 mcg of levothyroxine every 24 hours was given and was continued 100 mcg next day.

On the eighteenth day, there were no symptoms and no pericardial effusion. The patient was discharged with 100 mcg of *levothyroxine*.

A month later, the patient came for routine follow up. Her laboratory tests were normal. Pericardial effusion was also absent. Levothroxine 100 mcg of was continued.

## Discussion

Hypothyroidism can affect many organ systems with a wide variety of clinical symptoms. Effects on cardiovascular include abnormality of heart contractility, heart rate, conduction system, ventricle function, vascular resistance, and endothelial system, which makes hypothyroidism a predisposition factor for arrhythmia, heart failure, high blood pressure, and pericardial disease. [5]

The pathophysiology of pericardial effusion in hypothyroidism is increased vascular permeability, increase pericardial capillary permeability and decrease of albumin drainage into lymphatic vessels lead to the increase of pericardial colloid pressure and

decrease the colloid osmotic pressure gradient between pericardium and its cavity, thus lead to accumulation of fluid in pericardial cavity. Increased permeability of albumin is related to histamine release induced by low level of thyroid hormones or direct effect to endothelial layer of pericardial capillary vessels. Disruption of lymphatic drainage may be caused by pulmonary hypertension induced by hypothyroidism and the increase pressure of right ventricle. Decrease of catecholamine in the circulation induced by hypothyroidism, also presumed to play a role. [2, 3] Cardiac tamponade caused by hypothyroidism is rarely happens, but are reported on severe hypothyroidism cases or myxedema. (6-8)

Clinical manifestation of pericardial effusion in hypothyroidism are mostly asymptomatic. Classical symptoms are dyspnea especially with activities which develops to orthopnea, chest pain, or fullness in chest. Other physical examination may be normal, except the presence of hemodynamic disturbances in cardiac tamponade. [1, 9]

Hypothyroidism is a condition decrease in production of thyroid hormone or disruption of thyroid hormone effects on target tissue. [10, 11] Hypothyroidism can be suspected as a cause for pericardial effusion or cardiac tamponade after ruling out other secondary causes [1, 9]

In hypothyroid patients with pericardial effusion, heart rate is significantly lower compared to euthyroid patients. [12] Therefore, hypothyroidism should be considered in cases of cardiac tamponade without the presence of tachycardia. Low voltage is found on 42-50% of patients.[2] T-wave flattening and inversion are also commonly found. [3, 12]

Pericardial fluid in most cases of hypothyroidism is exudative. However, diagnostic pericardiocentesis mostly not necessary. Diagnostic pericardiocentesis is important to be done if there is a strong suspicion in bacterial infection or malignancy. [1, 3, 13, 14]

An important management is treating the cause. Levothyroxine is the drug of choice. Levothyroxine should be started at 1.6 µg/kg in overt hypothyroidism with TSH > 10 uU/mL and lower dose (25–50 µg every day) if TSH level 5-10 uU/mL. TSH levels should be checked every 4-6 weeks and levothyroxine dose may be adjusted 12.5–25 µg every day if needed. This is done until TSH reaches normal levels (0.4–4.0 mIU/L). If the target has been reached, TSH should be reevaluated 4-6 months after, and then every year if TSH remains on target. [3, 15]

Thyroid hormone therapy without pericardiocentesis can be done in several cases of cardiac tamponade caused by hypothyroidism with hemodynamically stable [12] The use of levothyroxine in acute cases like in pericardial effusion, pericarditis, or other acute diseases, dosage modification should be done. [3] Around 80% cases of massive pericardial effusion and cardiac tamponade occur in severe hypothyroidism or myxedema. On myxedema cases, levothyroxine can be started with initial dose 200-400 µg given through intravenous bolus or NGT, followed by oral dose of 1.6 µg/kg/day, decreased by 25% if given intravenously. [11, 16] In some case reports, initial dose is varied, starting from 25mcg/day to 400 mcg/day, depending on thyroid function examination results and other presenting comorbidities. [17-20]

## Conclusion

Cardiac tamponade in patient with hypothyroid is rare condition. Pericardial effusion induced by hypothyroidism are often underdiagnosed. Early detection of hypothyroid symptoms and prompt treatment can prevent complication. Its management consists of hormone replacement therapy and pericardial drainage in case tamponade happen.

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# Deep Vein Thrombosis and Diabetes Mellitus Type 2 as Complications of Psoriatic Arthritis: A Case Report

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## Abstract

A woman, 45 years old, complained of low back pain that improves with activity or analgesic and reoccurs when resting. The patient also complained of swollen joints, fever, shortness of breath, flaky skin and fragile nails. Physical examination found multiple inflamed joints, thickened and fragile nails, fever, limited range of movement of knee and ankle and positive Homan sign. Workup found negative anti-rheumatoid factor, increased blood sugar level, increased erythrocyte sedimentation rate, increased C-reactive protein, bacteria in urine, increased D-dimer, and imaging of lumbar spondylosis and sacroiliitis, and deep vein thrombosis in the left inferior limb using Doppler ultrasonography. The patient was diagnosed as psoriatic arthritis, deep vein thrombosis, type 2 diabetes mellitus, and urinary tract infection. The patient was given sulfasalazin, fondaparinux, warfarin, insulin, and antibiotic. On the 15th day of treatment, the patient experienced sudden shortness of breath and chest pain. The patient died due to suspected cardiovascular event.

***Keywords:*** *Cardiovascular Event, Deep Vein Thrombosis, Diabetes Mellitus, Major Adverse Cardiovascular Events, Psoriatic arthritis*

## Introduction

The prevalence of psoriatic arthritis (PsA) ranges from 0.06 to 1%.(1) PsA has been associated with an increase (55%) in the incident of cardiovascular events and 43% due to cardiovascular disease.(2) The risk of venous thromboembolism (VTE) in PsA is 2.5% and type 2 diabetes mellitus (T2DM) is around 43%. The pathogenesis is based on inflammation mediated by the immune system, genetics, and environmental factors. Systemic inflammation occurring is a potential risk factor for VTE and T2DM.(3)(4)

This case is presented to improve aware of psoriatic arthritis and its comorbidities and understand the management of psoriatic arthritis and deep vein thrombosis (DVT) so that morbidity and mortality can be decreased.

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## Case Report

History Taking

A woman, 45 years old, complained of pain in her left leg since 2 months ago accompanied with swelling, pain and redness. Both knees were painful resulting inability to walk so that she often stayed in bed. The joints in the fingers were swollen, painful, reddish. She also had fever, shortness of breathing, loss of appetite, decreased body weight 1 week before admission. White patches were found on abdominal and back skin 3 months before admission and dandruff

since 1 year before admission. The skin peeled off like wax when it was scratched. The toe nails were white and brittle. The patient also complained of low back pain, worsened with resting and waking up, improved with activity since 6 months before admission.

The patient previously was diagnosed as DM without taking any drugs.

The patient has past history of dermatitis since childhood. There was no history of autoimmune disease or similar complain in her family.

Patient visited alternative medicine to get her swollen joints massaged. Analgesic improve the complaints temporarily.

**Physical Examination**

Physical examination found tachycardia (105 beats per minute), tachypnea (28 times per minute), fever (38.2˚C), anemic conjunctiva, increase in jugular vein pressure (5+4 cm $H_20$), scaly skin, psoriatic plaque, thickened toes. The proximal digital interphalangeal II, III and IV, knee and pelvic joints were swollen, red and has limited flexion range of movement (ROM). The Homan sign was positive. Wells score was 6.

**Workup**

Laboratory examination found abnormal coagulation (APTT 27.2 (24) seconds, PPT 11.8 (10) seconds, abnormal renal function test (BUN 51 mg/dL; SK 1.41 mg/dL), hyperglycemia (426 mg/dL), hypoalbuminemia (2,9 g/dL), high ESR (25 mm/hour), high CRP (25 mm/L), and high D-dimer (2,6 µg/mL) were found.

Using compression ultrasound (CUS) found partial compressible in the left common femoral vein, left proximal femoral vein, left popliteal vein. Color coded partially fills in the left common femoral vein, left proximal femoral vein.

Lumbosacral plain radiography showed lipping on 1$^{st}$ to 5$^{th}$ lumbar spine and narrowing sacroiliac joint, indicating sacroilitis and lumbar spondylosis.

**Diagnosis**

The patient was diagnosed as PsA, DVT, acute kidney injury (AKI), anemia, hypoalbuminemia, T2DM and DVT in left leg.

**Therapy**

Therapies given were intravenous insulin 3 times 4 IV until blood sugar level (BSL) < 250 mg/dL, tramadol 100 mg tid and paracetamol 500 mg tid, subcutaneous levemir 12 IU qd, sulfasalazin 500 mg bid, rivaroxaban 15 mg bid, warfarin 4 mg, and albumin 20% 100ml. Stocking was applied to the left leg.

**Disease Progression**

On the 15th day of treatment, the patient underwent sudden shortness of breath and chest pain, leading to dead due to a cardiovascular event

## Discussion

Spondyloarthropathy (SpA) is a group of chronic inflammatory arthritis diseases associated with HLA-B27 with clinical features, inflammatory back pain due to sacroiliitis, enthesitis, tendinitis, oligoarthritis, and extrarticular manifestations.[5]

In patients with complaints of low back pain more than 3 months and the onset of the patient's age less than 45 years, the diagnosis of axial SpA is confirmed if the complaint is accompanied by signs of sacroiliitis on imaging and 1 or more SpA features. The diagnosis can also be made if the patient is accompanied by a positive HLA-B27 and more than 2 features of SpA. Those features of SpA are inflammatory back pain, arthritis, uveitis, enthesitis, dactylitis, psoriasis, inflammatory bowel disease, good response to nonsteroidal anti-inflammatory drugs (NSAIDs), family history of SpA, HLA-B27 or elevated CRP.[6]

According to ASAS 2009, typical low back pain is sufficient to determine the diagnosis of SpA. Typical low back pain in SpA are more than 3 months with a minimum of 4 of those following 5 criteria: onset

before 45 years, slow progressive pain, improved with physical activity, not improved with rest, and worsened at night. These criteria have a sensitivity of 77% and a specificity of 91.7%.[7]

In patients with peripheral symptoms, including arthritis, ethesitis, or dactylitis, the diagnosis is made when accompanied by uveitis, psoriasis, inflammatory bowel disease, preceding infection, HLA-B27, or sacroillitis on imaging. Other than that, peripheral SpA can be diagnosed if at least 2 findings of arthritis, enthesitis, dactylitis, history of low back pain, or family history of SpA are found.[6]

The patient had complaints of low back pain since 6 months before admission, worsened after waking up and resting, improved with activity and analgesic. Physical examination revealed swelling of the fingers of the right and left hands. Laboratory test showed an increase in CRP and ESR. Sacroiliitis was found on radiological imaging. Therefore, it met the axial SpA criteria.

Psoriatic arthritis (PsA) is inflammatory arthropathy related to psoriasis. The typical efflorescence of psoriasis vulgaris is reddish plaques and silver-like scales on the extensor surfaces of the elbows, knees, scalp, ears, and presacral area. Nails affected by psoriasis becomes pitting, ridging, cracking, brownish color, and rough surface and overcomes onycholysis and subungual keratosis.[8] The diagnosis criteria according The Classification for Psoriatic Arthritis (CASPAR) in 2006 had a sensitivity of 91.4% and a specificity of 98.7%. The criteria include joint, lumbago, enthesitis accompanied by 3 of the following 5 criteria: evidence of psoriasis, psoriatic nail dystrophy, negative rheumatoid factor, dactylitis, and radiological juxta-articular image of new bone formation or ossification.[9] No laboratory test can diagnose PsA. Several studies reported an increase in inflammatory markers, such as leukocytes, CRP, LED, P-selectin in PsA.[10] Radiological examination showed asymmetric joint involvement, enthesis with spurs formation, and asymmetrical spinal involvement that was lighter than Ankylosing spondylitis.[11]

This patient met the CASPAR criteria with a total score of 5 due to inflammatory back pain since 6 months before admission, psoriatic plaque in the inferior extremities, thickened toenails, swelling of the joints, and negative anti-rheumatoid factors.

According to the Group for Research and Assessment of Psoriatic and Psoriatic Arthritis (GRAPPA), the treatment for PsA is decided based on the symptoms and severity of PsA. Phototherapy psoralen and ultraviolet, disease-modifying conventional antirheumatic drugs (DMARD) are given to local PsA. Peripheral arthritis was administered NSAIDs, conventional DMARD or biological agents. PsA with dactylitis, enthesitis, and spinal involvement are performed by physiotherapy, NSAIDs or biological agents. Systemic steroids should be used carefully because it may cause flare-ups of the skin lesions while tapering off.[12]

PsA according to Wright and Moll has 5 forms: arthritis in the distal interphalanx joint (DIP), asymmetric oligoarthritis, symmetric polyarthritis resembles RA, spinal and sacroiliac joint involvement, and arthritis mutilans. Those forms may change during the disease. The simpler forms currently used are oligoarthritis, polyarthritis, and axial arthritis.[13]

The patient complained of symmetrical swelling of the joints of the fingers, psoriatic skin, thickened toenails, radiological features of lumbar spondylosis and sacroiliitis. The therapy given is paracetamol, tramadol as analgesic and sulfasalazine as DMARD.

Deep Vein Thrombosis (DVT) is a subtype of VTE. DVT in PsA is caused by systemic inflammation facilitating molecular adhesion and infiltration of activated monocytes resulting in vascular damage. In addition, inflammation increases the circulation of microparticles from endothelial cells and platelets triggering a prothrombotic pathway resulting in systemic hypercoagulation and inflammation.[4]

Cardinal signs of DVT include asymmetric swelling, warmth, and pain in the extremities. The Wells criteria has a sensitivity of 77-98% and a specificity of 38-58%. The criteria includes active cancer, current immobilization of the lower limb, recently bedridden for ≥ 3 days, or major surgery in the previous 12 weeks requiring general or regional anesthesia, stiffness localized along the distribution of the deep venous system, swelling of whole leg, swelling of the calf at least 3 cm larger compared to the healthy calf measured 10 cm below the tibial tuberosity, pitting edema confined to the symptomatic side of the leg, collateral superficial non-varicose veins, history of DVT, alternative diagnosis less likely than PE. The score is classified into low probability (-2-0), medium probability (1-2), high probability (3-8).[14]

The D-dimer aims to assess fibrin degradation products as fibrinolytic responses to the thrombus formation. This test has high sensitivity (75-100%) but low specificity (26-83%) for DVT. Duplex ultrasound is the first-line diagnostic test for proximal DVT. DVT is classified into acute (<14 days), subacute (14-28 days), and chronic (>28 days). Duplex ultrasound in acute DVT shows a thrombus floating in a blood vessel, thrombus formed by red blood cells and fibrin, low level of ecogenicity, homogeneous, thrombus extending the vein diameter, no collateral vein, non-compressible vein, and no recanalization in the vessels. In opposite, chronic DVT shows a thrombus adhering to the vessel wall, is formed by fibrin, covered by the endothelium, high level of ecogenicity, heterogeneous, shrinking vein diameter, collateral vein, partially compressible, and recanalization in blood vessels.[15] Contrast venography is the gold standard. However, it is not always available, contraindicated to allergy or impaired renal function, causes patient discomfort, and has inadequate visualization.[16]

In this case, the patient has immobilization of both legs, stiffness, swelling of the entire left leg, swelling of the calf 3 cm greater than the right side, pitting edema of the left leg, and increased D-dimer leading to the Wells score of 6 (high probability). Duplex ultrasound results showed the characteristic of chronic DVT.

The American College of Chest Physicians recommends direct oral anticoagulants (DOAC). Alternatively, vitamin K antagonist (VKA), low molecular weight heparin (LMWH) or unfractionated heparin (UFH) is given.[17] DOAC or VKA is recommended for at least 3 months.[16][18] DOAC have long half-lives making them less suitable for inpatient care and is contraindicated in patients with impaired liver or kidney function. DOAC is not recommended in cases of active malignancy, thrombocythemia, or a high risk of bleeding due to lack of research.[16]

DVT therapy given to this patient was rivaroxaban 15 mg bid for 7 days, then fondaparinux 7.5 mg sc for 5 days accompanied by warfarin 4 mg until INR ranged between 2 and 3.

DM is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion. Inflammation in PsA results in insulin resistance by inhibiting insulin substrate receptors, causing DM.[10]

The criteria for diagnosis of DM are examination of fasting BSL >126mg/dL, BSL of > 200mg/dL 2-hours after oral 75 g glucose tolerance test, BSL > 200mg/dL with classic complaints or HbA1c of > 6.5%.[19]

This patient had random BSL of 426 mg/dL and fasting BSL of 263 mg/dL so that she was diagnosed with T2DM The therapy given is in the form of long acting insulin 0- 0-12 ui sc.

PsA is often related to a risk of major adverse cardiovascular events (MACE), such as myocardial infarction, cerebrovascular accidents, and cardiovascular death.[20] Systemic inflammatory conditions result in endothelial dysfunction resulting in stiffness of blood vessels. The cascade triggers atherosclerosis which causes cardiovascular disease[4]

The patient complained of sudden shortness of breath on the 15th day of treatment accompanied by chest pain that immediately caused death. The patient was concluded died with cardiovascular event.

Conclusion

PsA is challenging to be diagnosed and treated. It has similar symptoms with other autoimmune disease. In addition, PsA may cause complications, such as DM, DVT and MACE. PsA is also related to death due to cardiovascular event or pulmonary embolism. However, early diagnosis and prompt treatment improve morbidity and mortality caused by PsA.

**Conflict of Interest:** no conflict of interest.

**Ethical Clearance:** Not required for a case report.

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# The Effects of MISUKE For Underweight Children

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## Abstract

**Background** : Malnutrition problems such as underweight was unhealthy (pathological) conditions that arise from not enough eating or consuming less energy and protein for a certain period of time. The results of monitoring the nutritional status of toddlers (0-59 months) in 2018 from Basic Health Research with WAZ Index, 17,6% children in underweight status. MISUKE (vegetable oil, milk & soybean) is a nutrient-dense supplement made from vegetable oil, skim milk and soybeans which are a high source of energy and protein but in small volume and in powder form.

**Objective** : The aims of this study was to determine the effect of MISUKE formula on the nutritional status of underweight children.

**Methods** : This research design was quasi-experimental research with pretest and posttest approaches. Samples taken by 30 underweight children under five. This research was carried out in working area of Sudiang Raya Health Center in Makassar city.

**Results** : The results showed that there was an increase in body weight before and after consume MISUKE around 0.25 grams with a p value of 0,000, besides that there was a significant increase in energy and carbohydrate intake after giving misuke with p values respectively (0,000 and 0.001), and for There was also an increase in fat protein intake, but not significantly with p values (0.56) and (0.51), respectively. Thus, MISUKE can be used as an alternative food for malnourished children.

**Conclusion :** MISUKE formulation used consists of 12 grams of vegetable oil, 6 grams of skimmed milk, 12 grams of soy bean flour, 4 grams of granulated sugar. The nutritional value of MISUKE is 190.8 kcal energy, 6.5 grams of protein, 14.5 grams of fat, and 10.7 grams of carbohydrates, while the change in nutritional status for weight before ranging from 10.48 grams and the increase of 0.25 grams after MISUKE treatment with an average body weight range of 10.73.

***Keywords****: MISUKE, Nutritional Status, Toddler, Underweight, Vegetable Oil*

## Introduction

Nowadays, nearly three of ten toddlers are malnourished, caused by insufficient eating habits containing lack of calories and protein, which will cause protein and calorie deficiencies or combination of both. Various policies and strategies involved to reduce malnutrition in children such as additional feeding but have not provided optimal results. One of the right solutions to meet the nutritional needs of children that there is no malnutrition is to prepare

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nutrient dense foods[1].

The result of Riskesdas (2018) the number of children who are still malnourished (WAZ Index) is 13.8%, while in South Sulawesi province the number of malnourished children (WAZ Index) is 18.6%[2]. The results of Nutritional Status Monitoring (PSG) Makassar province of South Sulawesi in 2016 the number of children who are still malnourished (WAZ Index) as much as 28%[3]. This suggests that the prevalence of malnutrition is still above the national prevalence Previous research has shown that nutrient-dense foods in the form of food formulas are very well given to malnourished children. That Formula contains high nutrition, small volume which is easy to give to malnourished children and accelerate to weight gain[1]. One of the formula foods is oil, milk, nuts in the form of nutrient dense additions with a small volume and in the form of powders whose composition consists of: vegetable oil, skimmed milk, tolo beans also plus granulated sugar and mineral mix with nutrient energy content of 175 kcal[4].

In this study was carried out the manufacture of nutrient dense formula with modification of vegetable oil, skimmed milk and soy beans (MISUKE). The based reason of this study because soybeans are the local food that contains the highest plant protein among other pods. The composition of MISUKE used in this study is vegetable oil 12 grams, skimmed milk 6 grams, soybeans 12 grams and granulated sugar 4 grams.. The aims of research to find out the effect of MISUKE formula on the nutritional status of malnutrition children.

## Materials and Methods

The research design was quasi ekxperiment with pretest and postest design,conducted in Sudiang Raya as working area of Public Health Center in Makassar City. The subject of the study was all toddlers who were underweight based on WAZ Index who were live in the working area of Sudiang Raya Health Center in Makassar City. Total sample in this research was 30 underweight children. The types of data collected is age, body weight and nutritional intake. Before given treatment toddlers are assessed their nutritional status and intake based on food recall 24 hours. Next steps was MISUKE treatment for one month (30 days). After the treatment completed the sample was reassessed their nutritional status and intake. Processing data by displaying basic status data in the form of average maximum and minimum values. The effect of MISUKE treatmeny by analysis data with Paired T Test if the data is distributed normally and if not distributed normally then wilcoxon test is carried out at alpha 5%.

## Result

The production of MISUKE formula consists of the composition of vegetable oil 12 grams, skimmed milk 6 grams, soy bean flour 12 grams and granulated sugar 4 grams, all ingredients weighing 34 grams. The nutritional value of misuke in one serving weighing 34 grams consists of 190.8 kcal energy, 6.5 grams of protein, 14.5 grams of fat and 10.7 grams of carbohydrates.

### 1. Characteric sample

The sample in this study was underweight children in Sudiang Raya as work area of Public Health Center with 30 samples selected by randomly simple.

**Tabel 1: Distribution Based on Age of Underweight Children in working area of Sudiang Raya Health Center**

| Karakteristik Sample | N | % |
|---|---|---|
| Age (Year) | | |
| 1 year old | 7 | 23.3 |
| 2 years old | 10 | 33.3 |
| 3 years old | 8 | 26.7 |
| 4 years old | 5 | 16.7 |

Source : Primary Data 2020

Based on table 1, distribution age of 30 samples shows that most of the sample of underweight children is 2 years (33.3%), and the least is from the sample of underweight children is 4 years old (16.7%.).

**2. Body Weight changes before and after MISUKE treatment**

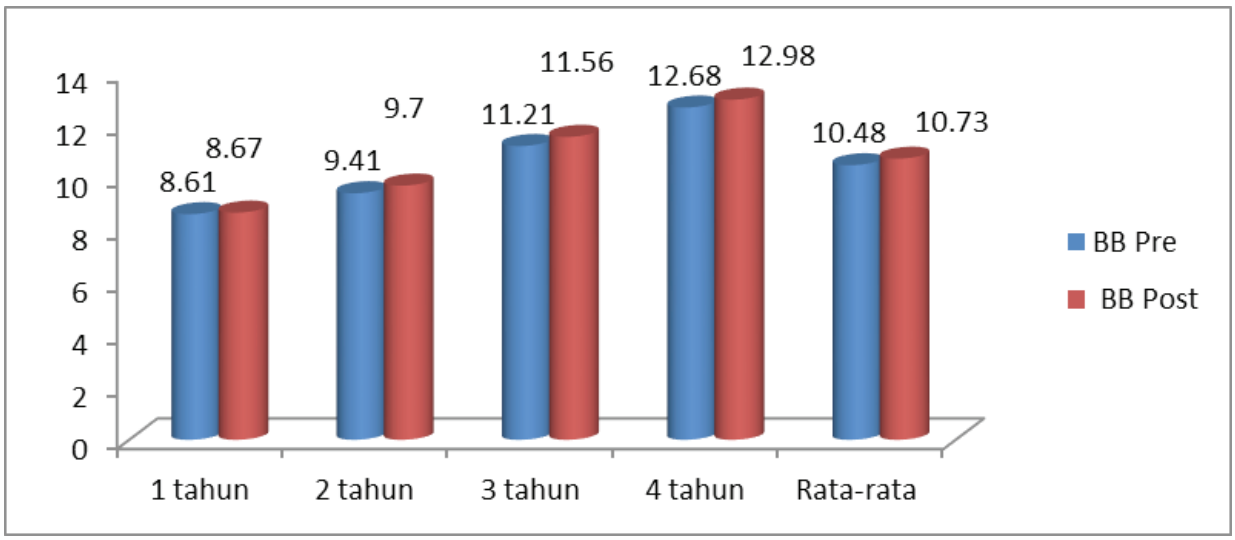


**Figure 1. weight changes before and after the administration of misuke**

In figure 1, shows that body weight before and after treatment of MISUKE. First, doing the normality test and shows that the data is not distributed normally. The next test to see the changes before and after MISUKE treatment was to use *Wilcoxon* which showed that the average weight of the sample before and after the intervention occurred a significant increase (0.000 < 0.05). The average of initial body weight of about 10.48 kilograms increased to 10.73 kilograms and the average increase ranged from 0.25 kilo grams within 1 month (30 days) of MISUKE treatment.

**3. Changes in nutrient intake before and after of MISUKE treatment**

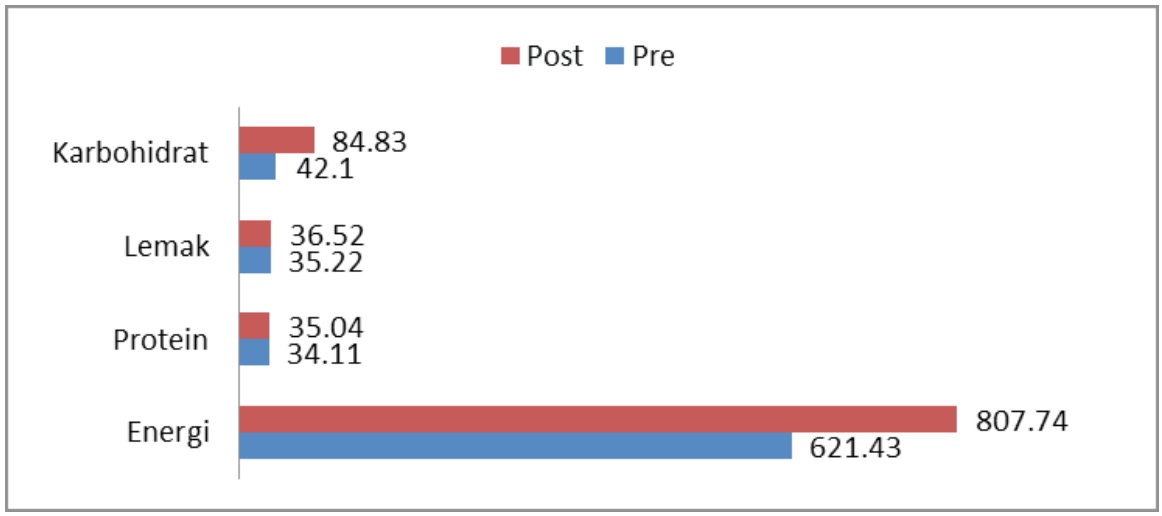


**Figure 2. Average nutrient intake before and after the administration of misuke**

In figure 2 shows nutrition intake of energy before and after MISUKE treatment. Firstly, test normality data and shows that the data is distributed normally. The next test to see the changes before and after treatment was to use the Paired T Test which showed that the average energy intake of samples before and after MISUKE treatment there was a significant increase (0.001 < 0.05). The average increase in energy intake before that was only 621.43 kcal, and increased after MISUKE treatment to 807.74 kcal

Intake protein before and after MISUKE treatment, firstly test normality and shows that the data is distributed normally. The next test to see the changes before and after treatment was to use *paired t-tests* that showed that the average protein intake of samples before and after MISUKE treatment had no significant changes (0,56 > 0.05). For fat intake before and after MISUKE treatment, firstly test normality and shows that the data is not distributed normally. The next test to see the changes before and after treatment was to use *wilcoxon* which showed that the average fat intake sample before and after MISUKE treatment had no significant changes (0,51 > 0.05).

In figure 2 above shows carbohydrate intake before and after MISUKE treatment, firstly normality test and shows that data is not distributed normally. The next test to see the changes before and after treatment was to use *wilcoxon* which showed that the average intake of carbohirathad a difference in samples before and after treatment increase in carbohydrate intake (0.00< 0.05).

## Discussion

MISUKE is an intersting food made from certain ingredients where the main raw material is soy beans in the form of flour, with comparisons from various other mixed ingredients such as oil, skimmed milk, and sugar that obtain high nutritional value. Consuming MISUKE can be one alternative to increasing the weight of underweight toddlers. The nutritional value of MISUKE itself has energy of 190.8 kcal protein 6.5 grams, fat 14.5 grams, and carbohydrates 10.7 grams with a weight 34 grams/portions.

The results of this research, show that MISUKE products are able to increase body weight of children by 0.25 kilograms within a month (30 days). This research is in line with research conducted by Niyibituronsa, Kyallo and Mugo, 2014[5] which provided pure soy flour intervention in malnourished children in

Rwanda with an average weight gain of 0.5 kilograms within three months of intervention. A considerable increase in weight with the treatment of MISUKE compared to the treatment of pure soy bean flour due to the manufacture of MISUKE products in addition to using the basic ingredients of soybean flour, also using oil, skimmed milk, and granulated sugar.

Modification of skimmed milk, oil, and granulated sugar adds nutrients to the product where soy flour provides protein, and oil and milk contribute to the fulfillment of fat sources. Based on AKG, 2019 the standard weight for children aged 1-3 years is 13 kilograms, and for 4 years is 19 kilograms[6], the treatment of MISUKE can increase the body weight so that it is blinded a longer time to reach normal weight. For energy intake, the results of this study showed an average change between the intake before and after the treatment of MISUKE from 621.43 to 807.74 kcal. The change in body weight due to the increase is due to the additional foods misuke has qualified in terms of type, quantity, and nutritional value. This is in line with the research conducted[7] by giving a mixture of soybeans with corn that was made as an additional food for malnourished toddlers, in addition to increased calorie intake, carbohydrate intake also had a significant difference before and se already given. This research is in line with research conducted by Fatmah, 2018 which shows that there is a correlation between weight gain and increased carbohydrate intake, after the treatment of soybean biscuits with a mixture of dates[8].

For protein and fat intake also increased, but the increase was no difference between before and after given MISUKE. Protein intake only increased by 0.9 grams and fat intake increased by 1.3 grams. The increase in protein value in the study occurred due to the protein content in MISUKE about 6.5 grams, but malnourished children are less usually less consuming protein and have not met the adequacy of AKG for children aged 1-4 years about 20-25 grams per day[6].

Changes in body weight before and after treatment of MISUKE there was a significant increase. According to the theory of weight gain experienced by children aged 1-3 years is 2-2.5 kg/year and for toddlers aged 4-6 years range from 0.7-2.3 kg/year[9]. In this study attainment of weight loss 0.25 kilo grams in amonth, if an upgrade occur for a year then the increase will be around 3 kg/year so that this increase indicates higher weight than the theory. This research was aligns with research by Pradhita, 2012 that gave PMT with a month-long trial of 17 tuberculosis toddlers aged 12-59 months. The results showed changes in weight before and after intervention in children aged 12-59 months. There is a change in nutritional status based on WAZ Index before and after treatment MISUKE due to the content contained on MISUKE suitable for malnourished toddlers in increasing their body weight[10].

## Conslusion

The results showed changes in weight before and after intervention in children aged 12-59 months. There is a change in nutritional status based on WAZ Index before and after treatment MISUKE due to the content contained on MISUKE suitable for malnourished toddlers in increasing their body weight.

### Suggestions

It is recommended to give MISUKE more than 1 (one) month to see the maximum body weight gain results. It is also necessary to provide nutritional child feeding to change the behavior of the mother in child feeding.

### Acknowlegment

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# Evaluation of the Effect of the Activity of Gum Arabic Aqueous Solution and Ozonated Water on the Chemical and Organoleptic Properties of Locally Produced Soft Cheese in Baghdad City

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## Abstract

Evaluated the effects of Gum Arabic (GA) combined with ozonated water (OW) at refrigeration (4ºC) storage temperature on the chemical and organoleptic properties of bovine locally produced Cows and buffalos', Fifty Soft cheese (25 for each kinds ) were collected from homemade local producers in the different regions of Abu-Ghraib villages/Baghdad city. Soft cheese samples were analyzed for Moisture percentage %, Total solids, pH levels, Protein, Fat as dry matters and ash contents before and after immersion in mixture decontamination solution of (GA) at concentration of (3% and 5%) with ozonated water (0.5) ppm during (30) minutes of exposure time at refrigeration (4ºC) temperature. The results showed that the moisture, fat as dry matter and ash content increased, while total solids (TS) and protein decrease after subjecting to natural decontamination solution. pH value was non significantly affected by the washing treatment. In conclusion evaluation of sensory properties showed that local cows soft cheese demonstrates higher values for sensory parameter compared to local buffalo's soft cheese.

***Keywords*** *Ozone, Gum Arabic, Cheese, Coating, Yeasts and molds.*

## Introduction

Cheese is food that increasingly consumed in all over the world characterized by elevated in the nutritional values with good taste and flavor [1]. This dairy product can be prone to both physical, chemical, and biological damage [2]. Development the hurdle technique to control the microorganism's survival without affecting in the nutritional and sensory properties of food; Physical and chemical stressor such as heat treatment, refrigeration, ozonation treatment and antimicrobial methods could be used under this approach [3,4]. Ozone is powerful antimicrobial agent that is used as alternative to other sanitizers , its effective even at low concentrations without toxic by-products, used in the food as rinsing ,dipping without noticeable effects in the qualities of food **[5]**, Low temperature increase both of solubility and stability of ozone resulting increasing in the ozone efficacy [6].Gum Arabic (GA) is polysaccharide exudate , acacia trees are source of this substance used in the food industry due to its adhesive, viscosity and the stabilization, emulsification properties **[7].** GA as an edible coating that can protect food from microbial

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alteration [8]. The aim of this study was to evaluate the effect of GA combined with OW at refrigeration (4°C) temperature on the chemical and organoleptic properties of bovine locally produced soft cheese samples in Baghdad.

## Materials and Methods

Fresh Soft cheese samples were purchased from local producers in the different regions of Abu-Ghraib villages and transported in sterile ice-cooled box to the laboratory of Veterinary Public Health for the evaluation chemical and sensory analysis. GA powder (Elnasr/Sudan) obtained from local market.

**Ozone generation** ozone generator ($A_2Z$, USA) as gas bubbling in the sterile water at concentration (0.5) ppm, Sterile large plastic containers were used for the ozonation washing process by inserting the stone inside the sterile distal water at refrigeration temperature (4°C) [9]

**Ozonated water with Gum Arabic treatment**; GA powder at level of (3% and 5%) w/v were dissolved in the ozonated water (0.5 ppm), mixed by glass rod until dissolving completely with stirrer, both of locally produced bovine soft cheese cubes (2.5 cm) were submerged in the mixture of ozonated water (0.5ppm) and GA (3% and 5%) solution for 30 minutes as a contact time at refrigeration (4°C) , both treated and untreated soft cheese samples were analyzed for chemical and organoleptic properties.

### Chemical analysis of soft cheese

The fat contents were determined by Gerber method [10]. protein content by Kjeldhal method [11]. Both of moisture and total solids were determined by drying in oven at 102°C [12]. The ash content by burning in furnace at 550°C for (three)hr [13]. pH was determined by using digital pH meter [14]. With triplicate.

### Sensory analysis of soft cheese

The organoleptic properties of locally produced cows and buffalos' soft cheese were evaluated before and after subjecting to combined treatment of GA (5%) with ozonated water (0.5) ppm for (30) minutes at refrigeration temperature (4°C). Five panelists from the department of veterinary public health to access the reaction by rating on the (nine points) scales. Scores range from 1 (poor) to the 9 (excellent), color, odor, taste, and overall acceptability were the sensory tests that performed in the current study with triplicate.

**Statistical analysis** Data was performed using SAS (Statistical Analysis System - version 9.1). One ways ANOVA with Least significant differences (LSD) post hoc test were performed to assess the significant differences among means. unpaired t test was used for detecting the significant difference between two groups ($P < 0.05$) is considered statistically significant.

## Results

The moisture contents in both type of soft cheese samples was increased significantly ($P<0.05$) with increased concentration of GA in combination with ozonated water at (0.5)ppm. The moisture content increased from (68.61±0.49 and 55.24±0.05) in cows and buffaloes control soft cheese samples to (72.50±0.50 and 56.32±0.02) respectively after exposed to combination of GA (5%) and ozonated water (0.5) ppm after 30 minutes. This could be attributed to an increase in the water retention capacity. The Chemical composition of cows and buffalos' soft cheese are shown in table 1 and 2. These results agreed with Ghamgui *et al* [15] Recorded that the addition of Almond Gum as a functional additive in the Sardaigne-type cheese has improved level of moisture, Lafta et al [16] reported that addition of Gum Arabic increase the moisture content of low-fat soft cheese. The total solids contents in both type of soft cheese samples decrease significantly ($P<0.05$) with increased concentration of GA combined with ozonated water (0.5). The total solid content decreased from (31.34±0.45 and 44.76±0.04) in cows and buffaloes control soft cheese samples to (28.01±0.01

and 43.67±0.01) respectively. This due to the high moisture contents in the local soft cheese samples. Similar results reported by Elkhidier [17] who study the physicochemical and sensory characteristics of soft white cheese made with different levels of Gum Arabic.

pH level of local cows and buffaloes soft cheese samples were slightly reduced after treatment, the reduction was non-significant (P>0.05). The pH level reduced from (6.27±0.005 and 6.47±0.06) in cows and buffaloes control soft cheese samples to (6.25±0.01 and 6.43±0.01) respectively after Gum Arabic (5%) and ozonated water (0.5) ppm treatment. The slightly variation in the pH value was not related to Ozonation affect, Cavalcante *et al* [18] demonstrated that sanitation of Minas Frescal cheese with Ozonated water did not change the pH of cheese. These results agreed with Murad *et al* [19] that studied the effect of Xanthan Gum in the low fat Karish cheese the pH value showed non-significant increase in cheese after using Xanthan Gum at different concentration.

The fat percentage as dry matter in the cheese samples slightly increased after submerged in the solution, fat content increased from (13.04±0.01 and 23.46±0.01) in the control to (13.75±0.01 and 23.76±0.05) respectively after exposed to a combination of Gum Arabic (5%) and ozonated water (0.5). these results were in -agreement with Shendi *et al* **[20]**. Kiiru *et al* **[21]** reported that the addition of Gum Arabic slightly diminishes in the fat contents in mozzarella cheese.

The protein contents in the cheese were significantly reduced ($P < 0.05$) with increase concentration of GA in combination with ozonated water. The protein content decreased from (16.42±0.01 and 20.78±0.05) in control to (13.05±0.04 and 18.01±0.02) respectively after exposed to a combination mixture. These results agreed with Uzun *et al* [22]. Reported that high concentration of ozone can affected the whey protein resulted in the protein oxidation. The reduced protein content could be attributed to an increase in moisture content or the low amount of protein in Gum [21].

Increase in ash contents is observed after treatment. The ash contents in the control samples increased significantly (P<0.05) from (1.22±0.005 and 1.69±0.02) to (1.81±0.03 and 2.38±0.06) after subjecting to sanitating solution. Sharafi *et al* [23] indicated that the direct effects of galactomannan and novagel gum can increased the ash content in the low-fat cheese. These results were disagreed with Murtaza *et al* [24] who reported that there was no effect in the ash contents after addition of Gum to the low-fat cheddar cheese.

**Table (1) Chemical composition of cow's soft cheese after subjecting to the GA (3% - 5%) and ozonated water (0.5 ppm) for 30 minutes at refrigeration temperature**

| N0 | Cows soft cheese | Moisture% | Total solid % | pH | Protein% | Fat% | Ash% |
|---|---|---|---|---|---|---|---|
| 1 | control | 68.61±0.49b | 31.34±0.45a | 6.27±0.005a | 16.42±0.01a | 13.04±0.01b | 1.22±0.005c |
| 2 | 3%+OW | 71.02±0.02a | 28.98±0.005b | 6.25±0.005a | 13.123±0.04b | 13.05±0.06b | 1.65±0.01b |
| 3 | 5%+OW | 72.50±0.50a | 28.01±0.01b | 6.25±0.01a | 13.05±0.04b | 13.75±0.01a | 1.81±0.03a |
| LSD | | 1.829 | 1.183 | 0.0318 | 0.0412 | 0.039 | 0.0318 |

Means with a different small letter in the same column are significantly different (P<0.05).

**Table (2) Chemical composition of buffalo's soft cheese after subjecting to the Gum Arabic (3% - 5%) and ozonated water (0.5 ppm) for 30 minutes at refrigeration temperature.**

| N0 | Buffaloes soft cheese | Moisture% | Total solid % | pH | Protein% | Fat% | Ash% |
|---|---|---|---|---|---|---|---|
| 1 | Control | 55.24±0.05c | 44.76±0.04a | 6.47±0.06a | 20.78±0.05a | 23.46±0.01b | 1.69±0.02c |
| 2 | 3%+OW | 55.81±0.01b | 44.21±0.01b | 6.45±0.02a | 18.59±0.05b | 23.49±0.04b | 1.81±0.01b |
| 3 | 5%+OW | 56.32±0.02a | 43.67±0.01c | 6.43±0.01a | 18.01±0.02c | 23.76±0.05a | 2.38±0.06a |
| LSD | | 0.039 | 0.0481 | 0.0486 | 0.0431 | 0.0318 | 0.0318 |

Means with a different small letter in the same column are significantly different (P<0.05).

**Table (3) Organoleptic properties of cows locally produced soft cheese that subjected to the Gum Arabic (5%) and ozonated water (0.5) ppm for 30 minutes at refrigeration temperature.**

| | | Organoleptic properties | | |
|---|---|---|---|---|
| | | Means ±SE | | |
| Groups | Overall acceptability | Taste | Odor | Color |
| Control | 7.80±0.37a | 7.80±0.37a | 6.60±0.24a | 7.40±0.24a |
| Panelists | 7.80±0.20a | 7.40±0.24a | 6.80±0.48a | 7.60±0.24a |
| P-value | 1.00 | 0.47 | 0.09 | 0.09 |

*Organoleptic properties of Cows locally produced soft cheese after subjecting to dipping in the antimicrobial solution was statistically non-significant at (P>0.05) (Unpaired t test).

**Table (4) Organoleptic properties of Buffaloes locally produced soft cheese that subjected to the Gum Arabic (5%) and ozonated water (0.5) ppm for 30 minutes at refrigeration temperature.**

| | | Organoleptic properties | | |
|---|---|---|---|---|
| | | Means ±SE | | |
| Groups | | | | |
| | Overall acceptability | Taste | Odor | Color |
| Control | 8.40±0.11a | 8.00±0.11a | 7.60±0.14a | 8.60±0.09a |
| Panelists | 7.40±0.10b | 7.40±0.09b | 6.20±0.08b | 7.80±0.12b |
| P-value | 0.04 | 0.05 | 0.03 | 0.03 |

* Organoleptic properties of Buffaloes locally produced soft cheese after subjecting to dipping in the antimicrobial solution was statistically in a significant at (P<0.05) (unpaired t test)

**Organoleptic evaluation**

The mean values of the organoleptic properties of soft cheese sample after subjecting to GA (5%) combined with ozonated water (0.5) ppm for 30 minutes at 4°C are shown in Table 3 and 4.The results showed that there was no significant differences (P>0.05), in the organoleptic properties (color, odor, taste and overall acceptance) between the treated cows samples and the control samples, there was no change in overall acceptability and there was improvement in odor and color of the treated samples, results were in -agreement with previous studies that deal with organoleptic properties of various kinds of cheese, another results indicate that controlled the Ozone concentration resulting no flavor defects or adverse effects were recorded [25]. Kiiru *et al* [21] illustrated that the use of GA up to a level of (3%) in mozzarella cheese improved the functional, nutritional, and sensory quality. Results of study of Cooke *et al* [26] showed that supplement of full fat cheddar cheese with tragacanth gum reduced in the hardness and increased in the softness during the period of production, there was no sign of adverse effects on the organoleptic properties of the cheese. Other researchers have reported the ability of Gum Arabic to encapsulate flavor and aromatic composition in the food products that explain the flavor retention in the food [27].Buffaloes cheese samples had a significant difference (P<0.05), in the sensory parameter after subjecting to treatment, this could be attributed to the fact that buffalo's cheese was made from milk in which the fat has been removed, fat in the food carries much of its flavor and satisfying characteristic of texture, improves appearance, good mouth-feel and overall acceptability, Several studies have shown that the fat content of cheese is essential for its overall acceptability [28].

## Conclusion

Gum Arabic and/or ozonated water as decontamination solution can enhance the chemical quality parameters of the locally produced cows and buffaloes' soft cheese. Subjecting local cheese to the natural antimicrobial solutions recorded that cows soft cheese show higher values for sensory parameter compared to local buffaloes' soft cheese, sold in the rural parts of Baghdad city.

**Conflict of Interest:** Nil

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**Ethical Clearance**: Obtained from Institutional ethical committee

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# Diagnosis of Fungi Associated with Wounds from Lying Patients at Al-Hussein Teaching Hospital in Dhi Qar Governorate

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## Abstract

About 30 samples were collected from burn areas from patients attending Al-Hussein Teaching Hospital in Dhi Qar governorate for the period between 2-4 for the year 2020. 7 types of fungi and yeasts, represented by the following fungi, have been isolated candida kruzei, candida tropicalis, and Rhizopus, candida albican, candida glabrata, Aspirgillus spp, rhodotorella. The results showed that *Aspergillus* spp. Was dominant over the rest of the fungi, with a rate of 36.36% over the rest of the fungi species, while *candida albican* yeast appeared with a frequency of 28.18% compared to the rest of the other fungi, and the rate of appearance of other fungi was about 12-3% compared to the rest of the other fungi. The fungi were distinguished by the fact that they are all of the opportunistic type, which begins to infect the body when there is a weakening of immunity or infection with the associated burns and ulcers.

***Keywords****: fungi. Aspergillus. Wounds. Iying patients. Rhodotorella*

## Introduction

Hundreds of people suffer daily burns of various types, ranging from moderate to light burns, blind burns, or so-called third-degree burns [(1)]. Burns are usually contaminated with a group of microorganisms, all of which are classified as opportunistic organisms, and fungi are the largest part of those organisms that are isolated and diagnosed according to the methods known to them. [(2)] Fungi have the advantage of being opportunistic organisms that may cause the occurrence of many diseases in humans, especially in people with burns and people with weak immunity[(3)]..

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## Materials and working Methods

1-**Samples collection**: About 30 samples were collected from burn patients who are hospitalized at Al-Hussein Teaching Hospital in Dhi Qar governorate during the period between 2 months to 3 months of 2020. Samples were taken from the wounds of patients with burns, using the swab, and the required studies were conducted on them.

**2-Diagnose Microscopy:** For the purpose of performing the microscopic examination, glass slides were prepared for the purpose of preparing a smear for microscopic examination from the fungal culture, which included placing a drop of lactophenol dye on the glass slide with mixing part of the colony on it and placing the glass cover. The slides were examined under a microscope to determine the fungal species and genera. Through the following equation, the most

frequent fungi were identified in the isolated samples:.

$$\text{Percentage Impression} = \frac{\text{The number of candida fungus isolates}}{\text{The number of total fungi isolates}} \times 100\%$$

**3-Diagnosis of fungi**: The classification keys were used for the purpose of diagnosing the fungi, in addition to using the phenotypic diagnosis to enhance the identification of the fungus type and the sex, according to Maroud in [4].).

: **4-Cultivation media**

A-Medium saproid acar: prepared by adding 40 g of dextrose sugar to 15 g of peptone, 20 g of acar-acar and 1000 ml of distilled water. Sterilize the medium in the autoclave for a period of a third hour under pressure of 121 lb / inch. According to the manufacturer's instructions.

**B- Acromo-gen agar**

The medium of chromoa used the diagnostic gene for Candida yeast which was prepared according to the manufacturer's instructions. The yeast was grown on the middle, and it was diagnosed according to the differential colors that appear on the middle as a result of the interaction of the enzymes of the yeast with the medium and according to the different types of yeast[5]

## Results and Discussion

Table No. (1) shows the fungi that were isolated from patients with burns, which were identified with approximately 9 fungal genera, which were represented by the following: *candida kruzei, candida tropicalis, and Rhizopus, candida albican, candida glabrata, Aspergillus spp, rhodotorella*, and the fungi are the opportunistic fungi group. Which was characterized by the recurrence of the genus Aspergillus, which is considered the most frequent fungi among the other fungi that were isolated, while the rest of the genera were distinguished by different frequency and according to the type and area of burning from which the fungus was isolated The study showed that the dominance of the genus Aspergillus among the rest of the fungal species, which ranged to 36.36%, is due to the ability of this fungus to grow in different environments, the ability to withstand harsh environmental conditions, and the ability to produce different enzymes that enable the use of nutrients in the fungus environment [6,7]. . The candida fungus comes in second place in terms of its recurrence among the other fungal species, which ranged to 28.18%, especially candida albican, which was distinguished by its frequency among the rest of the other types of candida, not only in burn sores in patients, but also by its appearance in other areas of wounds that It was characterized by the emergence of the opportunistic characteristic of this yeast, and it seems that the reason is due to the weak immunity of the Ramadi or due to the availability of nutrients for the yeast and its transformation from symbiotic organisms to parasitic organisms on the areas of ulcers and wounds. [8,9,] As for the rest of the other types of yeast and fungi, their appearance fluctuated between 12 - 3% from the rest of the other types, as shown in Table No. (1), and this is due to the nature of those yeasts and fungi, which have a lower ability to resist different environmental conditions [10,11].

**Table No. (1) shows the fungi that were isolated from the burn areas of the patients**

| no | Name of fungus | Fungus frequency |
|---|---|---|
| 1 | Aspergillus | 36.36% |
| 2 | Candidi albican | 28.18% |
| 3 | Candida kruzie | 12.2% |
| 4 | candida tropicalis | 8.4% |
| 5 | candida glabrata | 6.4% |
| 6 | rhodotorella | 5.4% |
| 7 | Rhizopus | 3.2% |

Rhodoterella candida spp Aspergillus spp

Through this study, it can be said that burns can be considered one of the ideal environments in which micro-organisms and opportunistic fungi grow in, which are a large part of these micro-organisms. Consequently, the area of wounds and burns should be taken care of and cleaned well, and the growth of microorganisms, especially in patients with BP, must be prevented.

## Conclusions

Aspergillus spp. Was dominant over the rest of the fungi, with a rate of 36 g 36% over the rest of the fungi species, while candida albican yeast appeared with a frequency rate of 28.18% compared to the rest of the other fungi, and the rate of appearance of other fungi was about 12-3% compared to the rest of the other fungi. The fungi were distinguished by the fact that they are all of the opportunistic type, which begins to infect the body when there is a weakening of immunity or infection with the associated burns and ulcers.

**Conflict of Interest –** Nil

**Source of Funding-** Self

**Ethical Clearance –** Not required

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# Investigation for Endemic Bacteria in the Intestine of Common Carp (*Cyprinus Carpio* L.) Feeding on Various Formulated Powders Fortified Diets

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## Abstract

The study was conducted in Fish Laboratory / College of Agriculture / University of Tikrit with the aim of investigating the effect of adding powders prepared from chicken meat and fish waste (mixed and carp) and added to diets on the performance of common carp *Cyprinus carpio* L. and the endemic bacteria in its intestine. 70 cuffs were used with an initial weight of 240 ± 1.60 g / fish and distributed randomly into 7 treatments (diets) at the rate of two repetitions for each treatment (5 fish per repeater) in 14 glass basins with dimensions of 30 x 40 x 30 cm. 7 experimental diets were prepared close to each other in protein and energy content. The parameters T1 included the commercial powder, T2 the powder prepared from raw chicken waste, T3 raw mixed fish waste powder, T4 common carp fish waste powder, T5 the chicken meat powder fortified with 1% lactic acid. , T6 fish powder mixed with lactic acid by 1%, T7 fish powder of common carp with lactic acid by 1%, the results of the statistical analysis showed a significant superiority (P≤0.05) for the parameters T5 and T7 in the percentage of protein and no significant differences in the percentage of fat were recorded for the treatments. T3, T4, T6 and T7, the treatment T4 recorded the highest percentage of humidity. The treatments T2, T3 and T5 recorded the highest rates of protein percentage in fish bodies after the end of the experiment, and there were no significant differences for the percentage of fat in fish bodies for the treatments T1, T2 and T4 and it reached (4.20, 4.20 and 4.12%), respectively. Several bacterial strains colonized in fish gut were isolated: 10 S.*aureus isolates*, 12 *Aeromonas hydrophilia*, 5 *Aeromonas* S isolates, 8 *E.oli bacteria*, 3 *Pseudomonas* bacteria, 5 *Bacillus* isolates, 7 isolates. *Flavobacterium isolates*.

***Keywords****: Mapk; Bacteria; Common Carp; Formulated Powders; Chicken meat powder; Fish powder carp*

## Introduction

Carp fish is regarded one of the most important fish found in many regions of the world [1]. The improvement in fish production results from preparing suitable forages through ingredients, technologies and processing that comply with international standards [2]. Fish feed must be contained. On a source of protein rich in amino acids [3], poultry waste, such as legs, heads, viscera, feathers and blood, was used on a small scale as animal fertilizer and the accumulation of these wastes has a harmful effect on the environment and public health [4]. The tendency by researchers and breeders to exploit these wastes in the manufacture of diets for farm animals, including in particular fish, as

they were used in the diets of common carp [5].

The most common types of bacteria that infect fish live in aquatic environments with fish or in them with the presence of stressful factors, whether natural, such as changes in temperature, dissolved oxygen in the water, or chemical factors such as chemical pollutants and mechanical factors such as handling and transportation of fish, or biological factors such as microbes present. In water as a result of organic or other fertilization that reduces and weakens fish immunity and resistance and works to activate bacteria that prevent the synthesis of bacterial cell proteins or their nucleic acids, and there are some of them that kill bacteria as it prevents the formation of the cell wall or cytoplasmic membrane in them [6].

The researchers [7] Indicated that the control of bacterial diseases that affect fish is done by using antibiotics usually with the aim of prevention before the appearance of disease symptoms or treatment when the disease appears of bacterial growth by affecting growth by inhibiting the biosynthesis of bacterial cell proteins and nucleic acids.

The current study aims to reduce the cost of diets and reduce environmental pollution by preparing powders from fish and chicken wastes fortified with lactic acid to improve the properties of the diets and to study growth parameters, endemic bacteria and biochemical measurements of the fish body.

## Materials and Methods of Work

Three types of protein powders were prepared from offal (legs and heads) of broiler of Ros308 type and cleaning residues (internal viscera) of five species of river fish (*Silurus triostegus* - *Carasobarbus luteus* - *Cyprinion kais* - *Cyprinion macrostomum* - *Planiliza abu*) and the guts of *Cyprinus carpio* L.pond-bred fish, which were obtained from the local markets of Tikrit city. The powders were fortified with lactic acid, and the diets were prepared using the raw materials (wheat, barley, yellow corn, bran, vitamins and minerals) from the local markets of Tikrit. Pellets are 4-5 mm in length and 2-3 mm in diameter. Then the feed was dried by air and on sunlight after formation for a period of 6-8 hours.

Common carp fish were brought from one of the private farms in the Uweinat area of Salah al-Din Governorate - Tikrit District, whose original source is the hatchery in the Tarmiyah area to the Fish Laboratory - College of Agriculture - Tikrit University and were sterilized with a 3% saline solution to get rid of any pathogen for a period of five It was indented for a week in ponds containing continuous ventilation devices until the start of the experiment, and the dead and sick fish were excluded. The fish were fed at a rate of 3% of their body weight, and in this experiment 60 Common carp weights were used at a rate of 240 ± 1.60 g / fish, with 5 fish per pond .. Environmental measurements were made for water in the ponds daily, which included measuring each of (water temperature, oxygen in Water, ammonia, pH (using specialized equipment). The experiment lasted for a period of 70 days. 70 cuffs were prepared with an average initial weight of 240 ± 1.60 g / fish and distributed randomly on 7 treatments (diets) by repetition of each treatment at a rate of 5 fish per replicate on 14 ponds. The fish were weighed weekly with a Chinese electronic sensor scale (the scale weighs two numbers after the sorter).

**Table (1) Chemical analysis% of the raw materials used in the preparation of diets**

| Soluble carbohydrates % | Crude fiber % | ash % | fat % | protein % | Humidity % | Forage material |
|---|---|---|---|---|---|---|
| 71.49 | 2.01 | 2.33 | 4.34 | 9.03 | 10.80 | Yellow corn |
| 71.27 | 3.35 | 1.96 | 2.02 | 11.70 | 9.70 | Wheat |
| 70.39 | 6.50 | 3.82 | 1.42 | 10.72 | 7.15 | Barley |
| 54.89 | 10.63 | 4.99 | 4.05 | 15.86 | 9.58 | Bran |

NRC)، 1993)

**Chemical Analyzes**

Standard methods were used to estimate the percentage of moisture, protein, fat and ash for each of the commercial protein centers in the manufactured feeds and the primary feed materials used in the preparation of these diets according to what was stated in [8].

**The Percentage of Humidity**

The percentage of humidity was estimated by using an electric oven at a temperature of 105 ° C for a period of 6 hours until the weight was stable.

Moisture weight g = weight of sample before drying - weight of sample after drying

Moisture percentage% = moisture weight / sample weight x 100

**The Percentage of Crude Protein**

The amount of nitrogen was according to the Microkjedal method

Crude Protein Percentage =) (F 1 - F 2) x 0.014 x 6.25 }/ sample weight (g) x 100

The amount of acid is 1 in the receiving beaker.

F2 The base amount consumed to titrate the excess amount of sulfuric acid in the receiving flask

**Fat percentage**

The percentage of fat was estimated using the Soxhlet device and the absolute diethyl ether solvent. The samples were placed in the device for 8 hours and the fat percentage was calculated according to the following method:

Fat percentage = weight of ether extract (g) / sample weight (g) x 100

The Percentage of Ash

The percentage of ash was calculated by burning the sample weight at 550 ° C in the MUFFLE FURNACE incinerator for three hours.

Ash percentage% = Ash Weight (g) / Sample Weight (g) x 100

Fibers Estimate

The fibers were determined by adding 1.25 N standard sulfuric acid for the purpose of digesting the sample for half an hour after it was boiled, then the sample was washed from the acid with hot distilled water and a 1.25 N standard NaOH was added for half an hour after boiling the sample and it was washed from the base with hot distilled water and then with acetone. Then, the dry and empty vessel was weighed, the sample was placed in it, and the oven was entered at a temperature of 60 ° C**.**

Percentage of crude fiber% = weight of fiber (g) / sample weight (g) x 100

Carbohydrates

Carbohydrates were calculated by difference by subtracting the percentage of nutrient components (moisture, protein, fat, and ash) out of a hundred.

Isolation and diagnosis of bacteria

The Nutrient agar medium used in the cultivation of bacteria was prepared by adding a certain weight of the medium according to the specifications of the company supplying it to a liter of distilled water, then its acidity was controlled using the acid function measurement device, and it was filled with the Autocalve device at a temperature of 121 m and a pressure of 15 pounds / inch 2 for 15 minutes and left to cool At a temperature of 45 ° C before pouring it into sterile Petri dishes.

Isolation of bacteria from fish intestine

The intestines of the cleaned and washed fish were cut with sterile scissors into small pieces and placed in a sterile container containing a special sterile dilution solution. The cut intestine was crushed well

and 1 gm of the fish sample was taken in sterile test tubes containing 9 g of sterile physiological solution. Transfer 0.1 mL of the appropriate dilution and spread onto nutrient (NA) agar media. The media was then incubated in an Incubator at 37 ° C for 48-72 hours.

The bacterial colonies were purified by taking a colony of each type of colonies with different phenotypically in shape, color and size and planted on the medium of nutrients and then incubated in the incubator for 38-72 hours. The bacteria were diagnosed by conducting phenotypic and biochemical tests according to [9].

## Statistical Analysis

The Statistical Analysis System -SAS (2012) program was used in data analysis to study the effect of different parameters on the studied traits according to a complete random design (CRD). The significant differences between the averages were compared with the [10] polynomial test on the level of probability ($P \leq 0.05$).

## Results and Discussion

Chemical composition of experiment feeds:

Table (2) shows the chemical composition of the experimental diets, and the results of the statistical analysis showed that there were significant differences ($P \leq 0.05$) between the treatments. It was noticed that the percentage of moisture increased in treatment T4, and the two treatments T5 and T7 outperformed the percentage of protein, which amounted to (36.21, 36.11) %. Respectively, and the highest percentage of ash was 6.72%, and this result is comparable to what [11] found, which is 6.68% when using fish meal in trout diets.

**Table (2) the chemical composition of the experiment diets**

| carbohydrates % | ash % | fiber % | fat % | protein % | moisture % | Transactions |
|---|---|---|---|---|---|---|
| 35.86±0.198 b | 6.72±0.034 a | 5.32±0.036 A | 8.73±0.032 b | 34.64±0.136 b | 8.73±0.030 B | T1 Commercial powder |
| 37.15±0.014 A | 6.54±0.017 A | 5.25±0.017 A | 7.313±0.020 c | 35.77±0.043 b | 7.97±0.011 C | Raw chicken raw meat powder T2 |
| 35.02±0.155 b | 5.86±0.416 b | 4.96±0.482 A | 9.87±0.047 a | 35.47±0.382 c | 8.82±0.041 b | Mixed raw fish powder T3 |
| 35.09±0.314 b | 5.63±0.525 b | 4.89±0.280 A | 9.67±0.200 a | 35.51±0.720 c | 9.21±0.244 a | Fish powder Raw carp T4 |
| 34.36±0.057 C | 6.69±0.1 A | 5.71±0.040 B | 8.66±0.055 b | 36.21±0.057 a | 8.37±0.274 c | Chicken meat powder with lactic acid T5 |
| 34.96±0.213 c | 5.90±0.043 b | 4.93±0.063 A | 9.76±0.040 a | 35.65±0.153 b | 8.8±0.0320 b | Fish powder mixed with lactic acid T6 |
| 34.54±0.536 c | 5.61±0.085 b | 4.85±0.332 A | 9.93±0.032 a | 36.11±0.401 a | 8.96±0.158 b | Powder of carp fish with lactic acid T7 |

* Similar letters, there are no significant differences.

** Different letters were significant at 0.05 level.

Table (3) shows the number of bacterial species that were isolated from the intestines of common carp fish, and it was by 50 bacterial isolates, in which they settled naturally and could, in certain circumstances, transform them into pathological bacteria or remain unsatisfactory, as it was observed that the bacteria *Aeromonas* sp form the highest number while bacteria *Psweudomonas* make up the lowest percentage. Several similar studies were found in the investigation of the bacteria, as [12] isolated in their study similar types of bacteria in freshwater fish of Basra city and included types of positive and negative bacteria for the cram stain [13], the bacteria isolated from the Acinetobacter species of fish found in the Konkaz River in Brazil. sp and Pseudomonas. sp *Micrococcus*. sp and *Aeromonas*. Sp and *Lactobacillus*. Sp and *Bacillus*. Sp and *Enterobacteriaceae*. [14] isolated 40 bacterial isolates from O.mykiss trout in freshwater lakes in Turkey, including Aeromonas .sp and Pseudomonas. sp, Salmonella and other bacterial genera, as identified by [15]. Different bacterial species from the tissues of tilapia fish found in the fish farms of the Fouqueta River located in Guinea include *Salmonella*. sp and *Corynebacterium*, sp and *Bacillus*. sp and Pseudomonas. sp and *Vibrio*. sp and *Streptococcus*. sp

**Table (3) bacterial genera isolated from the intestines of carp fish**

| Number | Isolators |
|---|---|
| 10 | S.areus |
| 12 | Aeromonas |
| 5 | Aeromonas hydrophelia |
| 8 | E.coli |
| 3 | Pseudomonas.sp |
| 5 | Bacillus |
| 7 | Flavobacterium |

Second - Chemical Analysis of Fish Bodies

**Table (4) shows the chemical analysis of fish bodies, where an improvement and high percentage of protein were observed for the treatments T2, T3 and T5 and reached (19.92, 20.15, 19.98%), respectively, compared to the control treatment T1 19.49%. This is confirmed by [16].**

| Ash % | Fat % | Protein % | Humidity % | Transactions |
|---|---|---|---|---|
| 5.43 | 4.11 | 19.80 | 70.85 | Fish Before the experiment |
| 5.28±0.017 b | 4.20±0.033 a | 19.49±0.02 d | 71.02±0.011 A | T1 Commercial powder |
| 5.33±0.017 b | 4.29±0.017 a | 19.92±0.017 a | 70.45±0.017 C | Raw chicken raw meat powder T2 |
| 5.18±0.017 c | 3.73±0.017 c | 20.15±0.017 a | 70.92±0.017 b | Mixed raw fish powder T3 |

***Cont...*** **Table (4) shows the chemical analysis of fish bodies, where an improvement and high percentage of protein were observed for the treatments T2, T3 and T5 and reached (19.92, 20.15, 19.98%), respectively, compared to the control treatment T1 19.49%. This is confirmed by [16].**

| | | | | |
|---|---|---|---|---|
| 5.24±0.023<br>c | 4.12±0.028<br>a | 19.74±0.028<br>c | 70.98±0.023<br>b | Raw Carp Fish Powder<br>T4 |
| 5.54±0.017<br>a | 3.82±0.017<br>c | 19.98±0.017<br>a | 70.64±0.017<br>b | Chicken meat powder with lactic acid<br>T5 |
| 5.54±0.023<br>a | 3.87±0.023<br>b | 19.56±0.028<br>d | 71.01±0.017<br>a | Fish powder mixed with lactic acid<br>T6 |
| 5.20±0.034<br>c | 3.92±0.028<br>b | 19.85±0.023<br>b | 71.02±0.028<br>a | Powder of carp fish with lactic acid<br>T7 |

Table (4) Chemical analysis of fish bodies

I don't have a moral clearness.

I don't have **Conflict of Interests**

**Self Funding**

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# The Role of Vitamin D in Metabolic Syndrome in Polycystic Ovary Syndrome

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## Abstract

**Objective**: The aim of this study was to assess the women complaining from polycystic ovarysyndrome and the relation of serum vitamin D levels according to the different phenotypes of the disease by a retrospective study.

**Methods:** Records for 242 infertile women diagnosed with PCOS have been examined. In this retrospective study, 100 of them have been randomly recorded for 4 PCOS phenotypes. 40 normal ovulation women with a male factor history were selectedas the control group.

In four phenotype types including: age, BMIs, the infertility duration, the hormonal profile and the serum vitamin D, the P value of 0.05 was found to bestatistically significant.

**Results:** In comparison with PCOS patients (P<0.001), the data showed a serum vitamin Dlevel of statistically importance in the control group. Also, four PCOS phenotypeshad no significant variation in the serum vitamin D levels.

**Conclusions:** There were no significant differences between the serum vitamin D levels of thedifferent PCOS phenotypes. The additional studies of large samples are recommended for determining the role of the serum vitamin D level in PCOS patients.

***Keywords:*** *Infertile women, Polycystic ovary syndrome, Rotterdam Criteria, Vitamin D.*

## Introduction

Polycystic ovary syndrome (PCOS) referred to as a condition affecting the endocrine system. Approximately 5-21 percent of women in reproductive age areat risk. The main symptoms of PCOS commonly are ovulatory failure, menstrual cycle defect, and hirsutism. PCOS is a metabolic syndrome of various types withother characteristics including hyperandrogenemia, dyslipidemia and insulin resistance ([1]).

The guidelines for the diagnosis of PCOS are Rotterdam criteria. PCOS is highly accepted to be diagnosed by these criteria. This disease is typically diagnosed in women with two or more of the three symptoms I; polycysts of ultrasound ovaries, II; hyperandrogenemia and III; oligo or anovulation ([2]).

The patient health and infertility risk can be increase by PCOS([3]). The term polycystic ovarian syndrome does not entirely or correctly reflect the complications of the disease. It covers a broad range of diseases and clinicalexpressions.

The indications of reproductive defects is high in women with PCOS, includingtype 2 diabetes mellitus, Insulin resistance, high blood pressure, high LDL,

lowHDL, anxiety and depression([4]).

PCOS in pregnant women have a significant risk of preeclampsia, gestational diabetes, small-for-gestational age infants, fetal macrosomia and perinatal mortality ([5]).

There have been major efforts to classify PCOS types over the last few decades. The initial data comprised the PCOS classification indicator for chronic anovulation and hyperandrogenism.

The data gathered were finally integrated into the Rotterdam criteria([6]). On the basis of the criteria in Rotterdam, four different PCOS phenotypes may be defined including chronic anovulation, polycystic ovary (PCO) and hyperandrogenism. polycystic ovaries and hyperandrogenism but ovulatory cycles; chronic anovulation but normal ovaries, and polycystic ovaries and chronic anovulation butno biochemical or clinical hyperandrogenism ([7]).

Recently, vitamin D supplementation are focused by the clinical researches as an adjuvant therapy for PCOS. Women with PCOS have been confirmed to be high in vitamin D deficiencies. Consequently, a correlation was established with several metabolic symptoms in patients suffering from serum vitamin D.

Recently, vitamin D deficiency has been suggested to be a cause of PCOS([8]).

Numerous studies have demonstrated an adverse correlation of the vitamin D biomarker, e.g. 25-hydroxy vitamin D (25 OH D), with waist circumference, fat level in the body and body mass index (BMI) ([9]-[11]).

A study that examines serum vitamin D in different PCOS phenotypes has been found in the literature review for this study. In this retrospective study, the level ofvitamin D in women with PCOS has been assessed by different phenotypes.

## Methods

In this retrospective study, the clinical and laboratory records of 2500 patients have been examined for a 30-month period from April 2017 to August 2019. PCOS was diagnosed in 250 infertile women aged 18-40. In each phenotype group (200/250 women), fifty cases were then randomly enrolled in the study. Fifty normal, ovulatory women with a male history have been chosen as the control group.

Standard ovary reserve (AMH > 1.2 ng/mL) and non PCOS in control group women with regulatory objectives based on selected Rotterdam criteria.

Criteria for inclusion: the diagnosis for polycystic ovary syndrome was made by women aged 18-40 years based on Rotterdam criteria.

The exclusion criteria were: women with a history of recurrent miscarriage, endometriosis, hyperprolactinemia and a severe male factor (PESA), oligospermia,testicular sperm extraction (TESE).

Based on the Rotterdam criteria, PCOS was diagnosed([2]). If at least two of the following criteria were fulfilled, PCOS would be diagnosed: Clinical and/or biochemical hyperandrogenism (defined by overall concentrations of circulating testosterone over a maximum of 35 days (0,481 ng/mL) are more than 95 percent of the levels detected in the women's group without clinical evidence of menstrual disorders or hyperandrogenism taking no hormonal medication), 'oligomenorrhea/anovulation' (defined as the delay in menses over 35 days or less than 8 spontaneous hemorrhagic episodes per year), polycystic ovary on ultrasonography (≥ 12 small follicles measuring 2-9 mm in at least one ovaryand/or the ovarian volume > 10 $cm^3$).

The patients were classified in the following defined criteria into four groups: polycystic ovary (PCO), chronic anovulation, and hyperandrogenism; polycysticovaries and hyperandrogenism but ovulatory cycles, chronic anovulation and hyperandrogenism but normal ovaries, and polycystic ovaries and chronic anovulation but no biochemical or clinical hyperandrogenism.

Data from the hospital records were collected. The ELISA kit and range of detection of this test was between 1.56 to 100 ng/ml for biochemical testing in order to determine vitamin D level. Specific ELISA kits were used for other hormones.

The data from patients included duration of infertility, age, hormone profile, body mass index BMI, and serum vitamin level D.

## Statistical Analysis

Using the Social Sciences Package 20.0 data were analyzed (SPSS, SPSS Inc, Chicago, Illinois). Continuous data was presented to the independent student t-test as a mean ± standard deviation (SD). A post-hoc LSD with analysis of variance (ANOVA) was used for comparison of the mean of pair parameters. Less than 0.05 p-value was regarded statistically important.

## Results

In this study, data were analyzed for 200 PCOS cases compared to 50 cases in control group for male factor infertility. Table 1 summarizes the patient characteristics. Both groups showed a similar average age and duration of infertility. The serum vitamin D was statistically significantly higher in the control group than in patients with PCOS ($P < 0.001$) on statistical analysis. Other factors such as BMI: body mass index; AMH: anti-Mullerian hormone; FBS: fasting blood sugar: FSH: follicle stimulating hormone; LH: luteinizing hormone were significantly different between the two groups (Table 1).

**Table 1 Comparison between PCOS and control characteristics of women**

| p-value | Control (n = 50) | PCOS (n = 200) | Variables |
|---|---|---|---|
| NS | 28.46 ± 4.18 | 28.19 ± 5.37 | Women's age (years) |
| NS | 5.44 ± 3.53 | 5.82 ± 3.78 | Infertility duration (years) |
| 0.001 | 29.08 ± 5.50 | 20.60 ± 9.22 | Vit D3 (ng/mL) |
| 0.001 | 3.76 ± 1.22 | 8.70 ± 4.07 | AMH (ng/mL) |
| 0.001 | 23.96 ± 2.63 | 28.54 ± 3.45 | BMI (kg/m2) |
| 0.001 | 5.36 ± 2.96 | 9.14 ± 5.51 | LH (IU/l) |
| 0.001 | 6.66 ± 1.69 | 5.42 ± 2.21 | FSH (IU/l) |
| 0.001 | 0.88 ± 0.44 | 1.97 ± 2.06 | LH/FSH ratio ≥2.5 |
| 0.001 | 97.56±14.12 | 99.16±17.76 | FBS (mg/dl) |

Note: Mean ± SD values are shown in a single way P-values obtained from adifference between media were tested on ANOVA for importance.

PCOS: polycystic ovarian syndrome; AMH: anti-Mullerian hormone; BMI: bodymass index; FSH: follicle stimulating hormone; LH: luteinizing Hormone;

FBS: fasting blood sugar.

The parameters have been compared between the PCOS phenotypes, as shown in Table 2. The serum vitamin D level of the four PCOS phenotypes did not differ significantly. The four phenotypes demonstrated a significant three-parameter variation of AMH, LH and testosterone. Major parameter differences and comparison results between the PCOS phenotypes pair showed by letters.

Important differences in the AMH-related phenotypes were noted between A and B (P-value = 0.02). Moreover, LH differs markedly between A and C (P-value + 0,007), as well as between A and D (P-value = 0,08). The difference in the FBS parameter between B and C was also marked (P-Value = 0.008) (Table 2).

The results of comparisons between vitamin D concentrations associated with the PCOS variables indicate that the serum vitamin D levels did not differ significantlyfrom the study parameters. (Table 3).

**Table 2 Study variables comparison in four PCOS phenotypes**

| p-value | Phenotype D (n = 50) | Phenotype C (n = 50) | Phenotype B (n = 50) | Phenotype A (n = 50) | Variables |
|---|---|---|---|---|---|
| **NS** | **27.20 ± 6.13** | **28.12 ± 4.81** | **29.44 ± 4.57** | **27.98 ± 5.71** | **Women's age (years)** |
| NS | 5.28 ± 4.04 | 6.00 ± 3.91 | 6.08 ± 3.40 | 5.90 ± 3.80 | Infertility duration (years) |
| NS | 19.97 ± 9.33 | 20.54 ± 9.53 | 20.92 ± 9.43 | 20.97 ± 8.80 | Vit D3 (ng/mL) |
| 0.001 | 9.71 ± 5.39b | 8.53 ± 3.33 | 7.13 ± 2.35a, b | 9.43 ± 4.19a | AMH (ng/ml) |
| NS | 28.27 ± 4.30 | 28.44 ± 3.57 | 28.43 ± 3.03 | 29.00 ± 2.75 | BMI (kg/m2) |
| 0.01 | 8.54 ± 5.46d | 7.61 ± 5.07c | 9.26 ± 4.87 | 11.14 ± 6.12c, d | LH (IU/l) |
| NS | 5.39 ± 2.26 | 5.19 ± 1.88e | 5.94 ± 2.62e | 5.16 ± 1.97 | FSH (IU/l) |
| NS | 2.16 ± 3.48 | 1.52 ± 1.06 | 1.73 ± 0.92 | 2.41 ± 1.66 | LH/FSH ratio |
| 0.01 | 98.04 ± 10.66 | 102.30 ± 21.85 | 91.86 ± 12.85 | 98.52 ± 16.73 | FBS (mg/dl) |
| 0.001 | 0.56 ± 0.28f | 0.75 ± 0.41 | 0.94 ± 0.35 | 0.91 ± 0.38f | Testosterone (IU/l) |

Note: Mean value ± SD is shown. The importance of ANOVA was tested in ranks by one way, P-values derived from the difference of means. A statistical difference for comparison between PCOS phenotypes is indicated in different letters: -

a: Significant between A and B phenotypes. c: Significant between A and C; d: Significant between A and D phenotypes; e: Significant between B and C phenotypes; b: Significant between B and D phenotypes; f: Significant between A and D phenotypes.

FSH: follicle stimulating hormone; LH: luteinizing hormone; BMI: body mass index; AMH: anti-Mullerian hormone; FBS: fasting blood sugar.

**Table 3 Comparison of the levels of PCOS-related vitamin D**

| p-value | Toxicity level (>100 ng/mL) (n = 0) | Sufficiency (30 to 100 ng/mL) (n = 74) | Insufficient (20 to <30 ng/mL) (n = 99) | Deficiency (<20 ng/mL) (n = 27) | Variables |
|---|---|---|---|---|---|
| **NS** | - | **28.04 ± 5.10** | **28.30 ± 5.91** | **28.15 ± 3.93** | **Women's age (years)** |
| NS | - | 5.36 ± 3.58 | 6.30 ± 5.82 | 5.26 ± 4.07 | Infertility duration (years) |
| NS | - | 8.61 ± 4.08 | 8.73 ± 3.80 | 8.84 ± 5.09 | AMH (ng/mL) |
| NS | - | 28.22 ± 2.86 | 28.76 ± 3.21 | 28.57 ± 5.35 | BMI (kg/m2) |
| NS | - | 8.74 ± 5.57 | 9.06 ± 5.41 | 10.51 ± 5.73 | LH (IU/l) |
| NS | - | 5.29 ± 2.09 | 5.44 ± 2.35 | 5.68 ± 2.22 | FSH (IU/l) |
| NS | - | 1.80 ± 1.14 | 2.09 ± 2.70 | 2.02 ± 1.20 | LH/FSH ratio |
| NS | - | 98.68 ± 12.91 | 97.05 ± 17.58 | 97.26 ± 20.59 | FBS (mg/dL) |
| NS | - | 0.85 ± 0.39 | 0.77 ± 0.36 | 0.71 ± 0.44 | Testosterone (IU/l) |

Note: Mean value ± SD is shown. Analysis of variance has been tested for significance in ranks by one-way P-values obtained from difference betweenmeans.

## Discussion

The focus of this study was on serum vitamin D and the prevalence of vitamin D deficiency among PCOS patients. The results showed that the total serum level of vitamin D and vitamin D deficit are significantly different for women with PCOS and controls. However, the serum vitamin D level of different PCOS phenotypes did not differ significantly.

As much evidence demonstrates that vitamin D plays an important role in reproductive activities, all patients were infertile in the current study([13]). Studies have shown that vitamin D receptors are present in many tissues, including endometrium, ovaries and placenta in the reproductive system([14]). Vitamin D deficiency has been shown to be associated with calcium dysregulation. This condition increases follicular arrest and leads to menstrual and fertility

disorders inPCOS-patient women.

According to the study, in women with PCOS (n=545) the concentrations of serum vitamin D were lower than in the control group (n= 145) and 25.7 and 32 ng/mL respectively. Many studies suggest that the levels of serum vitamin D in women with or without PCOS are similar([16]-[18]).

In the study for vitamin D-PCOS correlations, only one study found that women with PCOS had a significantly higher serum vitamin D level than control womenof similar age and BMI([19]).

In the literature on serum vitamin D levels, therefore, there are various results forwomen with or without PCOS. The paper about vitamin D assessment were published in 2018 by Newly Davis and cols. where in all PCOS cases and male

infertility as a control group in the intrauterine insemination cycle. They showed a lower level of vitamin D than other PCOS phenotypes in androgen excess([12]).

However, this finding did not show the androgen level to find excessive androgen, but we presented androgen as testosterone levels in 4 phenotypes. These results showed that androgen in patients with deficiency was less than other vitamin D categories, but that difference was not statistically significant (Table 3). In four phenotypes, They also presented fertility hormone levels and this was what made the study strong.

Kim and cols. reported that 2-month therapy of 1,500 mg calcium daily and 50,000 weekly unit vitamin D improved menstrual cycles in 7 of 9 cases in PCOS patients with vitamin D deficiency([18]). ) There have been other trials in BMI, body fat, insulin and hyperinsulinemia patients in PCOS that showed negative linkage between the levels of serum vitamin D and metabolic disorder. ([20]-[22]).

Studies showed that PCOS is correlated with other metabolic problems like, dyslipidemia, depression, anxiety, high blood pressure and chronic inflammation. Metabolic disorders in women with PCOS have also been confirmed([23]).

In their study, Hang Wun and cols stated that in Scottish women with PCOS vitamin D was highly prevalent and this rate was higher in the UK than ovular controls. In addition, vitamin-D deficiency has been shown to be correlated with metabolic risk factors, including insulin resistance and low HDL-C levels, regardless of obesity measures. ([17]).

Although no trial to evaluate serum vitamin D levels in a range of PCOS phenotypes was found in the literature review of the current study, some studies evaluated PCOS-Clinical correlations. Reza Ghadimi and cols were found, although PCOS patients had common hypovitaminosis D, it was not correlated to the clinical features to insulin resistance or obesity and complications. In PCOS patients, there has been correlation between the severity of vitamin D deficit and certain PCOS features and complications, including Obesity and Insulin resistance.([24]).

The study of 260 PCOS women (cases) and 221 Normo-ovulatory (checks) whohave been recruited to a clinic for reproductive endocrinology was performed byVakili and cols. They classified the cases according to their clinical and para- clinical characteristics into two groups of serious and mild PCOS phenotypes.

Adenosine was a genotyped PCR–RFLp method for guanine nuclear polymorphism (rs757343) of the VDR gene. They noted that there was no change in the distribution of genotypes and alleles among cases and checks showing a lackof correlation between the single nucleotide polymorphism (SNP) and increasing PCOS risk. However, PCOS phenotype severity was the responsibility of the SNP. The risk that a severe phenotype was present was 74% higher than in other patientsin the case of an allele. They also showed that the genetic version of the VDR was associated with the seriousness of the PCOS' clinical presence, but not PCOS risk([25]).They assessed the correlation between VDR and PCOS in Indian patients ina genetic study

by Dasgupta and cols. They identified a significant relationship between the VDR genotype and some PCOS events([26]).

In conclusion, we found no significant vitamin D differences in various PCOS phenotypes. Other studies recommend additional studies with larger samples to conclude that serum vitamin D is of importance in PCOS patients, including PCOS data phenotypes. The first restriction of insulin resistance and metabolic malformations associated with vit-D deficiency in PCOS was a retrospective study,and the lack of these data was limited by the manuscript.

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# The Multifactorial Causes of Neonatal Mortality: A Literature Review

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## Abstract

The reduction of neonatal mortality is part of the third goal of the Sustainable Development Goals that by the end of 2030 it is expected that neonatal mortality will decrease to 12 per 1000 live births. This study aims to organize risk factors for neonatal mortality through a traditional review. Information was obtained from previous studies around the world, from 2009 to 2021. The selection of articles was based on the researchers' justification, followed by a comparison of the articles and ending with a synthesis in order to answer the research questions. The study results show that the risk factors associated with pregnancy included: age, height, parity, interval between pregnancies, antenatal care, past pregnancy and childbirth (giving birth to dead babies, cesarean section, hypertension, abortion, bleeding), problems in pregnancy (anemia, tuberculosis, asthma, hyperthyroidism, hypertension, malnutrition) and pregnancy complications (hyperemesis gravidarum, abortion, degenation of trophoblast disease, ectopic pregnancy, prematurity, antepartum hemorrhage, premature rupture of membranes, fetal death in utero, pre-eclampsia / eclampsia, post date, meconeal membranes, fetal position abnormalities, multiple pregnancies and prolonged labor); while the factors related to childbirth were place, mode and assistant of delivery.

***Keywords****: neonatal mortality, risk factors, pregnancy, childbirth*

## Introduction

Neonatal mortality is the number of deaths of live births and deaths within the first 28 days after birth, which is formulated as the number of neonatal deaths per 1000 live births [(1)]. In 2017, UNICEF (2018) reported that 47% of all under-five deaths occurred in newborns. Globally, in 2018, 2.5 million children died in the first month of life. Of these, 7000 newborn deaths occur every day, i.e. about one third of deaths occur on the day of their birth and three quarters die within the first week of life. The first day and first week of life are critical life periods for a baby. Most of these deaths could have been prevented with relatively easy and effective interventions [(2)].

Globally, neonatal mortality has decreased, from 36 deaths per 1000 live births in 1990 to 19 deaths per 1000 live births in 2015, and 18 deaths per 1000 live births in 2018. The decline in neonatal mortality is slower than the mortality rate under five, namely 91 deaths per 1000 live births in 1990, to 41 deaths per 1000 live births in 2015 and 37 deaths per 1000 live births in 2018 [(2)].

The reduction of neonatal mortality is part of the efforts to achieve the world community towards the third goal of the Sustainable Development Goals

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(SDGs). In this case, it is hoped that by the end of 2030, the neonatal mortality rate will decrease to 12 per 1000 live births. However, it is estimated that if current trends continue, there will be 52 million children under 5 deaths worldwide between 2019 and 2030, and half of this number will occur in newborns [(2)].

Based on the background, it is necessary to know in detail the risk factors for this neonatal mortality, so that studies are needed to organize various risk factors for neonatal mortality through a literature review.

## Methods

This study was a literature review with a traditional review approach. Data and information were obtained from journals, proceedings, books and research reports from around the world, from 2009 to 2021. Referring to the traditional review principle, the selection of articles was based on the researchers' justification, with the consideration that the topic of this study is relevant to the field of expertise the researchers. Thus, no specific protocol was used for the selection and selection of appropriate articles. After the relevant articles were complete, then comparisons were carried out and were continued with synthesis in order to answer research questions.

## Findings

Based on the results of selecting articles based on justification, then the selected articles were compared with the results as shown in Table 1.

**Table 1. Selected articles on risk factors for neonatal mortality**

| Authors | Risk factors |
|---|---|
| Prabamurti, et al., 2008 (3) | Maternal age <20 years or >35 years, with a risk of 7.69 times; delivery with a helper not a health worker |
| Aini, 2014 (4) | Age is too old, with a risk of 1.93 times; the number of children is >2, with a risk of 1.11 times |
| Ouyang, et al., 2013 (5) | Maternal age <18 years or >35 years; history of giving birth to a baby who died, with a risk of 1.61 times; pregnancy with hypertension; fetal position abnormalities; preterm labor with a risk of 1.91 times |
| Mekonnen, et al., 2013 (6) | Maternal age <18 years, with a risk of 1.41 times; The interval between pregnancies was short (<2 years), with a risk of 2.19 times |
| Sun, et al., 2014 (7) | Maternal age <20 years or >35 years; meconeal amniotic fluid, with a risk of 10,995; multiparous; preterm labor with a risk of 32.3 times; cesarean section delivery |
| Ezeh, et al., 2014 (8) | Maternal age <20 years, risk: 4.07 times; the interval between pregnancies is short, with a risk of 2.19 times |
| Akinyemi, et al., 2015 (9) | Maternal age <20 years with 1.75 times the risk, or >35 years with the risk: 1.16 times; multiparous, with a risk of 1.28 times; less antenatal care |
| Syed & Kamathi, 2012 (10) | Maternal's height (too short) |
| Prawirohardjo & Wiknjosastro, 2016 (11) | Maternal's height is <145 cm; pregnancy with hyperthyroidism; pregnancy with hypertension; antepartum hemorrhage; premature rupture of membranes; fetal position abnormalities; pre-eclampsia and eclampsia; old labor |

***Cont...*** **Table 1. Selected articles on risk factors for neonatal mortality**

| Authors | Risk factors |
|---|---|
| Jain, et al., 2014 (12) | Maternal's height is <145 cm, with a risk of 1,153 times; history of pregnancy with bleeding, with low risk |
| Arendt, et al., 2018 (13) | The maternal's height is <145 cm, with a risk of 1.95 times |
| Abdullah, et al., 2012 (14) | The number of children is>2, with a risk of 5.5 times; antenatal care <4 times, with a risk of 7.3 times; pregnancy with anemia, with a risk of 32,818 times |
| Titaley, et al., 2010 (15) | Interval between pregnancies is short, with a risk of 2.08 times |
| Batieha, et al., 2015 (16) | No antenatal care, with a risk of 4.1 times; history of giving birth to babies dead, with a risk of 2.1 times; preterm labor with a risk of 23.8 times |
| Demitto, et al., 2017 (17) | History of giving birth to babies died, with a risk of 5.56 times; preterm labor with a risk of 682.47 times |
| Kolluru & Reddy, 2016 (18) | History of cesarean section delivery, with moderate risk; history of pregnancy with hypertension, with low risk; history of abortion, low risk; history of pregnancy with bleeding, with low risk |
| De, et al., 2015 (19) | History of pregnancy with bleeding, with low risk |
| Lumbiganon, et al., 2014 (20) | Pregnancy with anemia, with a risk of 3.8 times |
| Loto & Awawole, 2012 (21) | Pregnancy with tuberculosis |
| Kim, et al., 2015 (22) | Pregnancy with asthma |
| Bayou & Berhan, 2012 (23) | Pregnancy with hypertension, with a risk of 3.2 times; fetal position abnormalities with a risk of 11.6; antepartum bleeding with a risk of 12.2 times. |
| Sulistyoningsih, 2011 (24) | Chronic energy deficiency pregnancy |
| Ayu & Bagus, 2009 (25) | Complications in early pregnancy (hyperemesis gravidarum, abortion, degeneration of trophoblast disease, ectopic pregnancy); late pregnancy complications (preterm labor, risk of active bleeding, premature rupture of membranes, fetal death in utero, late pregnancy, pre-eclampsia and eclampsia) |
| Nakimuli, et al., 2015 (26) | Premature rupture of membranes; pre-eclampsia and eclampsia with a risk of 1.84 times |
| Engmann, et al., 2012 (27) | Multiple pregnancy; preterm labor with a risk of 2.84 times |
| Abdullah, et al., 2012 (28) | Home delivery, with a risk of 4.4 times |

## Discussion

Based on the search results, then these factors can be classified as factors related to pregnancy and factors related to childbirth.

### Factors Associated with Pregnancy

Maternal age at pregnancy is one of the factors associated with neonatal mortality. High-risk pregnancies are experienced by pregnant women aged less than 20 years or more than 35 years, with various empirical evidence [3-9]. Mothers under 20 years of age are considered to have a higher risk because their reproductive organs are not ready to accept pregnancy, and are also not psychologically ready to become pregnant. Meanwhile, mothers over 35 years

of age are physically weaker, and will be heavier if they already have many children.

Mothers with short stature are at risk for various obstetric complications such as cephalopelvic dysproportion and cessation of labor, intrauterine asphyxia, and intrauterine growth retardation [10]. Maternal height 145 cm is an indicator of risk factors for mothers who have potential obstetrical problems [11]. Various empirical evidence related to the risk of posture too short has been organized in the literature search results above [10-13].

Parity is also a risk of maternal mortality [4, 7, 9, 14]. Parity indicates the number of previous pregnancies that have reached the limit of viability and have been born, regardless of the number of children. A person with multiparous status (parity> 2) is a woman who has had two or more pregnancies that ended when the fetus had reached the limit of viability [29]. The optimal period of pregnancy is the second to the third pregnancy. The first and fourth pregnancies or more are very risky for both the mother and the fetus. This is because pregnancy in this condition is often accompanied by complications, such as fetal defect, antepartum hemorrhage and postpartum hemorrhage [30].

The interval between pregnancies is proven to be one of the risk factors for maternal mortality [6, 8, 15]. Babies born with a short gestation interval (less than 24 months) have a higher risk of becoming sick or dying when compared to mothers with a pregnancy interval of 2-4 years. This is because the physiological functions of reproduction and maternal health have not yet fully returned to optimal. Birth spacing that is too fast can lead to "maternal depletion syndrome".

Lack of antenatal care (ANC) has also been shown to be a risk factor for neonatal mortality [9, 14, 16]. ANC is conducted to determine the health condition of pregnant women and the fetus so that they can achieve optimal health, both physically and mentally to face childbirth. With good supervision, it can be known early on the various complications that may arise so that they can be addressed immediately and in the end can reduce the risk of maternal and infant mortality.

A history of giving birth to a dead baby is one of the risk factors for neonatal mortality [5, 16, 17]. According to WHO [31], stillbirth is a baby born without signs of life at or after 28 weeks of gestation. The main causes of stillbirth include: complications in childbirth, overdue pregnancy, maternal infections in pregnancy (malaria, syphilis and HIV), mothers with hypertension, obesity or diabetes, stunted fetal growth and congenital abnormalities. Nearly half of stillbirths occur while the woman is in labor (intrapartum).

Kolluru & Reddy [18] stated that mothers with a history of cesarean section delivery in a previous pregnancy were included in the moderate risk category for the occurrence of neonatal mortality.

A history of hypertension in pregnancy constitutes 5-15% of pregnancy complications and is one of the three main causes of maternal mortality and morbidity, due to unclear etiology and also care in childbirth which is still handled by non-health workers and due to imperfect referral systems [11].

According to Kolluru & Reddy [18] and also according to De, et al. [19] history of abortion is one of the risk factors for neonatal mortality and they include it in the scoring system they have developed, and fall into the low risk category.

Mothers with a history of bleeding in previous pregnancies are also one of the risk factors for mothers who have potential obstetrical emergencies [11], which could impact neonatal mortality. Jain, et al. [12], Kolluru & Reddy [18], also De, et al. [19] included history of bleeding in previous pregnancies in their developed scoring system, categorizing low risk to medium risk.

Empirical studies show that anemia during pregnancy is one of the risks of nenonatal mortality [14, 20]. Anemia in pregnancy is a public health problem especially in developing countries that can lead to

complications that threaten the life of the mother and the fetus [32].

Pregnancy with tuberculosis infection can increase the risk of perinatal death. The diagnosis of tuberculosis in pregnancy may be difficult to establish, since the initial symptoms are ascribed to pregnancy, and normal weight gain in pregnancy may temporarily offset the associated weight loss. Complications due to tuberculosis include spontaneous abortion, preterm delivery, low birth weight, and increased neonatal mortality [21].

Uncontrolled asthma can have a negative effect on a pregnant woman and her fetus. Asthma exacerbations increase the risk of preterm birth, low birth weight, perinatal death, and preeclampsia [22].

Pregnancy will cause changes in the structure and function of the mother's thyroid gland, making it sometimes difficult to diagnose the disease or determine the presence of thyroid abnormalities. A normal pregnancy will present a clinical condition similar to that of excess thyroxine, so that mild thyrotoxytosis may be difficult to diagnose [11]. Excess thyroxine can lead to spontaneous abortion and, if untreated, increases the risk of preeclampsia, heart failure and poor perinatal state [33].

Studies have shown that pregnancies with hypertension are at risk of neonatal mortality [5, 11, 23]. Hypertension in pregnancy is 5-15% complicating pregnancy and is one of the three highest maternal moratalities and morbidities [11].

Fetal growth and development is strongly influenced by maternal nutritional intake. Various complications can occur if the mother is malnourished, including bleeding, abortion, stillbirth, LBW, congenital abnormalities, mental retardation and so on, which can lead to neonatal death [24].

Ayu & Bagus [25] stated that some complications during early pregnancy are also risk factors for neonatal mortality, including hyperemesis gravidarum, abortion, degenation of trophoblast disease, and ectopic pregnancy. Meanwhile, complications during late pregnancy include prematurity [5, 7, 16, 17, 27], active bleeding [11, 23], premature rupture of membranes [11, 26], fetal death in utero, pregnancy with pre-eclampsia. and eclampsia [11, 26], as well as late pregnancy. In addition, meconeal amniotic fluid has also been reported as a risk factor for neonatal mortality [7]. Normally the color of the amniotic fluid is clear. Meconeal amniotic fluid has long been known to be one of the causes of respiratory distress in newborns, namely Meconium Aspiration Syndrome (MAS). Fetal defects are also recognized as a risk factor for fetal death due to prolonged labor or obstructed labor [5, 11, 23]. The complication of multiple pregnancy is LBW or IUGR. If labor in multiple pregnancies occurs in premature pregnancy, there is a risk of neonatal and perinatal death. Multiple pregnancy is also a risk factor for infant mortality [27]. Prolonged labor is also a risk factor for infant mortality as bacteria in the amniotic fluid penetrates the amniotic membrane and invades the decidua and chorionic vessels, causing bacteremia in the mother and fetus [11].

**Factors Related to Childbirth**

Factors associated with childbirth are conditions found during labor which are risk factors for neonatal death. This factor has generally been detected since the beginning of labor but can be found after delivery of complications.

Place of delivery is one of the risk factors for neonatal mortality. Mothers who give birth at home have a much greater risk of experiencing neonatal death [28].

The mode of delivery is also a risk factor for neonatal mortality. Sun, et al. [7] reported that delivery by cesarean section was associated with the occurrence of neonatal mortality.

In addition to the place and mode of delivery, birth attendants are also associated with the occurrence of neonatal mortality. In fact, in the field there are

still birth attendants who are not health workers. Prabamurti et al. [3] reported that deliveries assisted by traditional birth attendants have a much greater risk of experiencing neonatal death compared to deliveries assisted by health personnel.

## Conclusion

Based on the results of the study, it is concluded that there are 2 groups of risk factors for neonatal mortality, namely factors related to pregnancy including: maternal age, maternal height, parity, interval between pregnancies, antenatal care, history of previous pregnancy and childbirth (giving birth to a dead baby, cesarean section, hypertension, abortion, bleeding), problems during pregnancy (anemia, tuberculosis, asthma, hyperthyroidism, hypertension, malnutrition) and pregnancy complications (hyperemesis gravidarum, abortion, degenation of trophoblast disease, ectopic pregnancy, prematurity, antepartum hemorrhage, premature rupture of membranes, death fetus in utero, pre-eclampsia / eclampsia, post date, meconeal membranes, fetal position abnormalities, multiple pregnancies and prolonged labor); and factors related to childbirth, namely place, mode and assistant of delivery.

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# Malaria Infection Effect to Haemoglobin and Haematocrit in Pregnant *Mus Musculus*

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## Abstract

Malaria is a disease that is caused by Plasmodium Sp. And infected by Anopheles mosquitos. Kalimantan is one of endemic places for malaria. Prevalence of malaria is 2-76% depent to the places.Effect of malaria to pregnant women can be anemia, renal failure, cerebral oedema and death. Prevamence of anemia because of malaria in pregnant women is about 3-15%. This research was done by using animal model to show malaria in pregnant. Goal of this research was to analyze malaria infection effect to haemoglobin and haematocrit in pregnant Mus musculus. Research design was experimental using posttest only with control group design. It consisted of K0 for control (20 samples) and K1 for infected group (19 samples).Pregnant Mus musculus was injected P. berghei to infect plasmodium using 0,2 ml infected serum intraperioteal in the first day of pregnant. Mus musculus was terminated in 19$^{th}$ day of pregnant and took cardial blood to examine Hb and Ht of pregnant Mus musculus.The result was Hb level mean for K0= 12,69 gr/dl and K1=12,832 dr/dl. Mean of Ht for K0=38,070% and K1=38,495%. Statistical anayzes used U-Mann Whitney test, with p value for Hb level was 0,574 and Ht was 0,574. The conclusion was malaria infection could not effect yet to Hb dan Ht level in pregnant Mus musculus.

***Keywords:*** *Hb, Ht, malaria, pregnant.*

## Introduction

Malaria is caused by Plasmodium Sp. distribution of this disease is in tropical and subtropical countries. Kalimantan is one of malaria endemic region.[1,2,3]

Malaria can infect all of group of human, and pregnant women are high risk group to be infected. Malaria in pregnant is various in many places, between 2-76%.[4]Duka's research (2015) stated that 7 pregnant women suffered from malaria among 125 malaria patients.[5]

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Malaria infection in pregnant women can increase morbidity and mortality of mother and foetus. Effects of malaria to mother's health are anemia, renal failure, lung oedema, cerebral malaria and death. Effects of malaria to foetus are abortion, premature delivery, low birthweight, and infant death. Anemia in pregnant women because of malaria was about 3-15%, low birthweight was about 13-70%, and neonates death was about.[6]

Pregnant can cause hormonal changes. Progesterone increases in pregnant. Progesterone inhibits T-lymphocyte activation in fighting antigen stimulation. That immunity suppression in pregnant can cause malaria paracite enter to body. Malaria infection will destroy erythrocyte.[5]Damaged erythrocyte will make low Hb level an become

anemia. Low concentration of Hb level will influence Ht.

Until now, effect of malaria infection to mother and foetus' health is still in research. This research is a basic research that can be used to advanced research in prevention and treatment of malaria in pregnant women. So, this is a basic consideration to decrease morbidity and mortality mother and foetus. Malaria infection was given to Mus musculus and then effect of malaria was observed in haemoglobin (Hb) and haematocrite (Ht). This research's goal was to analyze effect of malaria to mother's Hb and Ht level.

## Method

This research used true experimental with posttest only with a control group design. Research groups consisted of K0 (control group) and K1 (infected group). Subjects of research were 20 femaleMus musculuscontrol group and 19 in infected group. Location of research was in biochemistry laboratory, Faculty of Medicine, Lambung Mangkurat University.

Independent variable was infection of malaria in pregnant. Dependent variables were Hb and Ht level. Health Mus musculus was healthty, 12 weeks, 200-250 mg, active, no wound, clear eyes.

Mus musculus was mated by injecting 5 IU PMSG intraperitoneal, next 48 hours was injected by 5 IU HCG intraperitoneal an then was mated by male ones. Next 17 hours they will be separated and called as day 0 of pregnant.Malaria infection was given by injecting $10^7$ Plasmodium berghei in 0,2 ml of blood in K1. Induction was on day 1 of pregnant. Mus musculus was terminated on day 19 of pregnant, and then blood from heart was taken to check Hb and Ht.

## Result And Discussion

This research was done on 20 Mus musculus of control and 19 Mus musculus of infected group. The results of Hb and Ht level is this table:

**Table 1. Hb and Ht level in Mus musculus pregnant**

| No | Variables | Group | Mean ± Sd | P Value |
|---|---|---|---|---|
| 1 | Hb level | K0 | 12.690 ± 1,3860 | 0,574 |
| | | K1 | 12,832 ± 1,8986 | |
| 2 | Ht level | K0 | 38,070 ± 4,1579 | 0,574 |
| | | K1 | 38,495 ± 5,6957 | |

*T*able 1 showes about Hb and Ht level. Mean of Hb level in K1 is higher than K0. Mean of Ht level in K1 is higher also than K0. Normality test was done and the results was not in normal distribution for K1, so the analyzes test used U-Mann Whitney Test with 95% significant level.

Both of variables showed > 0,05 p value. It meant there was no significanct different between K0 and K1. So, there was no effect of malaria infection to Hb and Ht level of pregnant Mus musculus.

Pregnant condition has weak immunity, and it makes malaria will infect easily. Malaria infection causes oxidative stress and inflammation, and then

it can damage erythrocyte. In this research, malaria infection could not effect Hb and Ht level statistically. Possibility, it is caused by mechanism of body to again infection. Good mechanism will produce enough anti inflammation and anti oxidant to again malaria infection. This condition can cause no significant effect to erythrocyte.

Some researches stated that antioxidant can decrease parasitemia and recover cells in spleen. Antioxidant also can manage inflammation in malaria infection.[7]This fact as a basic mechanism why there was no significant differences of data in this research.

Plasmodium that causes malaria infects erythrocyte. The effect is osmotic auto-hemolysis. Erythrocyte's duration of life become shorter and makes anemia.[8] Malaria infection makes body produces inflammation cytokine. High level of inflammation induces oxidative stress. This process damages erythrocyte's membrane and then it become rupture and induces anemia.[7]Low of erythrocyte number can induce its production. If production of erythrocyte is too much, it can increase haematocrit.[9]

## Conclusion and Acknowledgment

The conclusion of this research is malaria infection could not affect Hb and Ht level in pregnant Mus musculus.We would to say thank you for Faculty of Medicine, Lambung Mangkurat University, as the source of this research's fund.

### Declaration of Conflicting Interest

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

**Ethical Clearance:** This research has been declared ethically worthy by the ethics committee of the medical faculty of the Lambung Mangkurat University

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# Diagnostic Value of Mid Regional Proadrenomedullin as a Sepsis Biomarker in Pediatric Patients with Cancer-Related Chemotherapy

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## Abstract

The aim of this study was to analyze the diagnostic value of mid regional proadrenomedullin as a sepsis biomarker in pediatric patients with cancer-related chemotherapy. This cross-sectional observational study was located in General Hospital Dr. Soetomo Surabaya from September until December 2020. International Pediatric Sepsis Consensus Conference criteria was used to define sepsis term on 2 study groups. Serum mid regional proadrenomedullin has been drawn from 60 subjects (41 in-ward pediatric patients) and measured on sandwich enzyme-linked immunosorbent assay from Elabscience® Human Mid Regional Proadrenomedullin. Humareader Single Plus® was used to measure the optical density. ROC analysis was used to find out cut off value, sensitivity, specificity, positive predictive value, negative predictive value, negative likelihood ratio, positive likelihood ratio, and accuracy, respectively. Statistics declared significant if $p<0.05$ (95% CI). Between groups, temperature and heart rate were statistically different ($p<0.001$; 95% CI). The median difference of mid regional proadrenomedullin between groups was significant ($p<0.05$; 95% CI). Cut off value 2,88 nmol/L, sensitivity 60.0%, specificity 56.67%, positive predictive value 58.06%, negative predictive value 58.62%, positive likelihood ratio 1.38, negative likelihood ratio 0.71 and diagnostic accuracy 59.33% was obtained while area under curve was 0.707, respectively. Sepsis in children with cancer –related chemotherapy has not well diagnosed by serum mid regional proadrenomedullin.

***Keywords:*** *Cancer, chemotherapy, diagnostic, mid regional proadrenomedullin, pediatric, sepsis*

## Introduction

Sepsis is a life-threatening organ dysfunction which caused by dysregulated body response upon an infection[1]. There are many causes of sepsis, which one is immunity cells decrement of quality either quantity. One of condition causing the decrement is cancer or malignancy. Pediatric patients on cancer-related chemotherapy have often been suffered from sepsis[2,3]. Blood culture, as a gold standard of sepsis diagnosis, has long turn-around time and low sensitivity[4]. Therefore, many criteria and diagnosis guidelines has been established for diagnosis of sepsis, which included clinical sign, symptoms, with addition of several laboratory parameters[5]. However, many biomarkers have been used by clinician to diagnose sepsis in their daily practice, e.g., C-reactive protein, procalcitonin and presepsin[6]. Their results were useful to decide giving empirical antibiotics[7].

Adrenomedullin is known as a vasoactive peptide that firstly found in pheochromocytoma

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tissue[8]. Previous research showed this molecule had several roles in sepsis pathophysiology. As a prognostic biomarker, it has shown high concordance with SOFA, pediatric SOFA and PELOD score[9,10]. Unfortunately, this peptide has short half-life, which only 22 minutes in circulation[8]. Therefore, researchers measured its equivalent molecule, mid regional proadrenomedullin, which both are originated from the same precursor, proadrenomedullin[11]. Mid regional proadrenomedullin had varied diagnostic value as a sepsis biomarker in childhood population. Angeletti *et al*. and Debiane *et al*. showed this peptide had remarkable diagnostic value as a sepsis biomarker on adult, otherwise Agnello *et al*., proved an unsignificant result[9,12,13]. Kesik *et al*. and Al Shuaibi *et al*. has proven the peptide showed high value as a pediatric sepsis biomarker of leukemia-related chemotherapy[14,15]. As we knew, there were no published study about mid regional proadrenomedullin as a sepsis biomarker in childhood with chemotherapy. The aim of this study was finding the diagnostic value of mid regional proadrenomedullin as sepsis biomarker in children with cancer-related chemotherapy.

## Materials and Methods

This cross-sectional observational study enrolled 60 subjects from 41 patients into 2 groups: subjects with sign of sepsis as group 1, and subjects without sign of sepsis as group 2. We determined sepsis and non-sepsis according to IPSCC (International Pediatric Sepsis Consensus Conference) criteria[16]. All of the subjects were patients of the Pediatric Department of Soetomo General Hospital Surabaya, recruited from September 21 until December 30, 2020. The research population was 1 to 18-year-old children who have diagnosed with malignancy or cancer, who had informed consent been signed by their parents. The inclusion criteria for both groups were patients who had been diagnosed with cancer by hematology-oncology pediatrician based on bone marrow evaluation, radiology, or tissue biopsy. In addition, inclusion criteria for group 1 where subjects had at least 2 points on IPSCC criteria for SIRS, related to local infection they had suffered from. Subjects were willing to have their blood drawn for mid regional proadrenomedullin measurement and blood culture procedure. Exclusion criteria for both groups were impairment of renal function, diagnosed as pheochromocytoma, subjects transferred to the intensive care unit, and samples were hemolysis, lipemic or icteric. Subjects excluded if they declined or decided to drop out. Mid regional proadrenomedullin was measured at Immunology and Development Laboratory, Department of Clinical Pathology and Laboratory Medicine of Dr. Soetomo General Hospital Surabaya. A sandwich ELISA platform of human mid regional proadrenomedullin reagent kit from Elabscience® and ELISA reader Humareader® Single Plus were used. SPSS ver.17 was used to calculate sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio and diagnostic accuracy.

## Results and Discussion

Moreover, this study enrolled patients which were distributed into 83 subjects from both groups. Because of hemolysis, renal impairment, pheochromocytoma, and later intensive care unit admission, there were remaining 60 subjects from 41 eligible patients, which were 30 samples in each group.

**Table 1. General Characteristics.**

| Characteristics | Group | | p value |
|---|---|---|---|
| | Group 1 | Group 2 | |
| Sex | | | 0.605 |
| Male | 14 (46.7%) | 17 (56.7%) | |

***Cont...*** **Table 1. General Characteristics.**

| | | | |
|---|---|---|---|
| Female | 16 (53.3%) | 13 (43.3%) | |
| Diagnosis | | | 0.065 |
| ALL | 15 (50.0%) | 22 (73.33%) | |
| AML | 3 (10.0%) | 0(0%) | |
| Lymphoma | 8 (26.67%) | 7 (23.33%) | |
| Neuroblastoma | 1 (3.3%) | 0 (0%) | |
| Retinoblastoma | 3 (10%) | 1 (3.3%) | |
| Age (year) | 7 (1-15) | 8 (1-16) | 0.824 |
| Axillar temperature (°C) | 38.3 (38-39.1) | 36.75 (36.2-37.4) | <0.001 |
| Respiratory rate (per minute) | 24.0 (18-28) | 22 (18-28) | 0.081 |
| Heart rate (per minute) | 100 (88-124) | 92 (80-112) | <0.001 |
| Leukocyte (per mm3) | 3544.67-1365.61 | 4091.77±1029.74 | 0.085* |
| Immature granulocyte (%) | 4 (3.0-8.0) | 4 (3.0-6.0) | 0.092 |
| Neutrophil (per cmm) | 667.83±266.08 | 776.03±179.57 | 0.102* |
| Body weight (kg) | 22.83±10.07 | 24.63±8.13 | 0.449* |
| Body height (cm) | 119.5 (18-156) | 126 (75-160) | 0.790 |
| Sistolic pressure (mmHg) | 90 (80-120) | 100 (80-110) | 0.070 |
| Diastolic pressure (mmHg) | 60 (50-80) | 60 (50-70) | 0.671 |

Note: data in table showed in median (min-max)

*data showed in mean±standard deviation.

In group 1, there were 7 subjects (23.33%) had suspect respiratory tract infection, 7 samples (23.33%) with gastrointestinal tract infection, 6 samples (20.00%) had urinary tract infection, 5 samples (16.67%) with skin infection, 3 samples (10.00%) had oral infection, and 2 samples (6.67%) had eye infection. There were 9 subjects (30%) had positive blood culture. Bacteria identification results were *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella spp, Staphylococcus epidermidis, Staphylococcus haemolyticus* dan *Staphylococcus hominis*. There was no bacterial growth in Group 2.

The positivity of blood culture was 30%.

**Table 2. Median Difference of Mid Regional Proadrenomedullin Level Between Groups.**

| Group | N | Median (min-max) | Unit | p Value |
|---|---|---|---|---|
| Group 1 | 0 | 0.194 (1.63-11.34) | nmol/L | 0.006 |
| Group 2 | 0 | 2.51 (1.01-5.09) | nmol/L | |

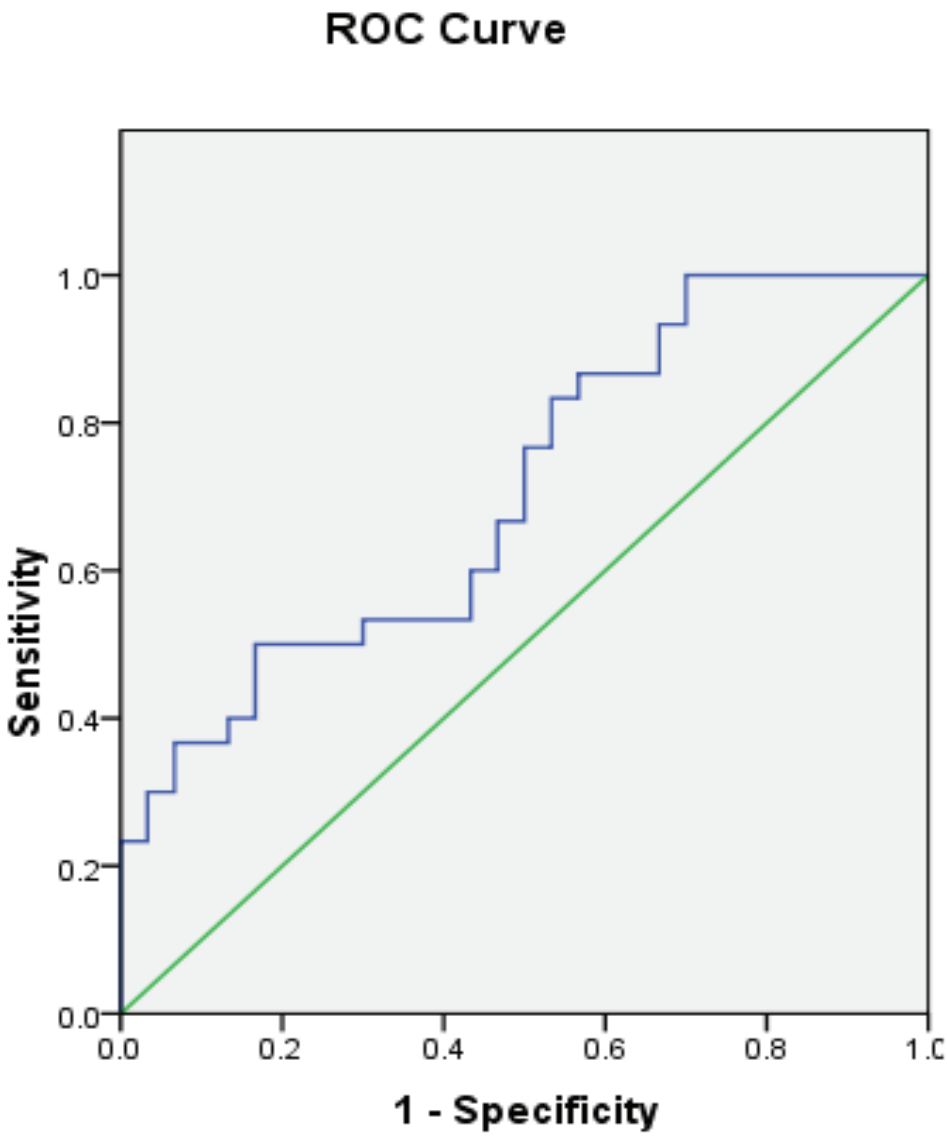


**Figure 1. Receiver operating characteristics curve of this study.**

ROC analysis showed area under curve 0.707 which p=0.006 (95% CI). It resulted some diagnostic parameters, they were cut off value, diagnostic sensitivity, diagnostic specificity, positive predictive predictive value, negative predictive value, positive likelihood ratio, negative likelihood ratio, and diagnostic accuracy. Cut off value of present study was 2.88 nmol/L. This result may tally with Kesik *et al*. which had cut off value 2.90 nmol/L from their study[14]. Diagnostic sensitivity of this study was 60.00% (40.60-77.34%; 95%CI) and diagnostic specificity was 56.67% (37.43-74.54%; 95% CI). This result was similar with study by Ribalta *et al*., which results were 66.7% and 52.94%[17]. This study has PPV and NPV 58.06% (45.58-69.60%; 95% CI) and 58.62% (45.26-70.82%; 95% CI).

This study had LR+ 1.38 (0.84-2.29; 95% CI), that agreed with Ribalta *et al*. (2020) and 0.71 (0.41-1.21; 95% CI)[17]. The LR- of this study was 0.71 (0.41-1.21; 95% CI). Diagnostic accuracy of this study was 58.33% (44.88-70.93%; 95% CI). This result was similar to the study by Al-Shuaibi *et al*.[15]

## Conclusion

In summary, this study has been conducted to analyze the diagnostic value of mid regional proadrenomedullin as a sepsis biomarker in pediatric patients with cancer-related chemotherapy. Although the AUC showed fair level at ROC, it is weak on sensitivity, specificity, PPV, NPV, LR+, LR- and diagnostic accuracy. This lacking value shows that sepsis in children with cancer-related chemotherapy is not well-diagnosed by serum mid regional proadrenomedullin level.

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

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**Ethical Approval**: This study was approved by the Health Research Ethics Committee of Dr. Soetomo Regional General Hospital, Surabaya, Indonesia (approval number: 0067/KEPK/IX/2020).

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# The Effect of Adiponectin Recombinant in Rattus Norvegicus with Polycystic Ovarium Syndrome Model on Anti-Müellerian Hormone Expression

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## Abstract

**Background:** Polycystic Ovary Syndrome (PCOS) is an important disease that causes various health problems in women. Ovulation - infertility disorders are found in 60-85% woman with PCOS. Anti-Muellerian Hormone (AMH), a protein from Transforming Growth-b Family, often increased in patient with PCOS and associated with severity and poor prognosis for assisted reproductive outcome. Dietary and lifestyle changes are the first-line therapies but consistent application of this method is difficult to attain for people with PCOS. Adiponectin, a product by adipose cells have a significant reduction in obese and women with PCOS. The purpose of this study was to see the differences in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant and mice with polycystic ovary model treated with placebo and control.

**Method:** This research is an experimental study with a Post Test Only Control Group design. The sample of this study was 3 months female Rattus norvegicus Wistar strain weighing 110-120 g. The sample size was 36, which were divided into three groups, K0 (control group of mice with polycystic ovary model), K1 (group of mice with polycystic ovary model that received placebo injection), and K2 (group of mice with polycystic ovary model that received 5 µg/ml injection of recombinant adiponectin). Polycystic ovary model made by injecting 10 mg/Kg body weight testosterone propionate for 14 days. The injection is carried out at the proestrous stage. The three groups of mice in a period of 3 weeks after treatment, were surgically removed according to the sampling procedure, then the ovaries were given a code and immunohistochemical staining was performed to see the AMH expression in the ovaries. The data were collected, then statistical analysis was carried out using SPSS software.

**Result:** The results showed that the AMH expression value in the K0 group was 3.3 (± 0.86), K1 3.1 (± 0.77) and K2 3.4 (± 0.81). The minimum score of the AMH expression for the K0 group is 2.2; with a maximum score is 5.1. For K1 group, the minimum score is 2,3 with a maximum score is 4.6. And for the K2 group, the minimum score of AMH expression is 2.2 and the maximum value is 4.6. From the results obtained, the homogeneity test was carried out with the Saphiro-Wilk test and showed

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that the data were normally distributed (control group p = 0.364; placebo group p = 0.09; adiponectin group p = 0.461). We then performed one-way ANOVA parametric test and found that the results of AMH expression in each group did not have a significant difference with a value of p = 0.651 (p> 0.05).

**Conclusion:** From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group. Further research is needed to study the effect of adiponectin in pathogenesis of PCOS.

***Keywords*** *: Polycystic Ovarian Syndrome, Anti-Müellerian Hormone, Adiponectin.*

## Introduction

Polycystic Ovary Syndrome (PCOS) is an important disease that causes various health problems in women. Bozdak (2016), through a global study, found that PCOS occurs in 6-10% of the female population[1]. Obesity is found in 75% of cases of PCOS, and insulin resistance is more often found in cases of PCOS with obesity (70-80%) than those without obesity (25%). In PCOS, ovulation - infertility disorders are found in 60-85%[2]. Anti-Muellerian Hormone (AMH), a protein from Transforming Growth-b Family, often increased in patient with PCOS and associated with severity and poor prognosis for assisted reproductive outcome[3,4,5,6]. Diet and lifestyle changes are the first-line modalities therapies, unfortunately this method is difficult to attain for people with PCOS. Adiponectin, a product by adipose cells, is a homotrimer protein. The synthesis and secretion of these proteins mainly expressed during adipogenesis and associated with differentiation and decreased levels of lipids. A significant reduction in adiponectin secretion was found in obese and women with PCOS[5,6,7,8,9]. The purpose of this study was to see the differences in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant and mice with polycystic ovary model treated with placebo and control.

## Method

This research is an experimental study with a Post Test Only Control Group design. The sample of this study was 3 months female Rattus norvegicus Wistar strain weighing 110-120 g, with the exclusion criteria that had been used previously as experimental animals in other studies. The sample size was 36, which were divided into three groups, K0 (control group of mice with polycystic ovary model), K1 (group of mice with polycystic ovary model that received placebo injection), and K2 (group of mice with polycystic ovary model that received 5 µg/ml injection of recombinant adiponectin). Polycystic ovary model based on previous study was made by injecting the testosterone propionate with dose of 10 mg / KgBW for 14 days. The injection is carried out at the proestrous stage. The three groups of mice in a period of 3 weeks after treatment, were surgically removed according to the sampling procedure, then the ovaries were given a code and immunohistochemical staining was performed to see the AMH expression in the ovaries. The data were collected, then statistical analysis was carried out using SPSS software. Ethical eligibility was obtained from the Animal Care and Use Committee (ACUC), Faculty of Medicine, Airlangga University, Surabaya.

## Result

The results showed that the AMH expression value in the K0 group was 3.3 (± 0.86), K1 3.1 (± 0.77) and K2 3.4 (± 0.81). The minimum score of the AMH expression for the K0 group is

2.2; with a maximum score is 5.1. For K1 group, the minimum score is 2,3 with a maximum score is

4.6. And for the K2 group, the minimum score of AMH expression is 2.2 and the maximum value is 4.6 (table 1 and figure 1). From the results obtained, the homogeneity test was carried out with the Saphiro-Wilk test and showed that the data were normally distributed

(control group p = 0.364; placebo group p = 0.09; adiponectin group p = 0.461). We then performed one-way ANOVA parametric test and found that the results of AMH expression in each group did not have a significant difference with a value of p = 0.651 (p> 0.05). The picture of AMH staining can be seen in figure 1 below.

**Table 1. Descriptive value for AMH expression in each group**

| Group | AMH Score | Minimum | Maximum | 95 % Confidence Interval | | p |
|---|---|---|---|---|---|---|
| | | | | Lower limit | Upper limit | |
| K0 | 3,3 ± 0,86 | 2,2 | 5,1 | 2,75 | 3,85 | 0,364 |
| K1 | 3,1 ± 0,77 | 2,3 | 4,6 | 2,63 | 3,60 | 0,09 |
| K2 | 3,4 ± 0,81 | 2,2 | 4,6 | 2,91 | 3,55 | 0,46 |

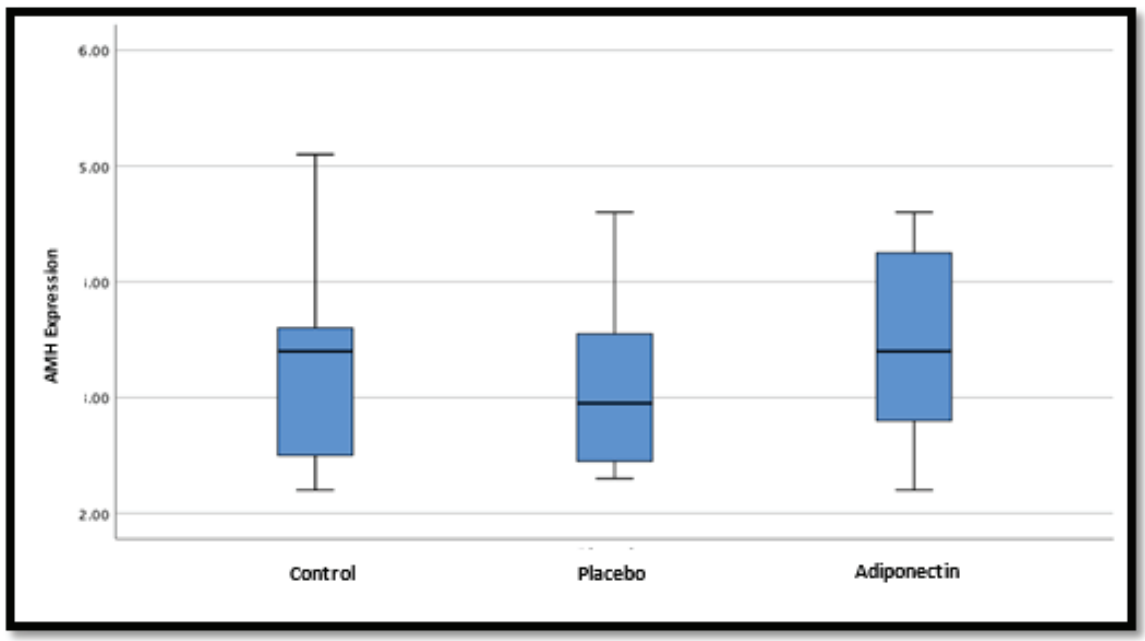


**Figure 1. AMH expression score data in each group.**

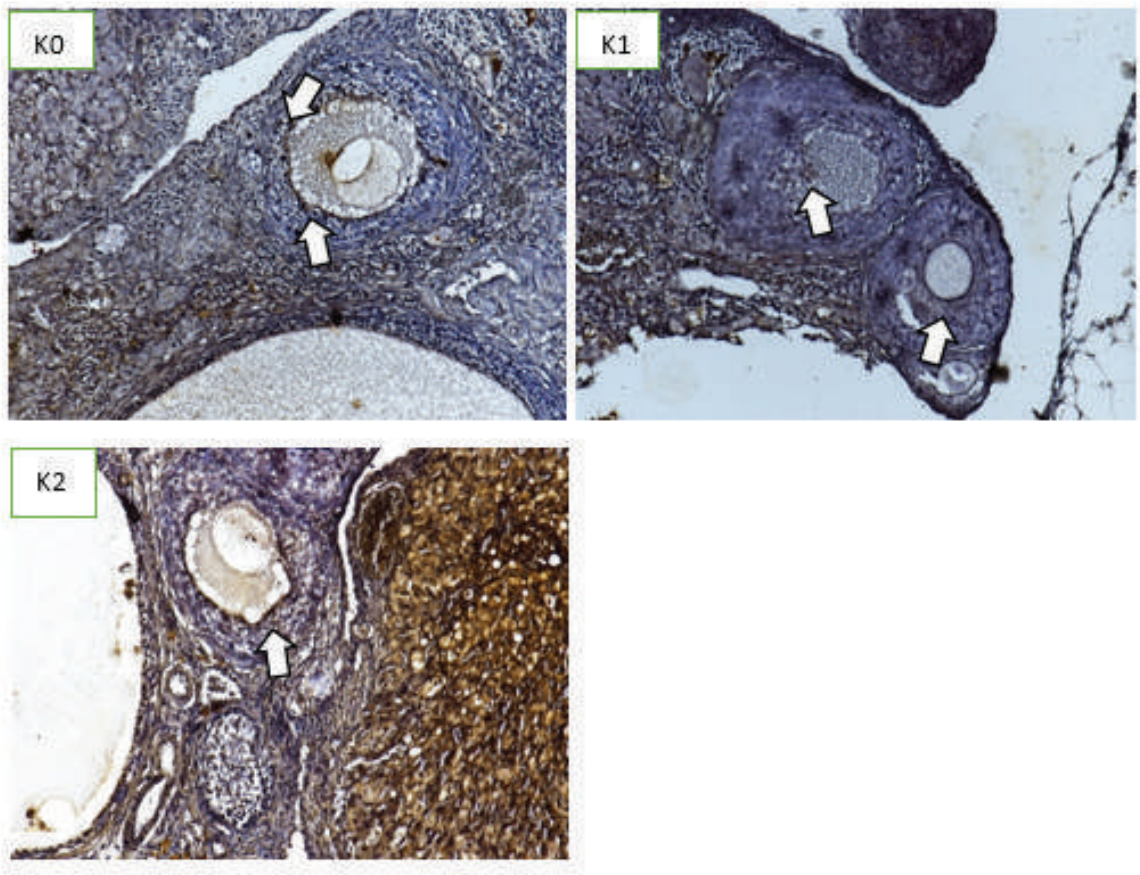


**Figure 2. Immunohistochemical staining pattern for AMH in each group. AMH expression on each group showed with brown staining on granulosa cell (white arrow).**

## Discussion

The results of our study showed that there was no significant difference in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant versus mice

with polycystic ovary control and placebo group. These results indicate that the administration of adiponectin in the polycystic ovary model, does not directly act on AMH expression. One of the possible mechanisms in explaining this phenomenon is that the change in PCOS, especially AMH in relation to folliculogenesis is complex with various hormonal and metabolic

mechanisms involved in it. The possible mechanisms involved are obesity and insulin resistance which play a major role in the pathogenesis of PCOS. Insulin resistance and increased levels of free fatty acids in obese patients with PCOS are one of the mechanisms that

cause this disease. The mechanism of obesity's effect on reproductive disorders is complex, but hyperinsulinemia with/without insulin resistance is thought to play a major role. Adiponectin can improve insulin resistance and cause a decrease in fatty acids in obesity[9,10,11,12,13]. Administration of adiponectin in previous studies will increase tissue sensitivity to insulin, reduce levels of free fatty acids, reduce LH secretion by the pituitary, reduce local production of androstenedione by theca cells and decrease the expression of LH receptors on theca cells all of which will result in decreased intrafollicular androgen production[13,14]. This decrease in androgen production will improve follicle growth thus decreased AMH

production. One of the drawbacks in our study is that in our experimental animals, obesity and insulin resistance were not found as metabolic factors for PCOS, so we could not determine the relationship between adiponectin and these factors on AMH expression. Administration of testosterone propionate for 14 days will result in polycystic ovary conditions. Research conducted by Muttaqin in 2009 shows that insulin resistance in mice is obtained after 28 days of testosterone propionate administration[16]. Based on our study, solitary hyperandrogenism condition without any underlying metabolic conditions e.g. insulin resistance or obesity did not produce significant changes in AMH expression. Another possible cause for the absence of differences in AMH expression in our study is the AMH polymorphism. Kaveenar (2007) showed that polymorphisms of genes responsible for AMH and AMH receptors coding affect AMH expression patterns in a person, especially those with PCOS[17]. It is currently unknown whether it is also found in mice, as from our study this polycystic ovarium model cannot induced this polymorphism, hence it is possible that no difference in AMH expression in the ovaries of the polycystic mouse model after administration of recombinant adiponectin in our study due to this factor. From the results of this study, we suspect that adiponectin does not result in changes in AMH expression via the hyperandrogenic pathway but possibly through the interaction between adiponectin and other factors, especially insulin resistance and obesity. From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group.

## Conclusion

From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group. Further research is needed to study see the effect of adiponectin pathogenesis of PCOS.

**Acknowledgement:** The authors would like to thank Universitas Airlangga, Surabaya, Indonesia for supporting this research.

**Ethical Clearance:** This study was approved by *Animal Care and Use Committee* (ACUC) Veterinary Medicine Universitas Airlangga Surabaya.

**Source of Funding:** Self.

**Conflict of Interest:** -.

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# Demographic Profile of Deaths Due to Drowning in and Around Vijayawada, Andhra Pradesh

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## Abstract

Drowning is a leading and preventable cause of death that has suffered an attention deficit. The World Health Organization identifies data collection as a key strategy underpinning effective interventions. The circumstances leading to drowning are complex. People interact with water primarily for recreation and often interact with water due to daily life or occupational endeavours. A Significant reduction the number of drowning deaths among young children in private swimming pools has been achieved through over 30 years of focused work on the epidemiology and risk factors for drowning in private swimming pools among young children. Proposed contributory factors for drowning in rivers include a lack of barriers controlling access to water, an absence of adult supervision for young children, poor swimming skills, minimal awareness of the dangers, the consumption of alcohol, transportation on water, a lack of safe water supply, and disasters related to flooding.

***Keywords:*** *Awareness, circumstances, collection, complex, drowning, data, proposed, reduction*

## Introduction

Drowning is a leading and preventable cause of death that has suffered an attention deficit. The World Health Organization identifies data collection as a key strategy underpinning effective interventions. It is among the top 10 leading causes of death worldwide, with the drowning death rates at least three times higher in the developing countries than the developed countries.[1 2] Despite the significant burden, drowning deaths continue to remain an invisible public health issue in most developing countries.[1,3] In continuing to bring action on drowning, this year, the WHO has released an implementation guide that provides practical steps for preventive measures to address the burden of drowning.[4] According to the World Health Organization (WHO), 0.7% of all deaths worldwide — or more than 500,000 deaths each year[5] — are due to unintentional drowning.[6]

The circumstances leading to drowning are complex. People interact with water primarily for recreation and often interact with water due to daily life or occupational endeavours. Prevention strategies, must therefore differ to suit the environment and the unique causal factors contributing to drowning risk in different contexts. Drowning occurs when an individual is unable to remain float in water. Complete submersion is not necessary. Submersion of nose and

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mouth alone for a sufficient period can cause death from drowning. Pathophysiological proof is often difficult or is even impossible to obtain. Drowning is the process of respiratory impairment from submersion or immersion in liquid and is considered a major global health problem [7] .

Drowning is a global public health issue, with the WHO estimating 372 000 people die from drowning annually. The true burden of drowning is likely to be higher due to under-reporting, as victims are not hospitalised or cases are not recorded because of a lack of death collection tools in many low and middle-income countries. Some data on drowning are also excluded because of reporting methods such as the use of International Classification of Diseases (ICD)coding frameworks that mean drowning may be classified elsewhere or excluded if it is related to transportation or disaster.1Drowning prevention interventions based on site-specific locations are likely to have a greater impact and prove more successful than general strategies aimed at preventing drowning. A Significant reduction the number of drowning deaths among young children in private swimming pools has been achieved through over 30 years of focused work on the epidemiology and risk factors for drowning in private swimming pools among young children.

Countermeasures intended to prevent or reduce drowning are most effective when evidence based. Research which delineates the nature and extent of the problem and identifies causal factors to be addressed via intervention is therefore an a priori step in countermeasure development [8.] For drowning, the type of body of water (e.g. swimming pool, river, ocean etc.) is an important consideration which informs prevention efforts as the populations and circumstances of different water sites vary. [9] In most countries, unintentional drowning occurs more frequently at natural water sites, compared to pools or bathtubs .[10]

Proposed contributory factors for drowning in rivers include a lack of barriers controlling access to water, an absence of adult supervision for young children, poor swimming skills, minimal awareness of the dangers, the consumption of alcohol, transportation on water, a lack of safe water supply, and disasters related to flooding.[1] Some proposed river prevention strategies include: community-based prevention; provision of safe places such as crèches for young children; basic swimming instruction for older children; increased public awareness of the vulnerability of children; legislation for safe boating; mitigation of flood risk; and continued research into priority areas.

## Materials and Methods

The study has been carried out in the year 2020 after approval from ethical committee of NIMRA Medical College/General Hospital. 176 cases were taken to study on epidemiological profile of the drowning deaths, excluding bodies in advanced state of decomposition. . The study was carried out over the period of April 2019 to May 2020, with the aim of studying various epidemiological parameters.. The factors taken to enumerate the study are age, sex, marital status, region, socioeconomic status and comorbidity conditions.

## Observations and Results

The cases taken were 176 and the study includes, only the dead bodies which were retrieved from water sources and having history of drowning, brought for post mortem examination. As per law of the land, consent of relatives is not required for carrying out the medicolegal post-mortem examination on the corpse of the deceased; hence it was not necessary to obtain consent from relatives or any other authority in this particular study. were present at the time of post-mortem examination, necessary information was collected regarding the personal, mental and behavioural aspect.

Table1 shows, the drowning deaths were commonly seen in age groups of 21-30 years (27.84%) followed by 31- 40 years (22.73%) and 41-50 years

(18.75%). Predominance of male was seen in all age groups in drowning death except in the age group 0-10 years age group and 50-70 year age group shows female predominance with 13.73% cases in both. Among the total cases, 71.02% victims were male and 28.98% were female. The male: female ratio was 2.45:1.

Figure 1 shows marital status, 46.59% victims were married and 32.95% were unmarried. Among the females 56.86% were married and (25.49%) were unmarried, and among the males 42.40% were married and were (36%) unmarried. And remaining cases status is not known.

In figure 2, it is observed maximum number of drowning deaths had occurred in urban region constituting 57.38% cases followed by rural region constituting 42.62% cases. In urban region, the male (59.20%) drowning deaths are more as compared to females (52.94%), whereas in rural region the females (47.06%) drowning death are more as compared to males (40.80%).

Table 2 shows, students were the most common victims in drowning deaths seen in 18.75%cases, followed by non-working victims in 17.62% cases, farmer in 16.48% cases and labourer in 11.36% cases.

Table 3, depending on the socioeconomic status, most of the victims of drowning deaths belong to upper-lower class seen in 35.23% followed by lower class in 20.45% cases and lower middle class in 14.77% cases, and 6.25% of the victims of drowning death belong to upper class of socioeconomic status.

Table4 shows, drowning deaths 21.02% of victims had familial and financial problems. The second most common history associated with drowning death was depression seen in 20.45% cases, followed by chronic alcoholism in 18.18% cases and chronic illness in 17.61% cases. Male predominance was seen in almost all of the associated history except psychiatric illness (7.38%) in which female outnumbered male in drowning deaths.

Figure 3 shows that the maximum number of drowning deaths occurred in rainy season (43.75%). This was followed by summer season (32.95%) and winter season (23.30%).

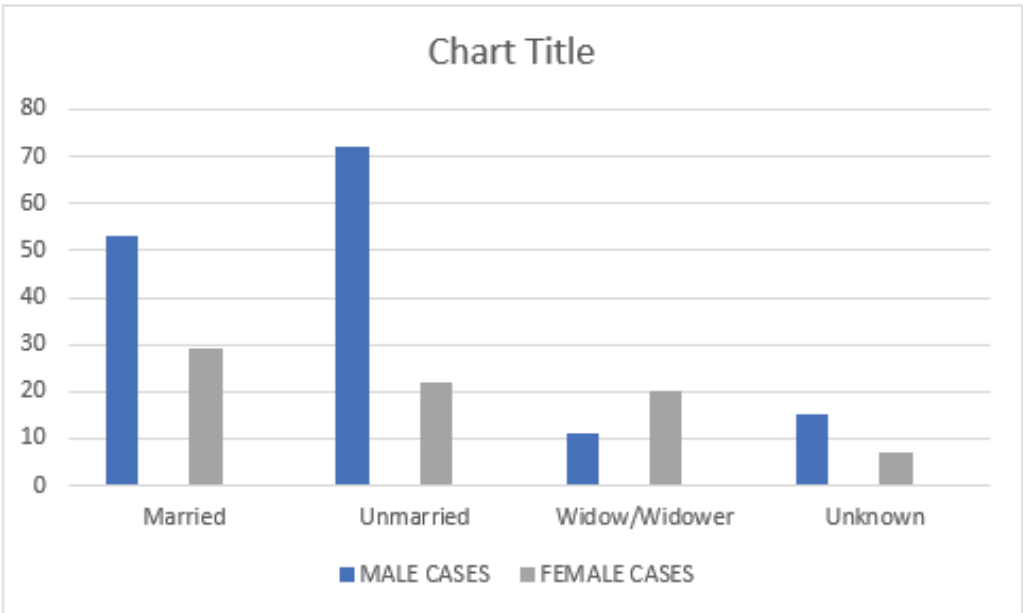


**Figure 1 Distribution of drowning deaths as per Marital Status**

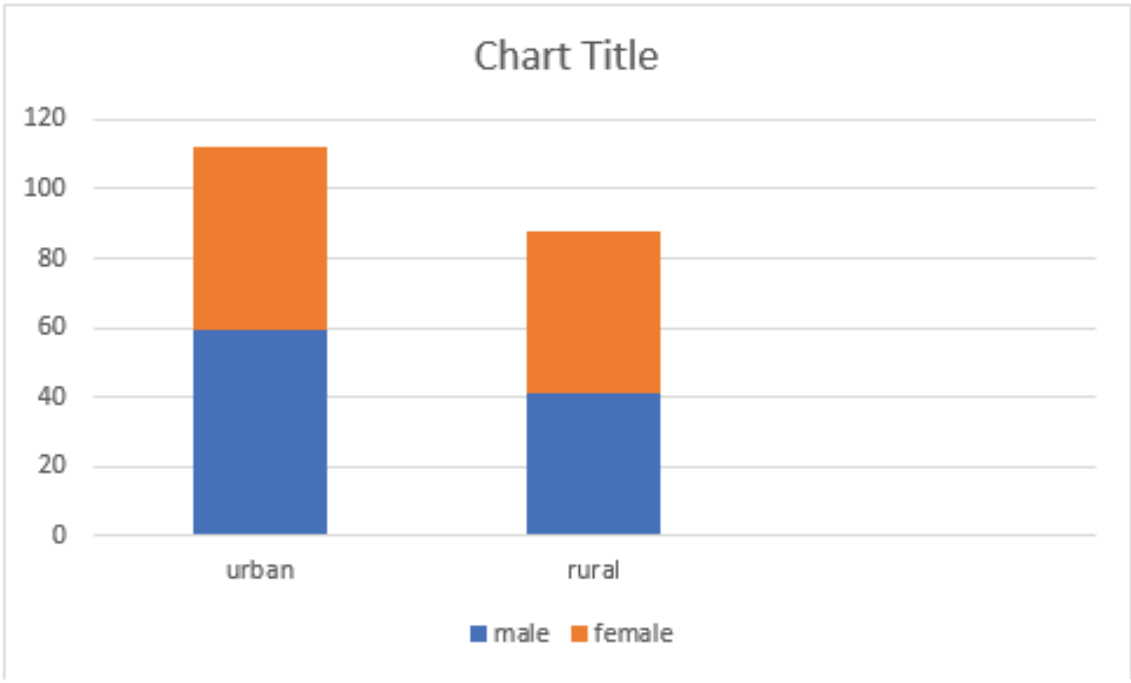


**Figure 2 Region of Occurrence**

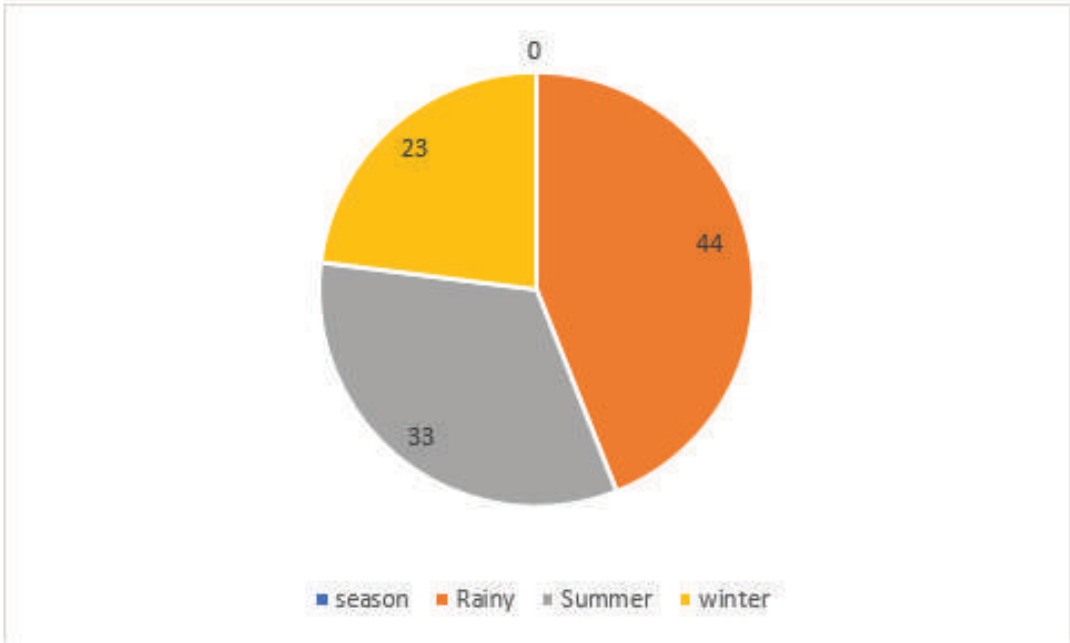


**Figure 3 Seasonal Variation of Drowning Deaths**

## Discussions

The drowning deaths were predominantly seen in male (71.02%) with male: female ratio of 2.45:1, they were commonly seen in age groups of 21-30 years (27.84%) followed by 31- 40 years and 41-50 years. Predominance of male was seen in all age groups except below 10 years and between 50 and 70 years of age. These findings are consistent with that of Auer, Quan, Suresh Kumar Shetty and Shetty, Pathak and Mangal and Saberi Anary et al.[12] The probable reason behind preponderance of 21-30 years age group in drowning is carelessness and adventurous nature usually seen in youngsters while swimming or doing recreational activities in or around water source leading to accidental deaths. This is followed by the age group of 31-40 years, it may be due to familial and financial problems arising in life and their inability to deal with them.

As per marital status, 46.59% victims were married and 32.95% were unmarried. Among the females 56.86% were married and (25.49%) were unmarried, and among the males 42.40% were married and were (36%) unmarried. And remaining cases status is not known. It may be due to over exposure of married and working people in and around water sources leading to accidental deaths. Also, they prefer to commit suicide by drowning due to inability to handle familial and financial problems. This finding is consistent with that of Gorea and Singh Ranga Rao et al.[13] who found 38% and 50% married victims respectively who died of drowning.

Maximum number of drowning deaths had occurred in urban region constituting 57.38% cases followed by rural region constituting 42.62% cases. In urban region, the male (59.20%) drowning deaths are more as compared to females (52.94%), whereas in rural region the females (47.06%) drowning death are more as compared to males (40.80%). These findings are in contrast to study carried out by Delmonte and Capelozzi and Murkey et al. This may be due to different region of study. The present study was carried out in urban region in contrast to Murkey et al [14] and Delmonte and Capelozzi which was conducted in rural region.

The most common victims of drowning deaths were the student seen in 18.75% cases. It may be due to carelessness and adventurous nature usually seen in youngsters while swimming or doing recreational activities in or around water source. This is followed by non-working victims in 17.62% cases, farmers in 16.48% cases and labourer in 11.36% cases; it is probably due to financial and familial problems,

Depending on the socioeconomic status, most of the victims of drowning deaths belong to upper-lower class seen in 35.23% followed by lower class in 20.45% cases, lower middle class in 14.77% cases upper class in 6.25% cases. In 13.07% cases, the socioeconomic status of the victims of drowning deaths was not known.

21.02% of victims of drowning death had familial and financial problems. The second most common history associated with drowning death was depression seen in 20.45% cases. This is followed by chronic alcoholism in 18.18% cases and chronic illness in 17.61% cases. Male predominance was seen in almost all the associated history except psychiatric illness (7.38%) in which female (15.68%) outnumbered male (4%) in drowning death. The present study is in accordance with Dietz and Baker[15], Auer, Fralick et al.

The maximum number of drowning deaths occurred in rainy season (43.75%). This is followed by summer season 32.95% cases and winter season 23.30% cases. This finding is consistent with Pathak and Mangal, Job , Ambade et al.[12] studies where maximum drowning deaths were found in rainy season.

## Conclusion

Drowning is a global public health threat, impacting both high income and low and middle income countries. Male predominance seen in drowning

deaths with highest incidence seen in third and fourth decades. Drowning deaths are most common in urban region followed by rural region. Students are the most common victims of drowning deaths followed by non-working, farmers and l. Understanding drowning risk factors aids in implementation of effective preventative strategies. Young children should receive swimming instruction and communities should implement daycares to ensure constant adult supervision, especially in the daytime. Additionally, small ponds and irrigation ditches should be encircled with fences or drained, and cisterns/wells covered by grates to prevent children from falling into them. Finally, programs to educate parents on the risks of drowning should be implemented and could help reduce the childhood drowning rate by 40%.[16] .These preventative strategies are especially important in rural areas where the risks of drowning are higher. The WHO in their Global Drowning Report also supports many of the recommendations included in this systematic review. Drowning literature is extensive and includes wide-ranging focus areas approached by multiple disciplines and various perspectives. This heterogeneity is both a challenge and a benefit. Standardisation in some areas would surely drive the science forward: consensus-based terminology and reporting practices would allow for richer data and improved comparisons across locations; a streamlined research agenda with identified objectives and prioritised questions to be addressed would propel prevention efforts towards the populations most in need; and established best practice for prevention program evaluation would ensure effective use of limited resources. Conversely, the disparate approach to drowning research in the past 60 years has allowed for creative investigation of essential, ground-level questions that has pushed boundaries and driven the field in new directions.

The next generation of drowning and safety science must build upon the advances of previous work by recognizing which areas have been studied thoroughly and where further attention is needed most. Coastal drowning researchers must address gaps in research from lower resourced settings and the lack of prevention strategy evaluation. The multidisciplinary nature of drowning research offers collaborative opportunities to advance science underpinning prevention efforts seeking to save lives and keep people safe.

**Ethical Clearance**: This study has been carried out in the year of 2020, from the ethical committee of NIMRA Institute of Medical Sciences and Hospital, Vijayawada, Andhra Pradesh

**Source of Funding**: Self

**Conflict of Interest** : Nil

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# The Role of K and Ca Channels in Hydrogen Sulfide Induced Relaxation in Arteries Feeding Human Colorectal Cancer

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## Abstract

This study was designed to find out the vasorelaxant effects of hydrogen sulfide ($H_2S$) on arteries feeding human colon cancer. In addition, it also included the study of the possible roles of potassium ($K^+$) and calcium ($Ca^{2+}$) channel types in $H_2S$-induced relaxation in the isolated arteries. Sodium sulfide ($Na_2S$) showed a potent dose-dependent relaxant effect on Norepinephrine ($1X10^{-5}$ M) precontracted arteries. The use of different specific $K^+$ channel blockers ($BaCl_2$, 4-AP, GLIB, and TEA) individually indicated $H_2S$-induced relaxation was affected by all K channel types participated to a various extent, except Kv channels. Both $Kca^{2+}$ and Kir channels played a major role in the induced relaxation, while $K_{ATP}$ played a minor and non-significant role. On the other hand, Kv channel played no direct role, and the induced response curve was very close to that of the control. Possible combinations of K channel blockers showed that some produced synergistic effect to different extent, whereas others produced mild and non-significant effect except at the highest doses used on dose-response curves. Thus, combinations of (GLIB+4-AP), (GLIB+$BaCl_2$) and ($BaCl_2$+TEA) caused a highly significant blocking in the induced response curve, while ($BaCl_2$ +4-AP) and (TEA+4-AP) produced a mild inhibition except at the highest doses used in which the inhibition was significant.

***Keywords:*** *Human Artery, colon cancer, $H_2S$, $K^+$ and $Ca^{2+}$ channels.*

## Introduction

Cancer describes a range of diseases that can affect different organs and tissues of the body. Colorectal cancer (adenocarcinoma), represents the third foremost identified malignant tumour and the second most common cause of cancer death [1,2, 3].

Arteries can be mechanically described as a long-range elastic element (elastin) arranged in parallel with a system of continuous collagen fibers that set the limit of extension. Tunica intima, tunica media and tunica adventitia are three recognizable layers in an artery [4]. The adventitia is the outermost layer of the vessel and consists of connective tissue created from elastin, collagen, fibroblasts, mast cells, macrophages, and nerve axons [5]. Tunica media, consists mainly of vascular smooth muscle cells (VSMCs), are in charge of generation of vascular tone (vasoconstriction). Depolarization of VSMC membrane evoked contraction mainly by opening of voltage dependent calcium ($Ca^{2+}$) channel which leads to an increase in $[Ca^{2+}]_i$ [6]. The inner layer is the tunica intima, which includes a single layer of cells pointed as the vascular endothelium [7]. Endothelium

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is the active inner monolayer of the blood vessels which forms an interface between circulating blood and the vessel wall. It plays a crucial role in vascular homeostasis [8], involved in many pathological and physiological processes, including the regulation of smooth muscle tone, control of thrombosis, inhibition of leukocyte, platelet adhesion and promotion of intra-arterial permeability [9]. The endothelium regulates the activity of smooth muscle fibers by synthesizing several vasoactive substances that affect the contractility of the arterial wall in response to various stimuli [10]. The most essential vasodilators are nitric oxide (NO), prostaglandin $I_2$ ($PGI_2$), and endothelium-derived hyperpolarizing factor (EDHF) [11]. The smooth muscle has a variety of selective and nonselective ion channels. Among the selective channels, are those transporting $Ca^{2+}$ or potassium ($K^+$) have been investigated most intensively, but chloride ($Cl^-$) and sodium ($Na^+$) channels are equally important [12].

Pathological angiogenesis is a hallmark of cancer and various ischemic, inflammatory diseases and the potent angiogenic factor vascular endothelial growth factor (VEGF) which is associated with metastasis in human colon cancer. Also, the proliferation rate of endothelial cells decreases when grown in conditioned media from human colon cancer cells with decreased VEGF expression [13].

The dynamic interplay of $Ca^{2+}$ and $K^+$ channels on the plasma membrane of VSMCs plays a pivotal role in modulating the vascular tone of small arteries and arterioles [14]. Potassium channels form the most plentiful with various classes of ion channels. These are membrane-spanning proteins allows efflux of $K^+$ through a $K^+$ selective pore. The activity of $K^+$ channels may be modulated by voltage, $Ca^{2+}$ and neurotransmitters [15]. These ion channels play an important role in regulating the membrane potential ($Vm$) of VSMCs [16]. Many types of $K^+$ channel have been recognized in endothelial and (SMCs); such as selective (ATP)-dependent $K^+$ channels ($K_{ATP}$), voltage-gated $K^+$ channels ($K_V$), inward rectifier $K^+$ channels ($K_{IR}$) and $Ca^{2+}$ activated $K^+$ channels ($K_{Ca}$) [17]. In excitable cells, the role of all $K^+$ channels is related to stabilization of the resting $Vm$ [18]. In contrary, $Ca^{2+}$ channels in many alternative cell types activate membrane depolarization and mediate $Ca^{2+}$ influx in response to action potentials and sub-threshold depolarizing signals. Calcium ions entering the cell through voltage gated calcium channels (VGCCs) initiate many different cellular events and serves as the second messenger for electrical signaling [19].

Hydrogen sulphide ($H_2S$) is a gaseous signaling molecule that mediate vasodilatory effects on the arterial tree. It is produced by the action of at least three enzymes, cystathionine beta synthase (CBS), cystathionine gamma lyase (CSE) and mercaptopyruvate-sulfur transferase (MST) [20,21]. Numerous physiological functions assigned to be exclusively or partly regulated by $H_2S$, some of which are vasodilation [22], and angiogenesis [23] via direct activation of $K_{ATP}$ channels [24].

## Materials and Methods

**Colon tissue collection** Colon tissues used in the current study were taken from patients with colon cancer. The 5 samples used were taken from colon cancer patients (including 3 female and 2 male) aged between 30 to 70 years in Duhok Province. Blood vessels and mesenteric artery branches were obtained from patients under-going colectomy that diagnosed as colon cancer. After operation, the mesenteric artery branches were isolated carefully from cancer tissue to avoid any damage in the blood vessels. The blood vessels with a part of removed tissue were kept in Krebs solution oxygenated with carbogen (95% $O_2$ and 5% $CO_2$), maintained at 4°C and transported within an hour from Vajeen Hospital (Duhok) to Advanced Physiology Research Lab, Department of Biology, Faculty of Science, University of Zakho. In the lab, the arteries were cleaned from connective and other unwanted tissues, cut into equal segments, each of about 2-3mm in length.

**Tissue preparation:** Blood vessel segments prepared for this study were immediately placed in a Petri jar with oxygenated Krebs physiological solution, maintained at 4°C, rinsed and freed from clotted blood. The mean time between harvesting and experimentation was about 60 minutes. The vessels were mounted to PanLab glass tissue chambers.

### Experimental Protocol

Each isolated arterial ring was mounted between two stainless steel hooks, connected from one end to the tissue holder at the base of 10 ml capacity tissue glass chamber containing physiological solution at 37ºC. From the other end, it was connected to a force transducer coupled to transbridge amplifier and PowerLab Data Acquisition System and the tissue was set at 5 grams resting tension. The preparation was left to equilibrate for 3-5 hours at 37ºC with changing of physiological solution at 15-minute intervals with maintain the pH at 7.4 by continuous aeration with carbogen gas.

The arterial segments were initially exposed to ($1X10^{-5}$) NE to test their functional integrity. Later, the bath medium was changed several times until a stable resting tone was recorded. The dose-response curve for sodium disulfide ($Na_2S$; 1-6 mM) was constructed against NE-precontracted rings. To study the role of different $K^+$ channel types in relaxation induced by $Na_2S$, the arterial rings were pre-incubated for 20 minutes with the following K channel inhibitors, tetraethylammonium (TEA; 1mM), glibenclamide (GLIB; 10 μM), barium chloride ($BaCl_2$;1mM) and 4-aminopyridine (4-AP; 1mM), which are the inhibitors of $K_{Ca}$, $K_{ATP}$, $K_{IR}$ and $K_V$ channels, respectively. These inhibitors were used individually or in combinations.

The dose-response curves were fitted with a Hill equation, from which the half maximal inhibitory concentration ($IC_{50}$) values were obtained as geometric mean. Maximum contractile responses to $Na_2S$ were calculated as a percentage of the contraction produced by NE and were expressed as the means ± standard error of the mean (SEM). The tension produced by NE was defined as 0% relaxation, and the baseline tension before addition of vasoconstrictors were defined as 100% relaxation.

## Statistical Analysis

The statistical analysis of the data was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out pair wise comparison between the same doses of different groups using GraphPad Prism software version 6.0 for Windows. P-values less than 0.05 ($P<0.05$) were considered as statistically significant. In all figures, the symbols (*, ** and ***) representing mean differences are significant at the 0.05, 0.01 and 0.001 levels, respectively.

## Results

### The role of $K^+$ channels in arterial $Na_2S$ induced relaxation

In arteries feeding colon cancer precontracted with NE, pre-incubation with TEA did not decrease the relaxant effect induced by $Na_2S$ significantly at low doses (1mM and 2mM), whereas at higher doses (3 to 6 mM), it reduced the relaxant effect of $Na_2S$ significantly ($P<0.05$ to 0.001). Thus, the $E_{max}$ value was significantly reduced from (108.00±7.89%) in the control to (40.94± 7.11%) in arteries feeding colon cancer pre-incubated with TEA (Figure 1A).

In arteries feeding colon cancer precontracted with NE, pre-incubation with GLIB caused a mild and non- significant reduction in the relaxation induced by $Na_2S$. Thus, the $E_{max}$ was also reduced from (108.00±7.89%) in the control to (91.35±18.16%) in arteries feeding colon cancer treated with GLIB (Figure 1B).

In arteries feeding Colon cancer precontracted with NE, pre-incubation with $BaCl_2$ did not decrease the relaxant effect induced by $Na_2S$ at low doses (1 to 3 mM), whereas, at higher doses (4 to 6 mM), it reduced the relaxant effect induced by $Na_2S$ at a

highly significant (P<0.001) level. Thus, the $E_{max}$ value was reduced at a highly significant level from (108.00±7.89%) in the control to only (41.97± 6.36%) in arteries pre-incubated with $BaCl_2$ as (Figure 1C).

In arteries feeding colon cancer precontracted with NE, pre-incubation with 4-AP did not affect vasorelaxation induce by $Na_2S$. Accordingly, the $E_{max}$ remain unchanged; it was (108.00±7.89%) in the control and (108.10±1.08%) in arteries treated with 4-AP (Figure 1D).

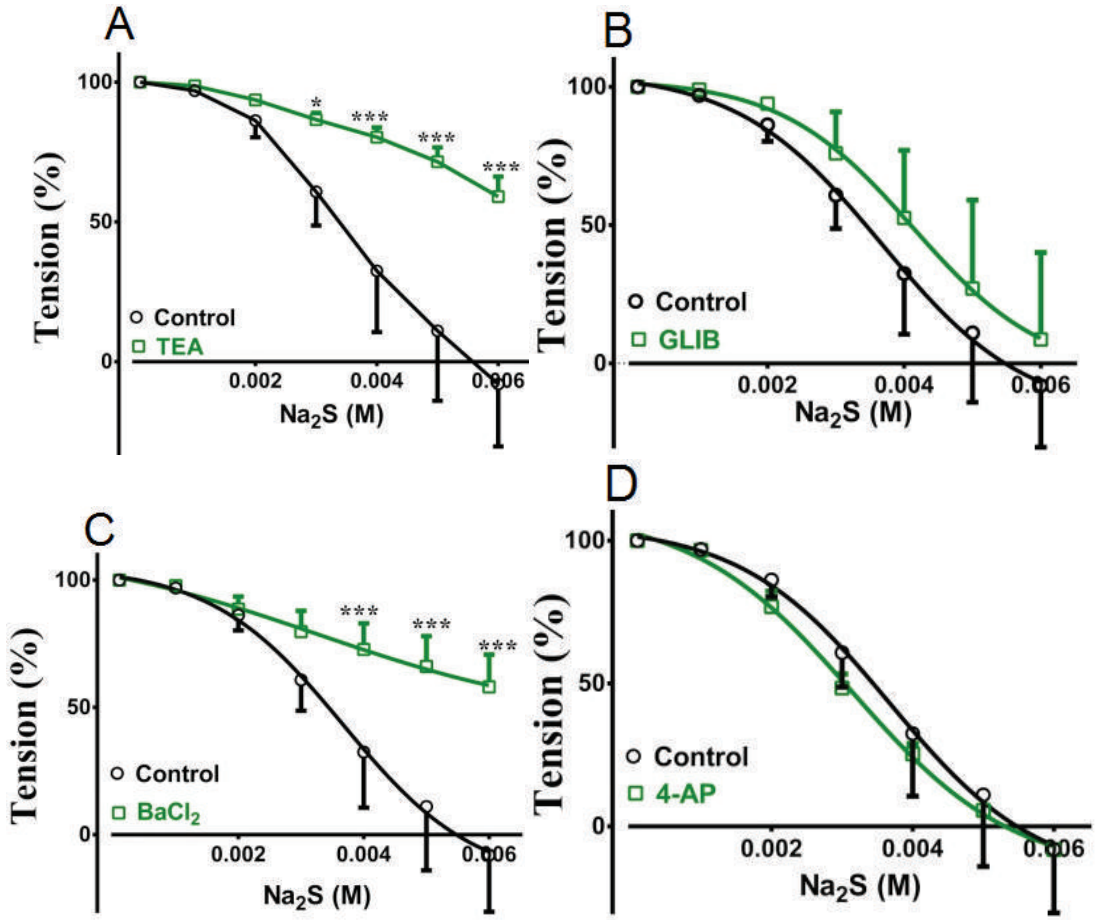



**Figure 1. Role of $K^+$ channels in the vasodilator effects of $Na_2S$ (1 to 6 mM) on NE-precontracted artery feeding colon cancer incubated in physiological solution containing (A) 1mM TEA, (B) 10µM GLIB, (C) 1mM $BaCl_2$ and (D) 1mM 4-AP for 20 min. All data are expressed as % of relaxation of NE-induced artery tone and are represented as the Mean±SEM. * P<0.05 versus control; *** P<0.001 versus control.**

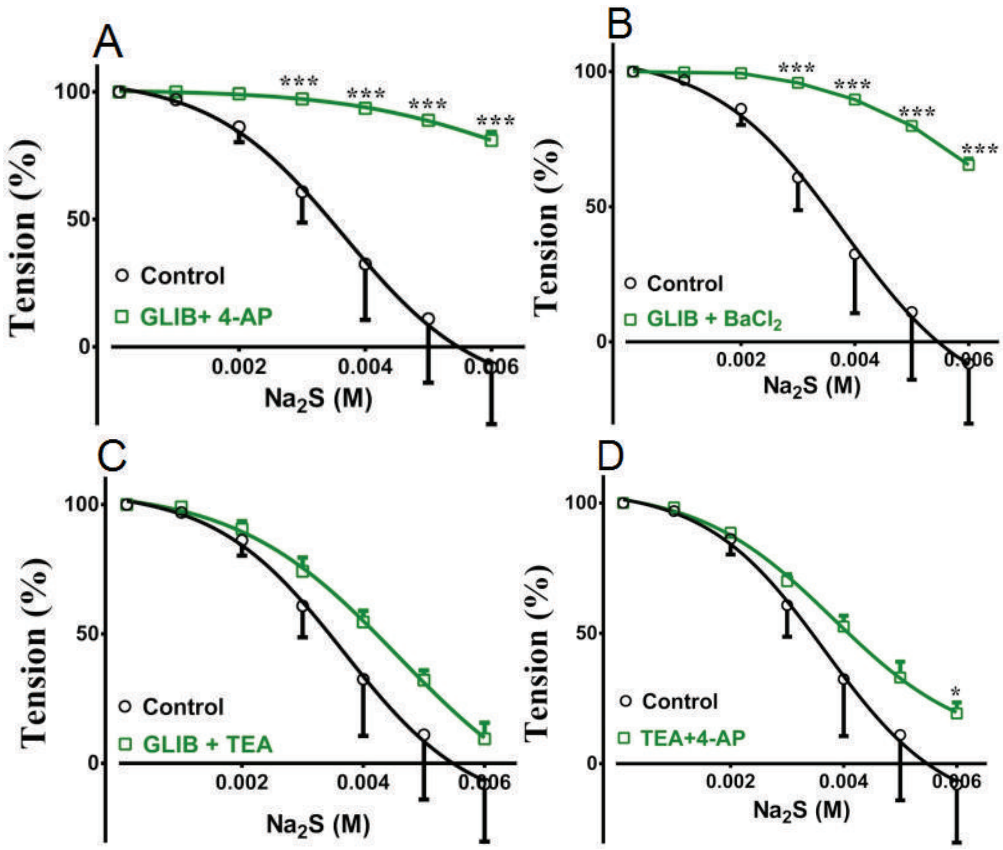
In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+4-AP) produced a highly significant (p< 0.001) decrease in the relaxation induced by $Na_2S$ at high doses (3 to 6 mM), whereas at low doses produced non-significant reduction. Therefore, the $E_{max}$ was reduced significantly from (108.00±7.89%) in the control to only (19.01± 3.20%) in arteries pre-incubated with a mixture of (GLIB+ 4-AP) (Figure 2-A).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+$BaCl_2$) decrease the relaxation induced by $Na_2S$ at a highly significant (p<0.001) level at high doses (3 to 6 mM), while, at lower doses, it produced a mild and non-significant reduction in the relaxant response. Thus, the $E_{max}$ was significantly reduced from (108.00±7.89%) in the control to (34.50±2.30%) in arteries pre-incubated with a mixture of GLIB+$BaCl_2$ (Figure 2-B).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of (GLIB+TEA) showed a mild and non-significant reduction in $Na_2S$ induced relaxation. The $E_{max}$ value was reduced non-significantly from (108.00±7.89%) in the control to (90.54±6.20%) in artery pre-incubated with a mixture of (GLIB+TEA) (Figure 2-C).

In arteries feeding colon cancer precontracted with NE, pre-incubation separately with a mixture of (4-AP+TEA) and (4-AP+$BaCl_2$) produced non-significant inhibitory effects at all doses used except the highest dose of each mixture in which the $Na_2S$ induced relaxation was significantly ($P<0.05$) reduced at a dose of (6 mM). The $E_{max}$ values reduced considerably from (108.00±7.89%) in the control to (80.72±4.20%) and (80.70±4.55%), respectively in arteries treated with (4-AP+TEA) and (4-AP+ BaCl2) (Figures 2-D and E).

In arteries feeding colon cancer precontracted with NE, pre-incubation with a mixture of ($BaCl_2$+TEA) decreased the relaxant effect induced by $Na_2S$ non-significantly at low doses (1 and 2 mM), whereas at higher doses (3 to 6 mM), it reduced the relaxant effect induced by $Na_2S$ significantly ($P<0.05$ to 0.001). Thus, the $E_{max}$ was reduced significantly from (108.00±7.89%) in the control to only (35.32± 4.51%) in arteries pre-incubated with a mixture of $BaCl_2$+TEA (Figure 2-F).



***Cont... Figure 2.***

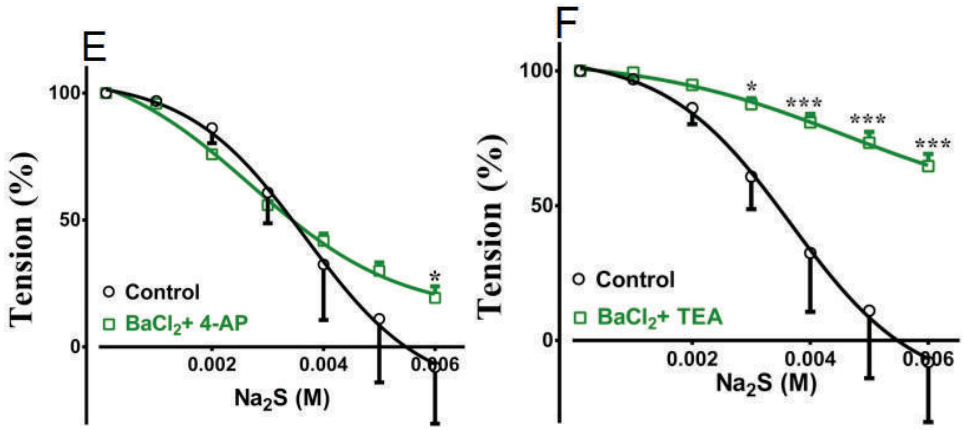



**Figure 2. Role of $K^+$ channel types in the vasodilator effects of $Na_2S$ (1 to 6 mM) on NE-constricted artery feeding colon cancer in Human incubated in physiological solution containing (A) GLIB+4-AP, (B) GLIB+$BaCl_2$, (C) GLIB+TEA, (D) 4-AP+TEA, (E) 4-AP+$BaCl_2$ and (F) $BaCl_2$+TEA for 20 min and then contracted with 10µM NE. All data are expressed as % of relaxation of NE-induced artery tone and are represented as the mean ±SE. * P<0.05 versus control; *** P<0.001 versus control.**

**The role of L-type Ca channels in $Na_2S$ induced arterial rings relaxation.**

In arteries feeding colon cancer precontracted with NE, pre-incubation with Nifedipine, produced a non-significant reduction in the relaxant effects of $Na_2S$. Thus, the $E_{max}$ values were very close (108.00±7.89%) in the control and (107.44±26.46%) in arteries treated with Nifedipine (Figure 3).

## Discussion

Relaxant Responses of Arteries Feeding Colon Cancer to $Na_2S$

**The Role of $K^+$ Channels**

The relaxation induced by $Na_2S$- in vascular smooth muscle is predominantly induced through the activation of potassium channels, efflux of $K^+$, hyperpolarization of SMCs and subsequent relaxation. The participation of several additional signaling pathways and mechanisms confirmed also including changes in intracellular pH or ATP levels as well as endothelium-derived hyperpolar-izing factors [25]. Several types of potassium channels have been reported to be the major molecular targets for $H_2S$ which resulted vasorelaxant effects. A rise in $K^+$ permeability normally hyperpolarizes cell membrane; and thus, inhibits $Ca^{2+}$ influx through voltage gated L-type $Ca^{2+}$channels resulting in muscle relaxation. The first clear connection between $H_2S$ and $K^+$ channels was indicated by [26] through a series of *in vivo* and *in vitro* studies. They showed that $H_2S$ induced relaxation of VSMCs occurs via the opening of $K_{ATP}$ channels.

The data of the current study demonstrate that $H_2S$ produced a dose dependent relaxant effect in human NE pre-contracted arteries feeding colon cancers. This effect is in line with that of [27] who showed that $H_2S$ donor (NaHS) caused relaxation in pre-contracted non-cancer human mesenteric arterial rings in a concentration-dependent manner.

The inhibitory effect of $BaCl_2$ on $H_2S$ induced relaxation, indicates that $K_{ir}$ channels play an important role in $H_2S$-induced dilation in arteries feeding Human colon cancer, is supported those of [28] who showed that $BaCl_2$ (a specific inhibitor of $K_{ir}$ channel) decreased the sensitivity of $Na_2S$-induced vasorelaxation in mouse aorta without reducing the maximum response. Also, it has been observed that $K_{ir}$ channels located

on the endothelium and gap junctions may mediate a conduction of $K_{ir}$ dependent hyperpolarization from endothelium to the smooth muscle cells [29]. Using the same vascular bed, [30] showed that $K^+$-mediated relaxations were endothelium-dependent. It has been reported that the mechanism of relaxation is mainly mediated by the activation of $K_{ir}$ channels and inhibition of $K_{Ca}$ channels [31]. Therefore, $K^+$ efflux through $K_{Ca}$ channel could act upon endothelium $K_{ir}$ channel which could amplify the endothelial cell hyperpolarization and thereby increase the magnitude of the electrical signal passing electroto-nically to the smooth muscle layer [32].

The present study showed that $Na_2S$ induced relaxation in human arteries feeding colon cancer reduced by $K_{Ca}$ channel blocker. This result suggests that a part of the relaxation of the arteries feeding colon cancer in human produced by $H_2S$ is due to the activation of $K_{Ca}$ channels. It is demonstrated that the activation of small and intermediate conductance $K_{Ca}$ channels by $H_2S$ cause relaxant effect [22]. Furthermore, both [33,34] reported that co-application of intermediate ($IK_{Ca}$ blocker) and small conductance ($SK_{Ca}$ blocker) reduced the extent of the $H_2S$-induced vasorelaxation. It has been demonstrated that $H_2S$ increased the frequency of $Ca^{2+}$ sparks in piglet cerebral arteriole smooth muscle cells causing an increase in the frequency of transient $K_{Ca}$ current; and thus, vasorelaxation [35]. In contrast, Tang *et al*., (2010) observed that different $K_{Ca}$ channels blockers failed to affect the vascular effect of $H_2S$ [36].

In the present study, 4-AP did not decrease the vasodilation of arteries feeding colon cancer induced by $Na_2S$ which evident that $Na_2S$ cannot activate $K_V$ channels.

The $K_{ATP}$ channel blocker (GLIB) did not decrease vasodilation of artery feeding colon cancer induced by $Na_2S$ suggesting that $K_{ATP}$ might not be responsible for $H_2S$ induced vasorelaxation. [37] showed that $K_{ATP}$ channels were not involved in mediating effects of $H_2S$ in rat coronary arteries. Other investigators have found that the relaxations caused by $H_2S$ in the guinea-pig ileum were not mediated by $K_{ATP}$ channels [38].

The results of the current study indicated that $H_2S$ induced relaxation in smooth muscle cells of the arteries feeding colon cancer varies with the types of blocker combination and arteries feeding tissues when preincubated with specific $K^+$ channels blockers. Thus, a combination of ($K_{ATP}$ and $K_{ir}$) channel blockers produced a synergistic effect on $Na_2S$ induced relaxation in AFCC since it produced a significant inhibitory effect as compared with the effects of the individual blockers included in the combination since showed no inhibitory effects on their dose response curves. The same thing is true for the effect of ($K_{ATP}$ and $K_V$ channel blockers) which also produced a synergistic effect on $Na_2S$ induced dose response curved.

However, a combination of ($K_{ir}$ and $K_{Ca)}$ channels blockers produced significant inhibition in $Na_2S$ dependent relaxation in the arteries feeding colon cancers. This result goes in parallel with the inhibitory effects produced by ($K_{ir}$ and $K_{Ca)}$ channels blockers when used individually.

The remaining combinations of $K^+$ channels blockers which included ($K_{ATP}$+$K_{Ca}$), ($K_V$+$K_{ir}$) and ($K_V$+ $K_{Ca}$) blockers produced antagonistic effects since they produced no significant inhibitory effects on the dose response curved as compared with the effects produced when using the blockers included in the above combinations individually.

These results suggest that $H_2S$ may have more than one target in vascular smooth muscle cells since the activation of these $K^+$ channels by $H_2S$ would leads smooth muscle cells hyperpolarization and subsequent vasorelaxation. Since no data are available to compare these novel results. Any way it has been found that co-application of the $K_{ATP}$ blocker with $IK_{Ca}/SK_{Ca}$ blockers abolished all $H_2S$-mediated vasorelaxation in rat mesenteric arteries [22]. Conversely, in the current study, ($K_{ATP}$ +$K_{Ca}$) channels blockers did not produce any effect on $H_2S$ induced vasorelaxation in

artery feeding colon cancer in human.

Role of $Ca^{2+}$ Channel

Nifedipine (L-type $Ca^{2+}$ channel blocker) neither reduced nor enhanced $H_2S$ induced vasorelaxation in arteries feeding colon cancer. Therefore, L-type $Ca^{2+}$ channel plays a secondary role in $H_2S$ induced relaxation in rings of arteries. This implies that vasorelaxation might result from a direct action of $H_2S$ on $K^+$ channels followed by lowering of smooth muscle calcium and independent on the blockage of $Ca^{2+}$ channel. Other proposed mechanisms of $H_2S$-induced relaxation include binding of the $Ca^{2+}$-calmodulin complex, its interaction with NO and/or endothelium-derived hyperpolarizing factor (EDHF), inhibition of phosphodiesterase (PDE), and elevation of intracellular $Ca^{2+}$ in endothelial cells.

## Conclusion

In arteries feeding colon cancer both $K_{ir}$ and $K_{Ca}$ played major roles in induced relaxation, whereas $K_{ATP}$ and $K_V$ played no significant role in $H_2S$ dependent relaxation. In AFCC, K channel blocker combinations including (GLIB+ $BaCl_2$), (GLIB+4-AP) and ($BaCl_2$+TEA) produced significant inhibitory effects on $H_2S$ dependent relaxation, whereas the remaining three combinations produced mild and non-significant inhibitions in the induced relaxation. L-type Ca channel blockers caused a mild enhancement. However, $H_2S$ induced relaxation at a high dose, L-type Ca channels were showed neither inhibitory nor enhancing effect.

### Conflict of Interest

The authors declare that there is no conflicts of interest regarding the publication of this manuscript.

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# The Influence of Safety Management Practices on Safety Performance in Nurses of Emergency Installation on The Government Hospital of Surabaya

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## Abstract

**Introduction:** Hospital is one of the high-risk workplaces in terms of safety and health. Nurses are the most vulnerable health workers to work accidents because they have the most number in a hospital and most often interact with patients. Safety performance is very important for the prevention of work accidents. This research aimed to analyze the influence of safety management practices on safety performance in nurses of the emergency installation at X Hospital of Surabaya.

**Methods:** This research was an observational research with the cross-sectional design. It was conducted in May to June 2019. The population in this study was 208 nurses and the sample obtained using a simple random sampling technique involving 68 nurses. In assessing safety management practices and safety performance, the researcher used questionnaires, observation, and interviews.

**Results:** The results presented that safety training which was a dimension of safety management practices affected on the safety performance with a p-value 0.000. Suggestions in this research, to improve safety performance, management can conduct specific and periodic safety training, holding safety briefing and safety talk on a regular basis, and implementing a safety award by applying a system of reward and punishment.

**Conclusion:** Hospital management still needs to schedule a specific and periodic safety training for nurses. Furthermore, to improve safety performance, management can holding safety briefing and safety talk on a regular basis, and implementing a safety award by applying a system of reward and punishment.

***Keywords:*** *Nurses, Safety management practices, Safety performance*

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## Introduction

The work accident is undesirable events and resulted in losses, as well as property damage and loss of process (Suma'mur, 2009)[1]. Work accidents occurred due to a random combination of many factors that can generally be categorized into unsafe conditions and unsafe action. Unsafe action of

employees of the highest causes of work accidents that occur in comparison with unsafe working conditions (Choudhry & Fang, 2008)[2].

Data from the Social Security Organizing Agency / BPJS Employment (2019) states that in Indonesia in 2017 there were 123,041 work accident cases. This number has increased in 2018 with 173,105 work accident cases[3].

One of the efforts to prevent work accidents is to improve safety performance. The term can refer to the safety performance of two concepts. The terms of safety performance can refer to safety results in an organization, such as the number of injuries or work accidents every year. In addition, the term safety performance can also refer to an individual's behavior related to the safety or can be called a safety behavior (Christian et al., 2009)[4]. Term safety performance used in this study refers to the behavior of safety. Safety performance by Neal & Griffin (2002) formed from safety compliance and safety participation. Safety compliance is safe behavior by employees in maintaining safety, while safety participation is the behavior of employees to participate in safety activities[5].

Vinodkumar & Bhasi (2010) found that safety management practices influences safety performance. Vinodkumar & Bhasi (2010) states that the safety management practices is the practice, the role and management functions designed by the company to improve the safety of employees, which consisted of six dimensions, namely management commitment related to safety, safety training, involvement of workers in solving the problems of safety, safety communications, rules and safety procedures, and safety promotion policy[6]. In this research, the term refers to safety management practices on employee opinions against six dimension in safety management practices.

Hospital is one of the high-risk workplaces in terms of safety and health. Nurses are the most vulnerable health workers to work accidents because they have the most number in a hospital and most often interact with patients. The results of the *National Safety Council (NSC)* report in 1988 showed that the workers in a hospital had 41% more risk of work accidents than workers in other industries. (Ministry of Health of the Republic of Indonesia, 2007)[7].

The X Hospital of Surabaya is a place that provides health services to the general public. Emergency installation at X Hospital of Surabaya is a unit of service is most vital in helping to save the lives of patients with urgency medical when it first entered the hospital, because the handling of the emergency should get a response time of rapid and appropriate action causes the nurses in this section are often exposed to various source of danger that may threaten life and health as well as greater risk of work accidents.

Safety management practices are estimated to have an influence on safety performance and can improve these safety performance in hospital nurses. Therefore, this research aimed to analyze the influence of safety management practices on safety performance in nurses of the emergency installation at X Hospital of Surabaya.

## Materials and Methods

This research was an observational research with the cross-sectional design. It was conducted in May to June 2019. The location of the study was the emergency installation at X Hospital in Surabaya The population in this study was 208 nurses and the sample obtained using a simple random sampling technique involving 68 nurses. The dependent variable was safety performance. The independent variable was safety management practices that include management commitment, safety training, employee involvement in safety, safety communication and feedback, safety rules and procedures, and safety promotion policies. In assessing safety management practices and safety performance, the researcher used questionnaires, observation, and interviews. The data analysis used was descriptive analysis, relationship

analysis by chi-square test, and influence analysis by logistic regression test.

## Results and Conclusion

Table 1 shows that most respondents stated all dimensions in safety management practices in good categories, namely management commitment (52.9%), safety training (58.8%), employee involvement in safety (83.8%), safety communication and feedback (75%), safety rules and procedures (82.4%) and safety promotion policies (72.1%). Table 2 shows that most respondents also had a good category in safety performance (54,4%).

Based on the results of the relationship analysis by chi-square test as shown in Table 3, the results obtained a p-value of management commitment variable of 0.017, safety training variable of 0,000 and safety communication and feedback variables of 0.035. This means that the three variables is qualified as a candidate Multiple Logistic Regression (p-value <0.25). The candidate variables and then put together in multivariable analysis with logistic regression test to determine which the most influential variables.

Based on test results with logistic regression and using the backward: wald, it is known that the most influential variables that safety training, with a p-value of 0.000. Value B indicates a positive sign, which means nurses with good safety training has an increasingly good safety performance as well. (Table 4).

**Table 1: Distribution of Safety Management Practices in Nurses of Emergency Installation at X Hospital of Surabaya, 2019**

| Safety Mangement Practices | Categories | n | % |
|---|---|---|---|
| Management commitment | Less | 32 | 47,1 |
| | Good | 36 | 52,9 |
| Safety training | Less | 28 | 41,2 |
| | Good | 40 | 58,8 |
| Employee involvement in safety | Less | 11 | 16,2 |
| | Good | 57 | 83,8 |
| Safety communication and feedback | Less | 17 | 25,0 |
| | Good | 51 | 75,0 |
| Safety rules and procedures | Less | 12 | 17,6 |
| | Good | 56 | 82,4 |
| Safety promotion policies | Less | 19 | 27,9 |
| | Good | 49 | 72,1 |

**Table 2: Distribution of Safety Performance in Nurses of Emergency Installation at X Hospital of Surabaya, 2019**

| Safety Performance | Categories | n | % |
|---|---|---|---|
| Safety Performance (safety compliance and safety participation) | Less | 31 | 45,6 |
| | Good | 37 | 54,4 |

**Table 3: Relationship between Safety Management Practices and Safety Performance in Nurses of Emergency Installation at X Hospital of Surabaya, 2019**

| Safety Management Practices | Safety Performance | | | | Total | | p-value |
|---|---|---|---|---|---|---|---|
| | Less | | Good | | | | |
| | n | % | n | % | N | % | |
| **Management commitment** | | | | | | | 0,017 |
| Less | 20 | 62,5 | 12 | 37,5 | 32 | 100 | |
| Good | 11 | 30,6 | 25 | 69,4 | 36 | 100 | |
| Safety training | | | | | | | 0,000 |
| Less | 26 | 92,9 | 2 | 7,1 | 28 | 100 | |
| Good | 5 | 12,5 | 35 | 87,5 | 40 | 100 | |
| Employee involvement in safety | | | | | | | 0,748 |
| Less | 6 | 54,5 | 5 | 45,5 | 11 | 100 | |
| Good | 25 | 43,9 | 32 | 56,1 | 57 | 100 | |
| Safety communication and feedback | | | | | | | 0,035 |
| Less | 12 | 70,6 | 5 | 29,4 | 17 | 100 | |
| Good | 19 | 37,3 | 32 | 62,7 | 51 | 100 | |
| Safety rules and procedures | | | | | | | 0,511 |
| Less | 7 | 58,3 | 5 | 41,7 | 12 | 100 | |
| Good | 24 | 42,9 | 32 | 57,1 | 56 | 100 | |
| Safety promotion policies | | | | | | | 0,319 |
| Less | 11 | 57,9 | 8 | 42,1 | 19 | 100 | |
| Good | 20 | 40,8 | 29 | 59,2 | 49 | 100 | |

**Table 4: The Results of Logistic Regression That The Influence of Safety Management Practices on Safety Performance in Nurses of Emergency Installation at X Hospital of Surabaya, 2019**

| | | B | S.E. | Wald | df | Sig. | Exp(B) | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Lower | Upper |
| Step 1a | Management commitment (1) | ,084 | ,909 | ,008 | 1 | ,927 | 1,087 | ,183 | 6,456 |
| | Safety training (1) | 4,380 | ,949 | 21,281 | 1 | ,000 | 79,834 | 12,417 | 513,288 |
| | Safety communication (1) | ,981 | ,968 | 1,026 | 1 | ,311 | 2,666 | ,400 | 17,784 |
| | Constant | -3,279 | 1,145 | 8,204 | 1 | ,004 | ,038 | | |
| Step 2a | Safety training (1) | 4,413 | ,882 | 25,011 | 1 | ,000 | 82,542 | 14,639 | 465,406 |
| | Safety communication (1) | ,963 | ,944 | 1,040 | 1 | ,308 | 2,619 | ,412 | 16,673 |
| | Constant | -3,238 | 1,044 | 9,616 | 1 | ,002 | ,039 | | |
| Step 3a | Safety training (1) | 4,511 | ,876 | 26,528 | 1 | ,000 | 91,000 | 16,351 | 506,439 |
| | Constant | -2,565 | ,734 | 12,218 | 1 | ,000 | ,077 | | |

## Discussion

The training is part of an educational process that is intended to improve the knowledge, skills and attitude. According to Cooper (2001), training is one of the most frequently used ways to change the behavior of workers that must be set properly [8]. Safety training is an activity of workers in acquiring knowledge about the dangers of work accidents, get new skills, educate workers to deal with the potential dangers so that workers have the behavioral attitude of safe work and care for the safety conditions in the workplace and can maintain safe behavior in a new environment (Statt, 2000)[9].

Based on the survey results revealed that the dimensions of safety management practices that most influence on safety performance is safety training. This is in line with research by Syamtinningrum (2017) which explains that there is a significant effect between the safety training and unsafe action, where the better the safety training of respondents, the lower the unsafe action[10].

## Conclusion

The evaluation of most of the nurses at the emergency installation in X Hospital of Surabaya for safety training was good. This is due to the management has been providing safety training in the

form of training related to standard precautions include hand hygiene, use of personal protective equipment, and waste management. Moreover, also conducted training related to occupational health and safety, among others, the use of portable fire extinguisher and handling of hazardous and toxic material.

This study concludes that there is an influence of safety management practices on safety performance. Through a given safety training can increase the knowledge and skills of nurses so as to improve safety performance at the nurse anyway. Suggestions in this study, hospital management still needs to schedule a specific and periodic safety training for nurses. Furthermore, to improve safety performance, management can holding safety briefing and safety talk on a regular basis, and implementing a safety award by applying a system of reward and punishment.

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# Immediate Postoperative Radiographic Assessment of Hip Arthroplasty

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## Abstract

Radiograph is the keystone in the assessment of hip arthroplasty, as it is available with no metal artifact , at a low cost, and facilitating longitudinal comparison. Pelvic radiograph is significant to assess post-operative hip arthroplasty. So we aim to assess the outcome of the operation through immediate radiography for hip arthroplasty and to determine the different types of hip arthroplasty. This prospective study were conducted at surgical specialized hospital and nursing home hospital / medical city complex / Iraq-Baghdad at radiological departments by seimens x.ray machines , in the period between ( October 2020 – march 2021) . Fifty patients were included . The results showed after radiographic evaluation of hip arthroplasty , 90.0% of cases was Equal Leg length. The higher percentage of vertical center was (90.0 %) for similar compared to lower percentage was(10.0%) non similar , (96.0%) of horizontal center was similar compared (4.0%) non similar .The higher percentage of acetabular inclination was (78.0%) for angle 45degree compared to lower percentage was(6.0%) for angle 50 degree , the higher percentage of acetabular anterversion was (30.0%) for angle 25 degree compared to lower percentage was(2.0%) for angle 30 degree. The percentage for femoral stem position was (100.0%) for neutral alignment . (90.0%) not used of cement mantle compared to (10.0%) used cement mantle , the thickness in range 2-3mm . (94.0%) was no any early complications compared to (6.0%) was Superficial infection.

***Keywords:*** *Radiography, Hemiarthroplasty , Total Hip Arthroplasty, Femoral neck fracture.*

## Introduction

The hip is a socket and a true ball joint surrounded by muscles, enabling a wide range of motion in several physical planes while also exhibiting remarkable stability [(1)] .The function of the hip joint is to provide dynamic support the weight of the body [(2)] .It is a complex anatomic structure composed of muscular ,osseous and ligamentous structures [(3)] .Avascular necrosis of the femoral head is due to disruption of blood supply to the proximal femur. There are approximately 10000 to 20000 new cases reported each year in the United States alone [(4)] . Hip fracture is common injury, especially in the elderly patient . It is also seen in young patients who perform in high energy trauma. Immediate diagnosis are required to prevent threatening joint complications [(5)] . Hip arthroplasty is described as one of the most successful orthopedic procedures, allowing for mobilization and early weight bearing, pain relief, and improved quality of life for many patients [(6)] . There are two major

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types of hip arthroplasty , Total Hip Arthroplasty and Hemiarthroplasty. These major types can be identified from radiographs [7] . The term hemiarthroplasty refers to the replacement of only the femoral , while The term total arthroplasty is used when the femoral head and acetabulum are both replaced [8] . Radiography is the mainstay of imaging assessment of hip arthroplasty [9] . There are seven key elements which should be evaluated hiparthroplasty . These include the leg length, horizontal center of rotation, vertical center of rotation , acetabular inclination, acetabular antevertion, femoral stem position and assessment of the cement mantle [10] . The leg length is measured by drawing a line transversely connecting the inferior borders of the acetabular tear drops - the pelvic reference line , must be less than 1cm [11] . The vertical center of rotation is assessed by measuring the vertical distance between the center of the femoral head and the transischial tuberosity line ,this distance should be similar to that of the contralateral hip [12] . The horizontal center of rotation can be asessed by the measurement of the distance between the femoral head center and the bottom of the corresponding acetabular teardrop and comparison with the normal contralateral hip. The distances should be equal bilaterally [13] . The inclination is the angle calculated by the line, traced between the medial and lateral margins of the acetabular cup with the intersection of a transverse pelvic reference line [14] . The inclination should measure between 30–50° [15] . Greater angles with greater risk of hip dislocation and Smaller angles are associated with reduced abduction [11] . Femoral stem positioning should be noted in a neutral alignment of the longitudinal axis of the femoral shaft [13]. The acetabular anteversion is assessed on lateral views. It is the angle between the acetabular axis and the coronal plane [14] .Normal ranges from 5° to 25° anteversion as this allows adequate flexion of the hip [10]. The optimal thickness of the femoral cement mantles should ideally be 2–3 mm thick and the acetabular cement mantle is 3 mm as this thickness has been proven to bear good long term radiographic and clinical outcomes [16] .

## Patients and Methods

This prospective study were conducted at surgical specialized hospital and nursing home hospital/ medical city complex / Iraq-Baghdad at radiological departments by seimens x.ray machines , in the period between ( October 2020 – march 2021) . 50 patients were included (31 male and 19 female), their age ranged from 25 – 75 years. The surgery done for them by one surgical team . The surgery done for them due to, avascular necrosis and femoral neck fracture . All patients undergo pelvic radiography during their stay and before discharge from the hospital , through AP supine, AP standing , lateral (cross table supine) to manually measure the leg length , vertical and horizontal center of rotation , lateral acetabular inclination , acetabular antevertion, femoral stem position , cement mantle if used and if there are immediate complications (fracture or dislocation or early infection).

**Inclusion Criteria :** Patients who undergone to surgery due to avascular necrosis of hip and femoral neck fracture.

**Exclusion Criteria** : We exclude patients with complex trauma involving acetabular fracture, patients with revision total hip, infected hip arthroplasty and congenital displastic hip.

## Results

Fifty patients have been included in the study age group ranging between 25 and 75 years. In table(1) and Figure (1) show distribution of type of hip arthroplasty by type of fixating techniques . The highest percentage of cases was (84.0%) total hip arthroplasty was Cement less according to fixating techniques , while (10.0%) of Bipolar was Cemented according to fixating techniques . The association between Type of hip arthroplasty and Type of fixating techniques was high significantly (P=0.000).

**Table(1): Distribution of Type of hip arthroplasty by Type of fixating techniques**

| Type of fixating techniques | | Type of hip arthroplasty | | Total |
|---|---|---|---|---|
| | | Bipolar | Total hip arthroplasty | |
| Cement less | No. | 3 | 42 | 45 |
| | % | 6.0% | 84.0% | 90.0% |
| Cemented | No. | 5 | 0 | 5 |
| | % | 10.0% | 0.0% | 10.0% |
| Total | No. | 8 | 42 | 50 |
| | % | 16.0% | 84.0% | 100.0% |

P=0.000 MCP< 0.01 : (HS)

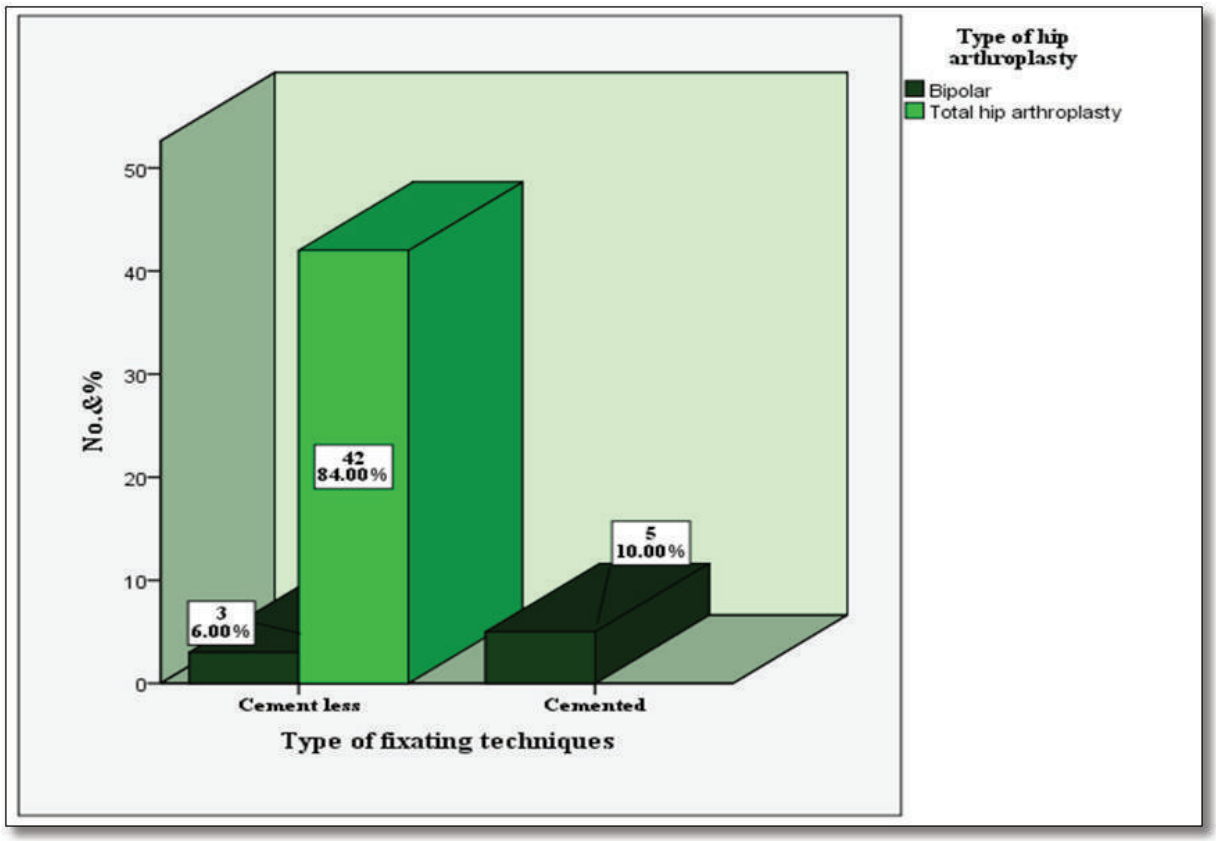


**Figure 1: Distribution of Type of hip arthroplasty by Type of fixating techniques**

Table (2) Show distribution of total hip arthroplasty by cement mantle. The higher percentage of cement mantle was(84.0%) among Total hip arthroplasty cases was no Cement mantle use, while (10.0%) of bipolar was 2-3mm compared to lower percentage was (6.0%) for bipolar was no Cement mantle use . The association between type of hip arthroplasty and cement mantle was high significantly (P=0.000).

**Table (2) : Distribution of Type of hip arthroplasty by Cement mantle**

| Cement mantle | | Type of hip arthroplasty | | Total |
|---|---|---|---|---|
| | | Bipolar | Total hip arthroplasty | |
| 2-3mm | No. | 5 | 0 | 5 |
| | % | 10.0% | 0.0% | 10.0% |
| NO | No. | 3 | 42 | 45 |
| | % | 6.0% | 84.0% | 90.0% |
| Total | No. | 8 | 42 | 50 |
| | % | 16.0% | 84.0% | 100.0% |

**P=0.000 MCP< 0.01 : (HS)**

Table (3) Show distribution of of total hip arthroplasty by acetabular inclination. The higher percentage was(92.9 %) of angle at 45degree compared to lower percentage was(7.1%) of angle 50 degree.

**Table(3):Distribution of Total hip arthroplasty by Acetabular inclination**

| Acetabular inclination | Total hip arthroplasty | |
|---|---|---|
| | No. | % |
| 45 | 39 | 92.9 |
| 50 | 3 | 7.1 |
| Total | 42 | 100.0 |

Table(4) Show distribution of of total hip arthroplasty by acetabular anterversion . The higher percentage was(35.7%) of angle at 25 degree compared to lower percentage was(2.4%) of angle at 30 degree.

**Table(4):Distribution of Total hip arthroplasty by Acetabular anterversion**

| Acetabular anterversion | Total hip arthroplasty | |
|---|---|---|
| | No. | % |
| 10 | 4 | 9.5 |
| 15 | 10 | 23.8 |
| 20 | 12 | 28.6 |
| 25 | 15 | 35.7 |
| 30 | 1 | 2.4 |
| Total | 42 | 100.0 |

In table (5) Show distribution type of hip arthroplasty and type of fixating techniques for Femoral Neck fracture. 63.6% from cases was Total hip arthroplasty with cement less as type for fixating techniques , while (22.7%) of bipolar was cemented as type of fixating techniques. Association between type of hip arthroplasty and type of fixating techniques of neck fracture was high significantly (P=0.002).

**Table(5): Distribution of Type of hip arthroplasty by Type of fixating techniques for Femoral Neck fracture**

| Type of hip arthroplasty | | Type of fixating techniques | | Total |
|---|---|---|---|---|
| | | Cement less | Cemented | |
| Bipolar | No. | 3 | 5 | 8 |
| | % | 13.6% | 22.7% | 36.4% |
| Total hip arthroplasty | No. | 14 | 0 | 14 |
| | % | 63.6% | 0.0% | 63.6% |
| Total | No. | 17 | 5 | 22 |
| | % | 77.3% | 22.7% | 100.0% |

**P=0.002 MCP<0.01 (HS)**

## Discussion

Several previous studies discussed the approach that used to radiographic assessment after hiparthroplasty . But the current study is one of the first study in Iraq that discussed immediate radiographic assessment after hiparthroplasty during staying and before hospital discharge by pelvic radiograph through AP supine, AP standing , lateral (cross table supine) , although in our study different variables such as age , gender and indication of hiparthroplasty were included. In our study ,all cases of Total hip arthroplasty was Cement less according to fixating techniques. In some studies, Cement less was performed with THA type [17] this is similar to our study . In our study, the cases of bipolar type was cemented more than cement less according to fixating techniques . In some studies, Cement less was performed with bipolar was more than cemented [18] and this was opposite to ours. The reason may be,cemented type make prostheses more stable , a spatially in elderly patient and because may be due to osteoporosis . In our study the association between type of hip arthroplasty and cement mantle was high significantly (P=0.000) . For the cement thickness in our study , the measurement was in the range (2-3mm) . In other studies , their measurement was in the range 2-5 [19] .According to most studies the acetabular inclination should be approximately 45degree [20] .In our study the higher percentage was(92.9 %) of angle at 45degree compared to lower percentage was (7.1%) of angle 50 degree. In some studies, radiographs reviewed within a range of 33.6 – 56.9 degrees [21] .This difference is due to some Variables such as surgical positioning, patient anatomy and surgical technique. In our study the range of angle between (10-30) degrees. In some studies, radiographs reviewed within a range of (8-28) degrees [22] . This difference is mostly due to some Variables such as patient anatomy specifically. In our study the association between type of hip arthroplasty and type of fixating techniques of neck fracture was high significantly , bipolar type was cemented more than cement less .Also some studies were cemented larger than cement less [23] .

This similar to our study because cemented is mostly used in elderly patient who have osteoporosis due to diabetes .In our study ,all THA type was cement less . But in some studies THA cemented was larger than cement less [24] . They note that elderly patients with FNFs treated with Cemented total arthroplasty show greater improvements in functional outcomes .

## Conclusions

Through our study , we showed and high light the importance of radiograph in assessment of hip arthroplasty despite the spread of modern devices such as MRI,CT,US . Through our research , we provide the radiologist systematic frame work which he can accurately assess the hip arthroplasty . Based on our study, we can explain three main things, namely, first the radiologist can read and evaluate the artificial hip joint, as well as the radiologist can criticize the surgeon in case there is any error in the operation and finally the surgeon can perform the operation accurately by depending on accurate measurements in order to extend the life of the artificial hip joint for a longer period possible.

**Ethical Clearance**: Taken from Middle Technical University ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

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# Phytotherapy in Renal Failure Due to Blood Pressure and Diabetes: A Systematic Review Study in Iram Ethnobotanical Documents

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## Abstract

Kidney failure is an irreversible condition and an important cause of morbidity and mortality in children worldwide. In conventional medicine, the methods of controlling these disorders and the existing treatments in many cases seem inadequate and sometimes ineffective. Due to the growing popularity of society in the use of knowledge and traditional medicine and herbal medicine, the potential of Iranian ethnobotanical knowledge can be used to find more effective treatment strategies. The aim of this study was to extract medicinal plants used in Iranian ethnobotanical knowledge in order to identify medicinal plants affecting renal failure. In this review study, keywords such as renal failure, renal impairment, hypertension, diabetes, herbs, ethnobotanics, and Iran were used. Databases such as ISI, Web of Science, PubMed, Scopus, ISC, SID and Google Scholar were used to review articles and resources. Finally, 23 articles containing ethnobotanical information for the treatment of renal failure were used to review the literature. Medicinal plants such as *Lamium album* L., *Origanum vulgare*, *Allium sativum* L., *Berberis vulgaris* L., *Ecbalium elaterium*, *Crataegus monogyna*, *Paliurus spina-christi* Miller., *Rheum ribes* L., *Olea europea*, *Silybum marianum* L., *Teucrium polium* L., *Nigella sativa* L., *Ziziphus jujuba* (L) H.Karst, *Centaurea bruguieriana* Hand. Mzt., *Juglans regia* L., *Brassica Napus* L. and some other herbs. are the most important medicinal plants used to treat kidney failure and disorders.

***Keywords:*** *Kidney, Kidney failure and disorder, Ethnobotany, Medicinal plants, Iran*

## Introduction

Kidney failure means the inability of the kidneys to excrete waste products. The kidneys are normally responsible for clearing the body of waste products, and if they fail, the waste products will accumulate and cause symptoms that vary in severity; Kidney failure can occur in two forms, acute or chronic [1-3]. Chronic

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renal failure refers to an irreversible renal failure that eventually leads to end-stage renal disease and requires one of the alternative treatments, including transplantation or dialysis [1, 2].These patients are unable to survive without kidney replacement therapy and are at serious risk of death [3(. One of the most important causes of kidney failure is diabetes and high blood pressure.The number of people with diabetes and high blood pressure is increasing worldwide.As a result, the number of people with kidney disease due to diabetes and high blood pressure, which is associated with high mortality, is also increasing [4,5]. Persistent high blood sugar in prolonged diabetes damages the small blood vessels inside the kidney. Initially, this damage manifests itself in the excretion of protein in the urine. Following high blood pressure, edema and other symptoms of kidney damage appear. Eventually the damage progresses and kidney failure develops [6, 7]. Severe kidney damage leads to sudden deterioration and reduction of kidney function and inability to excrete excess nitrogen products, regulate homeostasis, water and electrolytes, and acidosis [8]. The end result of chronic kidney disease, regardless of the type of kidney disease, includes progression to kidney failure, its complications and the development of cardiopulmonary disease, and bone problems that cause pain and suffering [9, 10]. Accumulation of uremic toxins and increased levels of parathyroid hormone in patients with chronic renal failure cause tissue resistance to insulin, especially in skeletal muscle, which is mainly due to damage in the processes after insulin binding to the receptor and disrupts glucose metabolism and glycogen production [11-13]. Its prevalence is 10 to 15% in the United States, 11.2% in Australia, 18.7% in Japan, 10.1% in Singapore and 18.9% in Iran [14-16]. This failure is a serious complication that occurs in 5 to 18% of patients admitted to the hospital and also in 30% of patients admitted to the intensive care unit (ICU) [17, 18]. Treatment of renal failure through permanent hemodialysis, in addition to high cost, has many physical and mental problems for the patient [19, 20]. Kidney disorders, including kidney failure, are common problems that cause general damage to the body. In conventional medicine, the control methods for these disorders and the existing treatments in many cases seem inadequate and sometimes ineffective. Studies have shown that medicinal plants have beneficial effects on human health due to their active ingredients and medicinal and antioxidant compounds. Herbs have a therapeutic effect on various organs and diseases, especially cardiovascular diseases, diabetes and hypertension [21-32]. Due to the growing popularity of the society to use traditional and complementary knowledge and medicine, the potential of Iranian ethnobotanical knowledge can be used to find more effective treatment strategies. The aim of this study was to extract medicinal plants used in Iranian ethnobotanical knowledge in order to identify medicinal plants affecting renal failure.

## Method

In this systematic review study, review of articles in Iranian ethnobotanical sources was used. Keywords such as renal failure, renal impairment, hypertension, diabetes, herbs, ethnobotanics, and Iran were used. Databases such as ISI, Web of Science, PubMed, Scopus, ISC, SID and Google Scholar were used to review articles and resources. In this study, 49 articles were searched and found. 4 articles also lacked full text. There were two duplicate articles that were deleted. Finally, 45 articles were reviewed to review the texts. Out of 45 articles, only 23 articles contained ethnobotanical information for the treatment of renal failure.

## Result

Based on the results of reviewing Iranian ethnobotanical texts, it was determined that medicinal plants such as *Lamium album* L., *Origanum vulgare*, *Allium sativum* L., *Berberis vulgaris* L., *Ecbalium elaterium*, *Crataegus monogyna*, *Paliurus spina-christi* Miller., *Rheum ribes* L., *Olea europea*, *Silybum marianum* L., *Teucrium polium* L., *Nigella sativa* L., *Ziziphus jujuba* (L) H.Karst, *Centaurea bruguieriana* Hand. Mzt., *Juglans regia* L., *Brassica Napus* L. and

some other herbs. Are the important medicinal plants used to treat kidney failure and disorders. The list of plants, families, organs used and the province in question is given in Table 1.

**Table 1. Medicinal plants affecting renal failure and additional information about the organ used, Persian name, region used**

| Scientific names | family | Organ used | Region used | Bioactive compounds | Chemical formula |
|---|---|---|---|---|---|
| Allium sativum L. | Aliaceae | Root | West Azerbaijan (33) | **Allicin** | C6H10OS2 |
| Berberis vulgaris L. | Berberidaceae | Leaves and fruit | Arasbaran (34) | Berberine | C20H19NO5 |
| Ecbalium elaterium | Cucurbitaceae | Root and fruit | Arasbaran (34) | kaempferol | C15H10O6 |
| Crataegus monogyna | Rosaceae | Leaves and fruit | Arasbaran (34) | Luteolin | C15H10O6 |
| Paliurus spina-christi Miller. | Rhamnaceae | fruit | Ilam (35) | Quercetin | C15H10O7 |
| Rheum ribes L. | Polygonaceae | Stem | Ilam (35) | Sitosterol | C29H50O |
| Olea europea | Oleaceae | fruit | North East Persian Gulf (36) | Oleoropein | C25H32O13 |
| Silybum marianum L. Gaertn. | Asteraceae | root | Khuzestan (37) | Silydianin | C25H22O10 |
| Teucrium polium L. | Lamiaceae | Aerial parts | Khuzestan (37) | Carvacrol | C10H14O |
| Tragopogon aureus Boiss. | Asteraceae | Leaves and fruit | Khuzestan (37) | n-hexadecanoic acid | C16H32O2 |
| Rumex pulcher L | Polygonaceae | Root | Khuzestan (37) | Inoleic acid | C18H32O2 |
| Arctium lappa L. | Asteraceae | Root, leaves | Khuzestan (37) | 1, 3cyclo-octadiene | C8H12 |
| Nigella sativa L. | Ranunculaceae | Seed | Sistan (38) | 9-eicosyne | C20H38 |
| Suaeda altissima Pall. | Chenopodiaceae | Leaves and stem | North Iran (39) | Alpha-Pinene | C10H16 |
| Silybum marianum (L.) Gaerth. | Asteraceae | Flower | Kazeroon (40) | Silydianin | C25H22O10 |
| Ziziphus jujuba(L) H.Karst | Rhamnaceae | Fruit | Mobarakeh (41) | Jujuboside B | C52H84O21 |
| Echium amoenum L | Boraginaceae | Flower | Mobarakeh (41) | Rosmarinic acid | C18H16O8 |
| Fumaria asepala Boiss | Fumariaceaea | Shoot | Marivan (42) | Protopine | C20H19NO5 |
| Rumex conglomerates Murr | Polygonaceae | Leaves and stem | Natanz (43) | beta-sitosterol | C29H50O |
| Falcaria vulgaris | Apiaceae | flowers and stem | Lorestan (44) | β-caryophyllene | C15H24 |
| Allium ursinum | Asteraceae | Shoot | Lorestan (44) | Thymidine | C10H14N2O5 |
| Anethum graveolens | Apiaceae | Shoot | Lorestan (44) | α-phellandrene | C10H16 |
| Centaurea bruguieriana Hand. Mzt. | Asteraceae | Leaves, flowers | Fars (45) | 9-octadecanoic acid | C18H34O2 |
| Urtica dioica L. | Utricaceae | Root, aerial parts | Maraveh Tappeh (46) | Hexahydrofarnes-ylacetone | C18H36O |
| Cichorium intybus L. | Asteraceae | Whole plant | Kohghiluyeh va Boyer (47) | Sochoside A | C12H22O11 |
| Haussknechtia elymaitica Boiss. | Apiaceae | Aerial part | Kohghiluyeh va Boyer (47) | trans-asarone | C12H16O3 |
| Astragalus fasciculifolius Boiss. | Fabaceae | Flower, root, gum | Kohghiluyeh va Boyer (47) | Isochoifolin | $C_{22}H_{26}O_{11}$ |
| Juglans regia L. | Juglandaceae | Leaves, bulb, fruit, root | Sirjan, Kerman province (48) | linoleic acid | C18H32O2 |

***Cont...*** **Table 1. Medicinal plants affecting renal failure and additional information about the organ used, Persian name, region used**

| | | | | | |
|---|---|---|---|---|---|
| Matricaria aurea (Loefl.) Sch.Bip. | Asteraceae | Leaf and flower | Sirjan, Kerman province (48) | n-Nonadecane | C19H40 |
| Centaurium tenuifolium (Hoffm. & Link) Fritsch | Gentianaceae | Flower, leaves | Hormozgan province (49) | Alpha-Pinene | C10H16 |
| Salvia mirzayanii Rech. F. & Esfand | Lamiaceae | Leaves | Hormozgan province (49) | 1,8-cineole | C10H18O |
| Dysphania botrys (L.) Mosyakin & Clemants | Amaranthaceae | Aerial parts | Mashhad (50) | Camphor | C10H16O |
| Vaccinium arctostaphylos L. | Ericaceae | Fruit | Mashhad (50) | Alpha-Pinene | C10H16 |
| Securigera securidaca (L.) Degen & Dorfl. | Fabaceae | Seed | Mashhad (50) | Cis-9-Octadecanioc acid | C18H34O2 |
| Althaea officinalis L. | Malvaceae | Flowers, roots, leaves | Sardasht, Western Azerbaijan province (51) | Isoquercitrin | C21H20O12 |
| Brassica napus L. | Brassicaceae | Root, seed, leaves | Sardasht, Western Azerbaijan province (51) | Isothiocyanate | C3H5NS |
| Tanacetum parthenium (L.) Schultz-Bip. | Asteraceae | Flower | Toiserkan (52) | Camphor | C10H16O |
| Alhagi pseudalhagi (M.B.) Desf. | Papilionaceae | Aerial part | Khabr and Rouchon (53) | Oxygenated sesquiterpenes | C15H24 |
| Medicago sativa L. | Fabaceae | Stem, Flower juice | Dehe-lolo, Kerman (54) | Alpha-pinene | C10H16 |
| Equisetum arvense L. | Equisetaceae | Whole plant | Zanjan (55) | kaempferol-3-O-sophoroside | C27H30O16 |

## Discussion

Kidney failure occurs due to factors and diseases such as hypertension, diabetes, kidney stones, kidney infection, glomerulonephritis, etc. Medications used to treat kidney failure are usually associated with serious side effects. Therefore, a new approach and strategy for the treatment of renal failure is the use of medicinal plants. Today, natural products obtained from living organisms such as herbs and secondary metabolites are used as one of the most widely used

complementary therapies and prevention in the treatment of renal failure [56]. In different parts of Iran, in the knowledge of traditional medicine, various medicinal plants are used to treat kidney pain. In Abadeh Shiraz (southern Iran) *Amygdalus oreintalis and Achila mellifolium* plants are used for cases of kidney pain. In Arasbaran region (northern Iran) of *Berberis vulgaris* L. and *Equisetum arvense* [57], in Ilam province in western Iran of *Alhagi persarum* and *Lycium depressum* [58], in Khuzestan in southwestern Iran of *Allium haemanthoides*, *Petroselinum crispum* and *Hyoscyamus orthocarpus* [59], In Sistan of *Malva neglecta* and *Alhagi persarum* [60], in Mobarakeh of Isfahan in central Iran from *Petroselinum crispum* and *Matricaria recutita* [61], in Hormozgan (southern Iran) of *Tribulus macropterus* and *Tribulus terresteris* [62] and In Hamedan (southwestern Iran) of *Ceratocephalus falcata* [63] and in Sistan in eastern Iran from *Malva neglecta*, *Zizyphus jujube* and *Alhagi persarum* [64] are used in cases of kidney pain. Some medicinal plants in different regions of Iran have an analgesic effect with medicinal plants in Shahrekord region. According to the results of this study and its comparison with the results of our study, and the emphasis of traditional medicine on the renal analgesic effects of medicinal plants, these plants can have a preventive effect on the treatment of renal disorders and insufficiency because some medicinal plants With renal analgesic effect, they have therapeutic effects on renal failure and disorder. Medicinal plants such as *Marrubium anisodo, Rosa hemispherica*, *Hippophae rhamnoides*, *Viola odorata*, *Haussknechtia elymaitica*, *Achillea wilhelmsii*, *Allium hirtifolium* Boiss., *Melilotus officinalis* (L.), *Silybum marianum*, *Rhus coriaria*, *Echium amoenum*, *Equisetum arvense*, *Ribes khorasanicum*, *Rheum turkestanicum*, *Rosa beggeriana*, *Camellia sinensis*, *Olea europaea*, *Allium sativum*, *Crataegus azarolus*, *Urtica dioica*, *Crataegus pontica*, *Rheum ribes*, *Mentha longifolia* and *Stachys lavandulifolia* are the most important medicinal plants affecting blood pressure in Iranian ethnobotanical documents [65]. In Iranian ethnobotany, medicinal plants such as *Urtica dioica* L., *Teucrium polium* L., *Trigonella foenum-graecum* L., *Centaurea bruguieriana*, *Citrullus colocynthis* (L.), Schrad., And Juglans regia L are used to treat diabetes. Comparison of the results of our study with another study by Asadi Samani et al. (2017) shows that the anti-diabetic plants mentioned in Iranian ethnobotanical studies can have a preventive effect on the treatment of kidney disorders and insufficiency because some medicinal plants have anti-diabetic effects. Therapies are in renal failure and disorder.The present study showed that Iranians from different cultures and in different regions use 40 species of medicinal plants from 25 families based in traditional medicine for the treatment of kidney disorder and this shows that traditional Iranian medicine is rich. Traditional Iranian medicine has long used natural resources to prevent and treat kidney disorders. There are various methods available to use herbs to treat diseases. Findings this study can be a comprehensive guide to the ideas and medicinal plants of different regions of Iran that are effective in treating kidney disorders.

**Authors' contribution**

All authors contributed equally to the manuscript.

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# The Effects of Chemicals Used For Suicide on Insect Succession, Diversity and Development: An Animal Model

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## Abstract

It has proven that the presence of different chemicals can affect the succession patterns of necrophagous insects. A comprehensive study was designed on the effects of nortriptyline, diazinon, and aluminum phosphate on arthropod's succession and diversity on cadavers.

Sixteen rabbits in two groups were used as a model in this study, 12 of them were treated with the drugs and were placed in two habitats (sun and shade) based on the study design. For each group, one cage was considered as control. Insects were collected twice a day from the cadavers and identified.

In total, 549 necrophagous insects from five families, were collected from all the carcasses. *Chrysomya albiceps* was the dominant insect on all cadavers, expect for one treated with diazinon, in which it was displaced by *Dermestes frischii*. The highest number of insects were collected on the sixth day for all cadavers. The majority were collected from the shaded cages.

Generally, the species diversity was higher for all the cadavers treated with the drugs compared to the control cadavers. The results showed that the presence of diazinon in the cadaver can repel necrophagous insects, on the contrary nortriptyline seems to attract more species/specimen.

***Keywords:*** *forensic entomology, arthropods succession, animal model, diazinon, aluminum phosphate, nortriptyline*

## Introduction

Cadavers attract and can be colonized by different developmental stages of arthropods, most notable of them being flies (Diptera), beetles (Coleoptera), mites and isopods (Isopoda). This creates a plethora of opportunities for utilization of forensic entomology, the study of insects and other arthropods of legal importance [1]. Forensic entomology gives us numerous methods to determine the time, place and in some rare cases the cause of death. They are two primary approaches for determination of minimum Post Mortem Interval ($PMI_{min}$), development based and succession based [2].

Aluminum phosphide (AlP), a solid fumigant which has been in extensive use since the 1940s, due to having properties such as, high toxicity and potency, insignificant impact on seed viability and not leaving

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behind considerable toxic residues [3]. Unfortunately, due to relatively easy access and lack of a proper antidote, its widespread use has been accompanied with an astounding rise in alphos poisoning, whether intentional or accidental [4, 5].

Nortriptyline, sharing the same class of pharmaceuticals with amitriptyline, is a tricyclic antidepressant occasionally prescribed in neuropathic pain treatment, which is recommended in European, UK, and USA guidelines [6]. Tricyclic antidepressants are usually lethal in high doses (causing cardiovascular and neurological toxicity) and have been frequently used as a method of suicide and their cure demands considerable therapeutic effort [7-9].

Diazinon (O,O-diethyl-O-[2-isopropyl-4methyl-6-pyrimidinyl]phosphorothioate) is a contact organophosphate (OP) insecticide, with numerous applications in agriculture and horticulture. Several countries have been using ectoparasiticidal formulations of diazinon for sheep and cattle, and in collars used for controlling parasites in pets. There have been multiple concerns about the diazinon`s effects on human health, which led to a restriction of its uses in the USA, but diazinon is still commonly used across the globe [10].

Several studies have been conducted regarding the effect of chemicals present in carcasses on the growth, development, survival of insect larvae and the accumulation in their bodies, which can be immensely useful in determination of PMI [11-14]. Based on the report (Unpublished data) of Iranian national legal medicine research center, organophosphorus pesticides (Diazinon is the most widely used phosphorus insecticide in the region) and some therapeutic drugs for patients (Nortriptyline with most local cases) and Phostoxin tablets (Which are very cheap, effective, and affordable) are the most common chemicals used for suicide in Iran.

In Iran, some studies have also been carried out on the fauna of forensically important insects on corpses (on indoor and outdoor human/animal corpses) in different parts of the country and different geographical conditions [15-17]. In this study for the first time, we have designed a study on the effects of the three commonly used chemicals in suicide on arthropod's succession, diversity and development in an animal model in Iran.

## Materials and Methods

### Study area

Shiraz is the fifth-most-populous city of Iran and the capital of Fars Province. Shiraz is located in the south of Iran and the northwest of Kerman Province. It is built in a green plain at the foot of the Zagros Mountains 1,500 meters above sea level. Shiraz's climate has distinct seasons, and is overall classed as a cold semi-arid climate, though it is only a little short of a hot semi-arid climate or a hot-summer Mediterranean climate. Summers are hot, with a July average high of 38.8 °C. Winters are cool, with average low temperatures below freezing in December and January. Around 300 mm of rain falls each year, almost entirely in the winter months.

This study was done in a garden behind the Shiraz school of health. Two less crowded places were chosen for the placement of cages, one under direct sunlight and the other in shade (29.593033°N, 52.560528°E; 29.592813°N, 52.561107°E).

### Chemicals

In order to simulate the suicide methods, different chemicals including diazinon (technical 98% was purchased from Ariashimi Company), aluminum phosphate (Phostoxin® 56% TB manufactured by QCC), nortriptyline (as hydrochloride 25 mg Tablets manufactured by Abidi Company) were purchased and used in this study.

### Rabbit cages

To prevent interference by scavenger animals, the corpses were placed inside eight custom made steel cages measuring 165 by 160 by 155 $cm^3$. Each

cage contained two corpses that had been killed by a specific chemical (in treatment groups) or method (in the control group). Four cages were placed under direct sunlight in an environment lacking dense vegetation while the other four were placed in shade. In the present study, we hypothesized that the presence of the aforementioned chemicals, exposure to sunlight and location of the rabbit carcasses would affect insect succession and diversity in two different habitats.

### Study design

This study was carried out between June and August 2019. The animal models used in this study were healthy inbred female rabbits (purchased locally from animal house) with the same age, and with an average weight of 650 ± 10 g. Rabbits were chosen for this study because of their suitable size and also as pigs are not available in Iran due to religious restrictions.

We used a total of sixteen rabbits in two groups, the first group (4 cages) under direct sunlight and the second (other 4 cages) in the shade. For each group, one cage was considered as control (this rabbit euthanized by applying blunt force trauma to head, care was taken to minimize external injury) [18] and three cages consisted of individuals were killed using prepared chemicals and considered as treatments. One milliliter (ml) of prepared solution of diazinon 60%, one aluminum phosphate tablet, and 30 nortriptyline tablets dissolved in 10 ml water were used orally per rabbit to kill animal models in the treatment groups.

Each cadaver was placed in a corner of the cage and spaced 1.8 meters apart. Subsequently the corpses were monitored daily (twice a day) for a period of two weeks. Fly larvae were collected and half were killed with almost boiling water and then preserved in 80% ethanol; they were identified using Szpila key [19]. The other half were reared till maturity, and the adult flies were then identified based on their morphological characteristics using valid identification keys. Adult coleopterans found on corpses were sampled and after that identified using the following identification keys [20, 21]. The other arthropods like ants that were not of forensic importance were ignored. Relative humidity and temperature of the sampling location were measured and recorded daily.

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [22].

### Statistical Analysis

All statistical analyses were performed using SPSS software, version 21. The description of the collected insects in terms of order, family, genus, species, abundance and time of collecting, descriptive statistics are provided. Differences between treatments and control were tested for statistical significance using the chi-square (or Fisher exact test) for qualitative variables, and the Student's t-test (or Wilcoxon test) for quantitative variables.

## Results

In total, 549 necrophagous/predatory insects from five families (419 Calliphoridae, 90 Dermestidae, 34 Histeridae, 5 Cleridae, and 1 Staphylindae), were collected from all the carcasses. The majority collected insects (459≈84%) were from the shaded cages and 16% of samples were collected from sunlight cages. More than half of the specimen were collected during night (52.3%). Of the total collected samples, 76.3 % belonged to the Diptera order and 23.7% were Coleopterans. Overall, 305 (55.6%) larvae and 244 (44.4%) adults were collected during this study.

Overall, 10 different species were collected from all carcasses. *Chrysomia alibiceps* with 68.1% frequency was the dominant species and *Creophilus maxillosus* (0.2%) was the least collected species (Table 1). Among all groups, number of collected insects in the nortriptyline-treated group was the highest (33.5%) and in the diazinon group this amount was the lowest (12.8%). In total, the largest number of specimens were collected on day 6, which is considered as the peak of necrophilic insects' activity on the tested carcasses in the geographical conditions

of the study site.

The effect of different chemical compounds tested on the carcasses showed that, the abundance of collected arthropods in control group had a significant difference with the diazinon (P value= 0.00) and nortriptyline (P value= 0.023) treated groups, but there was no significant difference (P value= 0.78) in the frequency of collected insects between the control and aluminum phosphate groups. Number of collected insects according to kind of treatment, time of sampling (day and night), species composition, and location of the corpses (in the sunlight and shade) are shown in figures 5-8.

More species were sampled from the shaded cadavers (Figure 1), except for *Lucilia sericata*, *Lucilia ampulacea*, and *Necrobia rufipes* that were often collected from the carcasses placed under direct sunlight. Most flies were collected during the day, while a considerable increase in the number of collected beetles was observed during night.

The aluminum phosphate-treated cadavers had the highest diversity among all carcasses with nine collected species (30%) followed by diazinon and nortriptyline-treated cadavers (8, 26.5%), but only five species (17%) were sampled from the control cadavers.

Percentage of all collected specimens from the two common necrophagous orders from each corpse is presented in Figure 2. The trend of the number of collected insects (specimen) in each of the treated groups based on days after treatment is shown in Figure 3. Also, number of collected samples in each of the treated groups by their families are shown in Figure 4.

On the first day blow flies were observed ovipositing near the mouth and eyes of rabbit cadavers, no fly larvae or adult beetles were collected on this day. Only two species of forensically important insects were collected on day 2, *Dermestes frischii* (Order Coleoptera, family Dermestidae) and *Chrysomya albiceps* (Order Diptera, family Caliphoridae). *Chrysomya albiceps* were collected from the diazinon-treated cadavers, on day 2, making them the first observed Diptera, while *Dermestes frischii*, the first observed Coleoptera, were collected from the aluminum phosphate-treated corpses on the same day; no insects of forensic importance were collected from the nortriptyline-treated and control corpses on this day.

*Dermestes frischi* was the dominant species on the diazinon-treated group, while *Chrysomya albiceps* was the most abundant insect in other treatment groups (Table 1). *Creophilus maxilosus* (Order Coleoptera, family Staphlinidae) was only collected from the diazinon corpses. No insects belonging to the Cleridae family were collected from the control group. *Lucilia silvarum* (Order Diptera, family Calliphordiae) was only collected from the aluminum phosphate-treated corpses. No *Lucilia ampulacea* was collected from the control corpses. The highest number of insects were collected on day 6 on all but the aluminum phosphate-treated corpse, making it our collection peak, after which a sharp decline was observed (Figure 3).

## Appendices:

**Table 1. Abundance of all collected necrophagous species from each corpse in different treatments groups**

| | Kind of species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Type of treatment | L. sericata | C. albiceps | L. illustris | L. ampulacea | L. silvarum | D. frischii | C. maxillosus | Hister spp. | N. rufipes | E. strobelli | Total | Percentage (%) |
| Diazinon | 0 | 17 | 4 | 2 | 0 | 32 | 1 | 4 | 1 | 9 | 70 | 12.75 |
| Nortriptyline | 3 | 129 | 3 | 5 | 0 | 33 | 0 | 1 | 3 | 7 | 184 | 33.51 |
| Aluminum phosphate | 9 | 97 | 6 | 7 | 3 | 18 | 0 | 1 | 1 | 4 | 146 | 26.59 |
| Control | 0 | 131 | 3 | 0 | 0 | 7 | 0 | 1 | 0 | 7 | 149 | 27.14 |
| Total | 12 | 374 | 16 | 14 | 3 | 90 | 1 | 7 | 5 | 27 | 549 | |
| Percentage (%) | 2.18 | 68.12 | 2.91 | 2.55 | 0.54 | 16.39 | 0.18 | 1.27 | 0.91 | 4.91 | | |

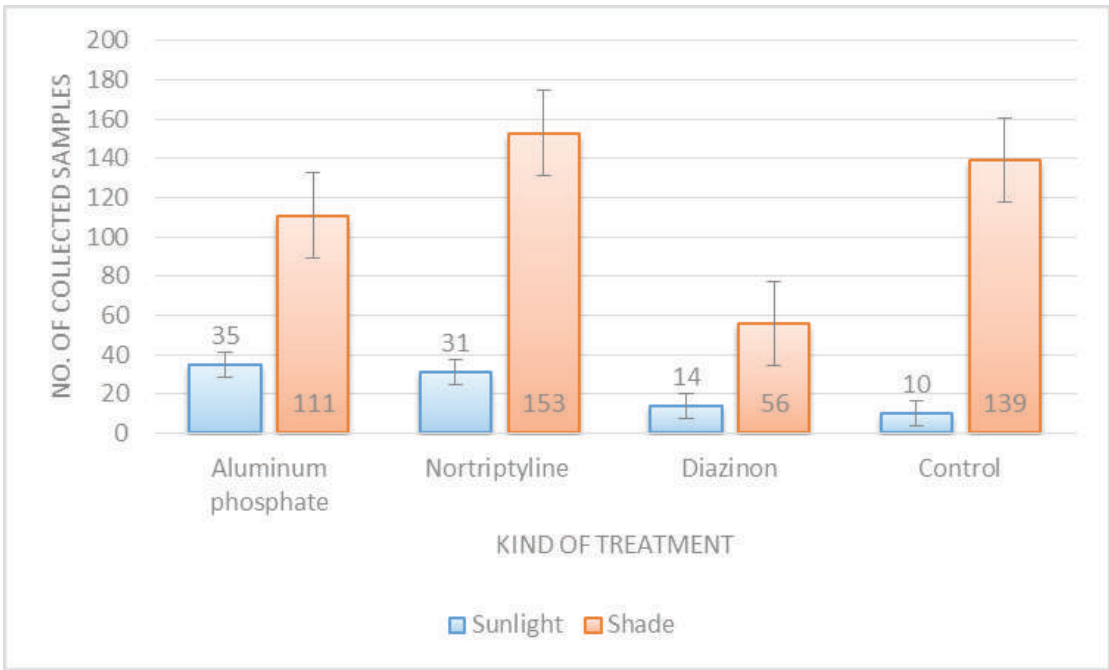


**Figure 1. Number of collected arthropods according to kind of treatment and location of the corpse (in the sunlight and shade)**

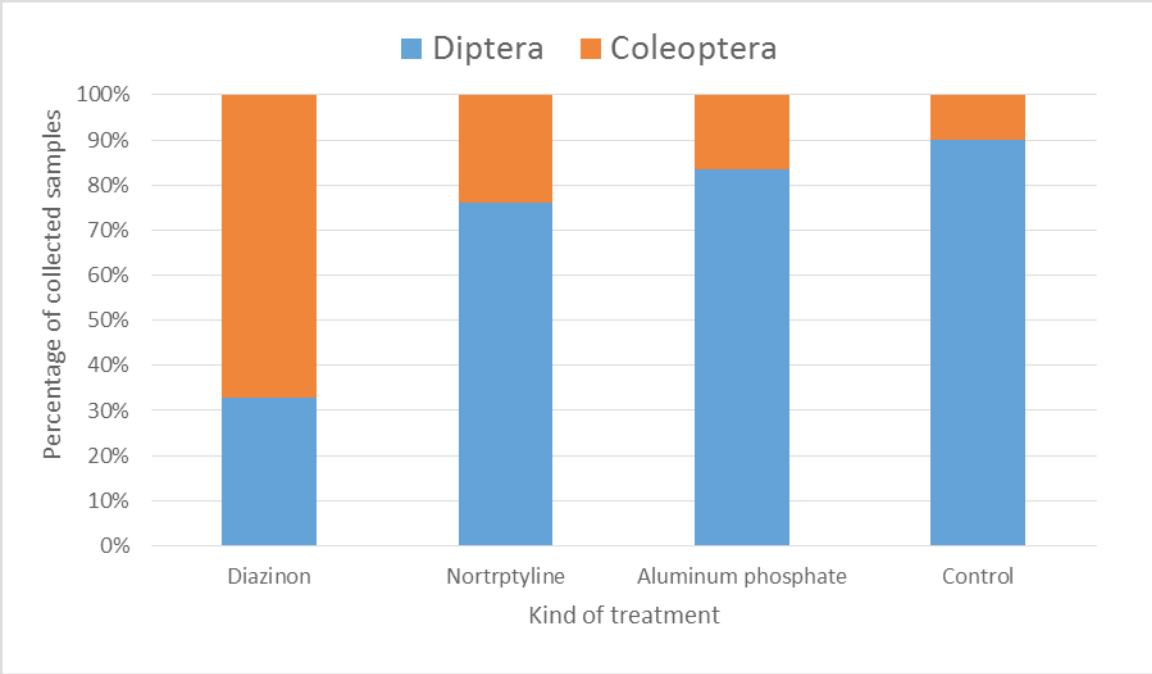


**Figure 2. Percentage of collected insect orders in different treatment groups**

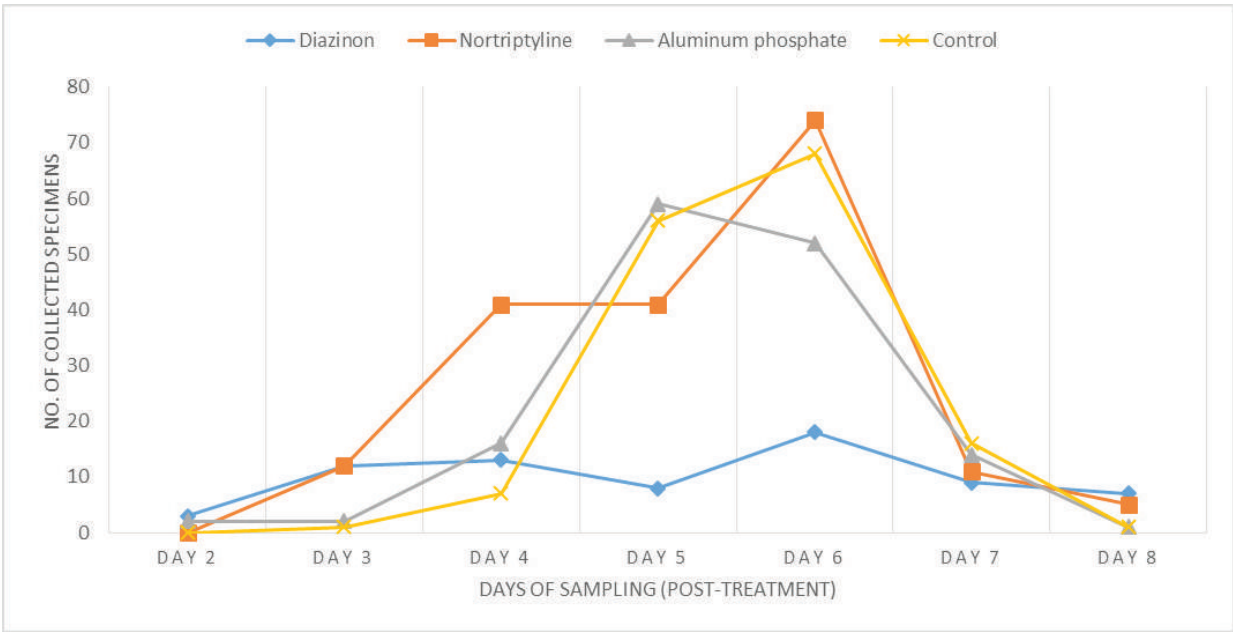


**Figure 3.** Number of collected insects from all tested groups according to post-treatment sampling date

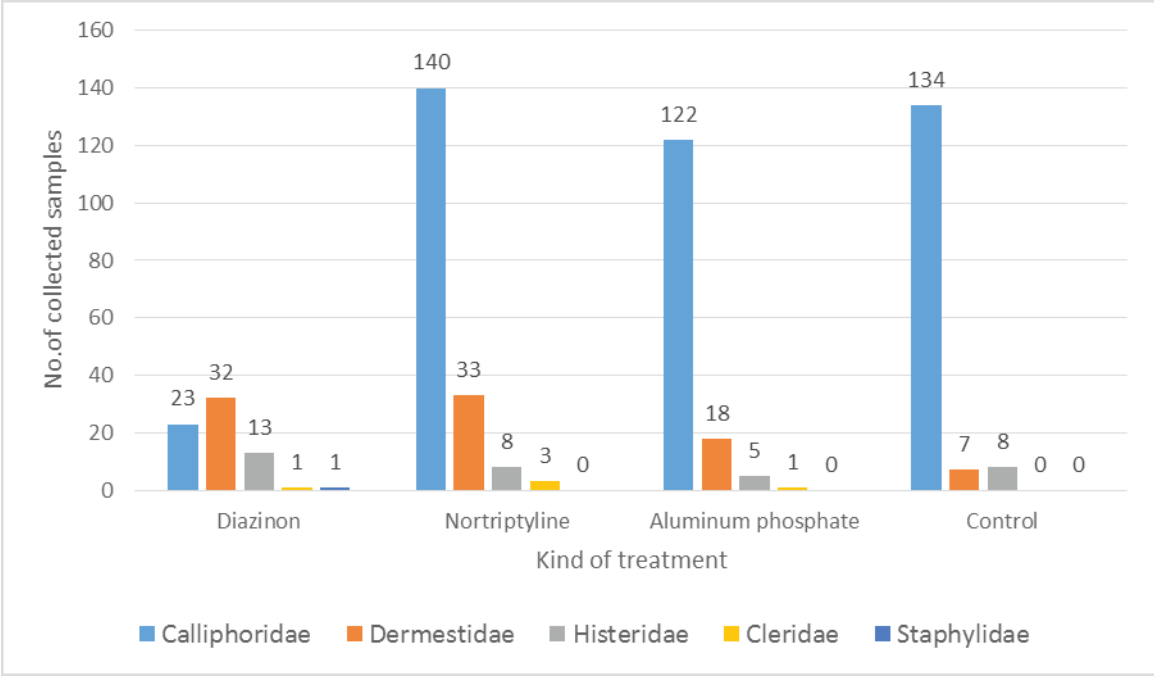


**Figure 4. Number of collected samples in each of the treated groups by their families**

## Discussion

Previous research has demonstrated that the oviposition site selection done by female blowflies can be influenced by the presence of chemicals such as albuminous protein, microbial products associated with larval feeding [23] and several other compounds released throughout the stages of corpse decomposition [24]. Furthermore, bait aging and the level of available nutrients have been proven to be key factors affecting colonization pattern [25].

In this study entomological data regarding the effects of 3 chemicals, which have been associated with a number of self-inflicted or accidental poisonings, on insect succession, diversity, and development was collected. 10 insect species belonging to 5 families were sampled. In our study, the collection peak between aluminum phosphate-treated corpses and other cadavers was different, which suggests the effect of this chemical on insects' activity peak as well as their succession pattern. Furthermore, the most diverse range of *lucilia spp.* were collected from the aluminum phosphate-treated corpses. The results showed that the presence of aluminum phosphate inside the carcass can hasten the insect activity peak and shorten their life cycle. No significant effect was observed on insect development by the other tested chemicals in this study.

The results of this study showed that, in general, diazinon poisoning has a repulsive effect and reduces the tendency of necrophagous insects to settle in the affected corpses. As a slight increase was detected in the number of the collected insects from the nortriptyline corpse, it is possible that this chemical or it`s metabolites can attract necrophagous insects and increased their abundance.

We observed a considerable difference between the number and diversity of the collected insects in the two sampling times (day and night). The majority of the coleopteran species were collected during the night, possibly due to their nocturnal behavior. During the day, non-treated corpses were visited by a high number of flies, similar to a study done by Singh [26], while other treated cadavers experienced different patterns.

Based on our results, in the control group, the frequency of collected species during day was higher than that of night. While, in treatment groups, the results were completely the opposite. These results reinforce the idea that the type of chemical present in the carcass can influence the behavior (diurnal and nocturnal activity) of necrophagous arthropods. Our results indicated that *Lucilia illustris* and *Chrysomya albiceps* were diurnal. But *Lucilia sericata*, *Dermestes frischii*, and *Euspilotus strobeli* were reported as nocturnal species. Other species showed equal diurnal and nocturnal activities. Based on the results, we can conclude that the type of toxicants can greatly affect the rate of insect activity during the day or night.

As *Chrysomya albiceps* managed to be the dominant species on all but the diazinon corpse, like results from a study done by Salimi [27], it is possible that the ingested diazinon had a repellent effect on this species, similar to an effect previously achieved when the outer surface of the carcasses was sprayed by the aforementioned chemical. [28] or perhaps attracted *Dermestes frischii*, a possible predator. As *Lucilia ampullacea* was collected from all but the control cadavers, it is possible that the addition of these chemicals acted as an attractant or hindered the rival species.

In all treated and control cadavers except the diazinon-treated group, the proportion of collected Diptera species was higher than that of Coleoptera (Figure 2). We can probably attribute this phenomenon to the possible attractant effect of diazinon on necrophagous coleopterans or perhaps other added chemicals increased the corpse's suitability as a possible oviposition site for Calliphorids.

Our results indicated that the type of chemical used to commit suicide influenced insect colonization. Diazinon attracted *Hister spp.*, *Euspilotus strobeli*, and *Creophilus maxillosus* species. Whereas, nortriptyline attracted only *Chrysomya albiceps*. Also, aluminum phosphate attracted *Lucilia sericata, Lucilia illustris, Lucilia silvarum,* and *Lucilia ampullacea*. Also, *Creophilus maxillosus, and Lucilia silvarum* are specific species respectively associated with diazinon and aluminum phosphate compounds. Another point to consider is that *Dermestes frischii* tends to be present in carcasses that are infused with various chemicals.

All corpses placed in the shaded cage experienced an increase in the number of collected Diptera and Coleoptera, compared to the corpses in the sunlight cage, expect the nortriptyline and aluminum phosphate-treated corpses, in which the sunlight cage managed to attract more coleopterans. The diazinon and control corpses placed in the shaded cage showed lower diversity than their counterparts which were exposed to sunlight, but this was reversed in the aluminum phosphate and nortriptyline-treated corpses, similar to the results of a study done by Goff [29]. Generally, 84 % of the samples were collected from shaded cages. This indicates that species abundance in shaded carcasses is 4 times higher than those placed in sunlight. This result indicates that carcass placement in roofed and shaded areas will increase the abundance of insects on cadavers under geographical conditions tested in this study.

The very first colonizers of the aluminum phosphate-treated corpses were the coleopteran *D. frischii,* conversely the diazinon corpse attracted three different species, two coleopterans, both members of the Histeridae family (*Euspilotus strobili* and *Hister spp.)*, and *C. albiceps*; with *C. albiceps* being the very first colonizer. A plausible explanation can be that the aluminum phosphate ingested by the rabbit prior to death created an unsuitable environment for oviposition of *C. albiceps*.

It was also found that the species composition of the collected insects by order was significantly different in the diazinon-treated group compared to the other tested groups. Diazinon often attracted Coleopterans (especially Dermestidae family). While in the other tested groups, the dominant species were necrophagous dipterans belong to Calliphoridae

family.

In this study, a notable difference between the number and species of insects attracted to each corpse was detected, which can be useful in forensic entomology. Furthermore, all the tampered corpses experienced a more diverse range of colonizing necrophagous arthropods (especially in the aluminum phosphate-treated group), proving the hypothesis that the presence of these chemicals can affect insect diversity, with nortriptyline affecting both the number and the diversity of insects.

Based on the results of this study, it can be concluded that without considering the effects of chemicals that cause death, forensic entomology studies alone cannot be reliable. In all calculations and interpretations used in forensic entomology, especially for estimation of the postmortem interval (PMI), the effects of different chemical constituents and their metabolites on the behavior, abundance, succession, diversity, and development of forensically important arthropods in different geographical conditions should be considered. Therefore, it is suggested that similar comprehensive studies should be carried out in each region and the effects of most commonly used chemicals for suicide should be precisely determined to better understand the main role of these compounds and their metabolites on different aspects of forensically important insect's life cycle.

**Declarations**

**Funding:** Not applicable.

**Conflicts of interest/Competing interests:** No conflict of interest to declare.

**Consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Availability of data and material:** All data are available.

**Code availability:** Not applicable.

**Ethical approval:** The project was done in accordance to the ethical principles and the national norms and standards for conducting Medical Research in Iran. The study was approved by Iran national Committee for Ethics in biomedical research (Approval ID: IR.SUMS.REC.1397.588).

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# Hippocampal Volume and Entorhinal Cortex Thickness in Alzheimer's Disease

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## Abstract

Magnetic Resonance Imaging (MRI) is the standard imaging evaluations in Alzheimer's Disease (AD). Information regarding hippocampus and entorhinal cortex sizes plays an important role in Alzheimer's disease. This study aims to determine hippocampal volume and entorhinal cortex thickness in Alzheimer's disease obtained from a group of patients who underwent head MRI.

This study was an observational study with retrospective approach in patients who were diagnosed with AD and had available head MRI examination results. A total of 14 patients were diagnosed by a neurologist with AD using MMSE, Hachinsky, and NIA-AA criteria.

From head MRI measurement in AD patients, we found that the mean volume of right, left, and total hippocampal was 1700±0.395 $cm^3$; 1.670±0.349 $cm^3$; and 3.370±0.725 $cm^3$, respectively. The mean thickness of right, left, and total entorhinal cortex was 1.821±0.459 mm; 1.463±0.369 mm; and 3.285±0.791 mm, respectively. There is a possible difference between the early and late stages of AD in the same patient. Further studies with larger cohorts are needed to examine these correlations.

***Keywords****: Alzheimer's Disease, Head MRI, Hippocampus, Entorhinal Cortex*

## Introduction

Alzheimer's disease is a progressive brain degenerative disease characterized by a gradual decline in memory or a disturbance in one of the highest intellectual functions[(1)]. Alzheimer's disease (AD) was the sixth leading cause of death in United States by Centers for Disease Control Prevention (CDC), accounting for 83,494 deaths in 2010[(2)].

Currently, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are the standard imaging evaluations in dementia. The most common MRI sequences are as follows: T1-Weighted Imaging (T1-WI), T2-Weighted Imaging (T2-WI), Fluid Attenuated Inversion Recovery (FLAIR), Diffusion Weighted Imaging (DWI), Gradient Echo (GRE) and acquisitions 3-Dimensional (3D) volume. Contrast imaging, Diffusion Tensor Imaging (DTI), Arterial Spin Labeling (ASL), and functional MRI might be useful but are not routinely performed[(3)].

Entorhinal cortex is a cortex that is partially covered by the rhinal (olfactory) sulcus. This cortex is the main part of medial temporal lobe, which plays an important role in memory system and the main connection between hippocampal formation and neocortex[(4)].

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Hippocampus is a cortical structure and is a part of medial temporal lobe where it is located inferiorly to lateral ventricle. This structure is involved in memory and neuroendocrine regulation. The hippocampus is a primitive cortex that plays a central role in memory processes with a relatively simple structure[4].

Information regarding hippocampus and entorhinal cortex sizes plays an important role in Alzheimer's disease. This study aims to determine hippocampal volume and entorhinal cortex thickness in Alzheimer's disease obtained from a group of patients who underwent head MRI.

## Materials and Methods

### *Study Design*

This study was an observational study with a retrospective approach that was conducted from July to December 2020 at a nursing home and a health care facility in Surabaya, Indonesia.

### *Study Subject*

The study subjects were patients who were diagnosed with AD and met the inclusion and exclusion criteria. The inclusion criteria were all patients who were diagnosed by a neurologist with AD using MMSE, Hachinsky, and NIA-AA criteria and had available head MRI examination results. The exclusion criteria included individuals with a history of head trauma within the last 6 months and was found features of infarct and/or hemorrhagic stroke and/or tumor on their head MRI.

### *Data Collection*

Demographic data and individual examination results were collected. In addition, data from individual head MRI examinations results were also collected.

The MRI machine used was General Electric (GE) Optima 360 1.5 T with GE Medical System software number 5394794-32. The MRI data used were taken from coronal slices of 3D-FSPGR MRI sequences. Entorhinal cortex thickness and hippocampal volume were measured with the following MRI settings: 3-Dimentional (3D) coronal slice, Fast Spoiled Gradient Echo (FSPGR), Time Echo (TE): 6, Time Repetition (TR): 13.2, Field of View (FOV): 24, slice thickness: 1.6, matrix: 320x192, bandwidth: 1563, and Inversion Time: 400.

Hippocampal volume and entorhinal cortex thickness measurements were performed manually using GE's Picture Archiving and Communication System (PACS) software with a single tracer. Hippocampal volume was measured in cubic centimeters ($cm^3$), while entorhinal cortex thickness was measured in millimeters (mm).

The assessment was conducted by two neuroradiology consultants, where each assessor did not know the patient's identity and data (double-blinded).

### *Data Analysis*

The obtained data and hippocampal volume and entorhinal thickness measurement results were analyzed using SPSS version 21.0 software. The data analysis used descriptive statistics.

## Results

### *Subject Characteristics*

A total of 14 patients were diagnosed by a neurologist with AD using MMSE, Hachinsky, and NIA-AA criteria. Most AD patients were women with nine patients (64.3%).

The youngest patient with AD who underwent MRI examination was 61 years and the oldest was 85 years. The mean age of AD patients who underwent MRI examination was 75.14±8.085 years. Alzheimer's disease patients were then divided into two age groups, which were under and over 65 years. The results showed that Alzheimer's disease patients were mostly found in the age group of over 65 years with a total of 12 patients (85.7%).

Alzheimer's disease patients were also divided into two groups based on their latest education background, which were non-undergraduate and undergraduate groups. The results showed that most patients with Alzheimer's disease were in non-undergraduate group with 10 patients (71.4%). Data on subject characteristics of are presented in Table 1.

**Table 1. Subject Demographic Characteristics**

| Characteristics | AD patients (N=14) |
|---|---|
| Mean age (years) | 75.14 (8.085) |
| Gender (Male/Female) | 5/9 |
| Latest Education Background (Non-undergraduate/Undergraduate) | 10/4 |
| Duration of symptoms (years) | 1.893 (1.318) |
| MMSE score | 19.07 (4.16) |

***Hippocampal Volume and Entorhinal Cortex Thickness in AD Patients***

Hippocampal volume and entorhinal cortex thickness measurements using head MRI were performed by two assessors. Kappa test was then performed on the first and second measurement results both on hippocampal volume and entorhinal cortex thickness. The kappa test values on hippocampal volume and entorhinal cortex thickness measurements were 0.714 and 0.851 respectively, with a significance value of 0.001 and 0.001. Results of the two assessors were then averaged, both for the hippocampal volume (right hippocampus, left hippocampus, total hippocampus) and entorhinal cortex thickness (right entorhinal cortex, left entorhinal cortex, and total entorhinal cortex).

Hippocampal volume and entorhinal cortex thickness measurements in AD patients were presented in the form of a box plot diagram to determine the existence of outliers. From the box plot diagrams on right, left, and total hippocampal volumes, no outlier was found (Figure 1a). Box plot diagrams on right, left, and total entorhinal thicknesses showed no outlier either (Figure 1b).

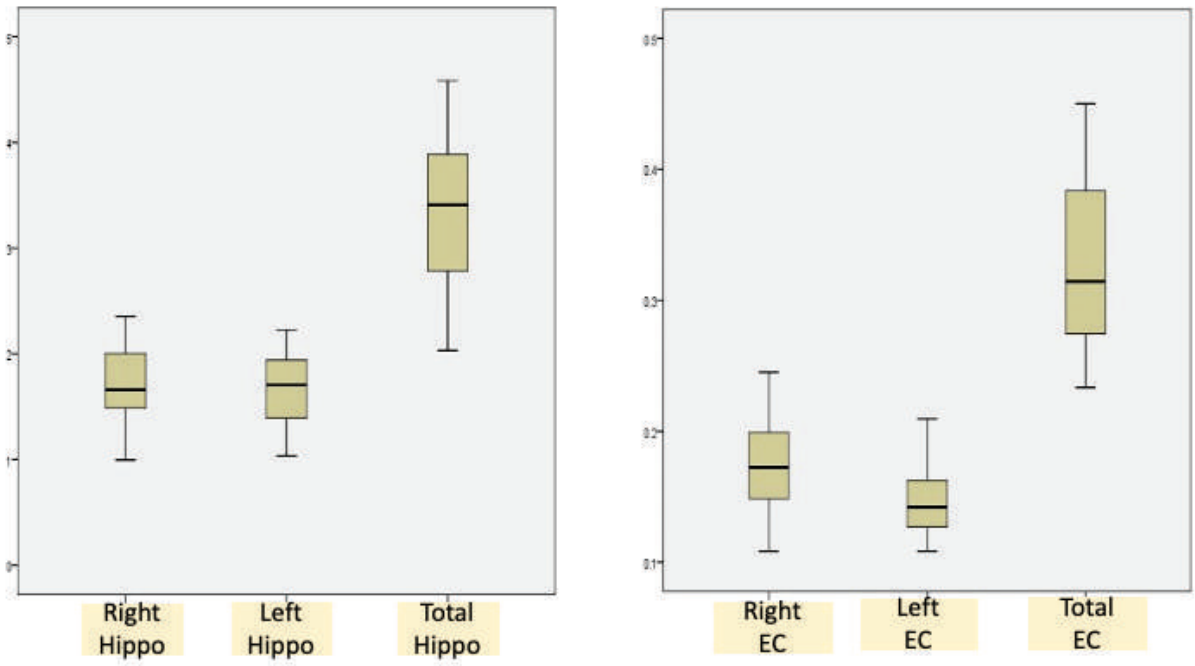


**Figure 1. a. Box plot diagrams on right, left, and total hippocampal volume, b. Box plot diagrams on right, left, and total entorhinal thickness.**

From head MRI measurement in AD patients, we found that the mean volume of right, left, and total hippocampal was 1700±0.395 $cm^3$; 1.670±0.349 $cm^3$; and 3.370±0.725 $cm^3$, respectively. The mean thickness of right, left, and total entorhinal cortex was 1.821±0.459 mm; 1.463±0.369 mm; and 3.285±0.791 mm, respectively. This data is presented in Table 2.

**Table 2. Mean Entorhinal Cortex Thickness and Hippocampal Volume**

| Mean Size | Alzheimer's Disease Patients (N=14) |
|---|---|
| Right Hippocampal Volume (cm3) | 1.700 (.395) |
| Left Hippocampal Volume (cm3) | 1.670 (.349) |
| Total Hippocampal Volume (cm3) | 3.370 (.725) |
| Right Entorhinal Cortex Thickness (mm) | 1.821 (0.459) |
| Left Entorhinal Cortex Thickness (mm) | 1.463 (0.369) |
| Total Entorhinal Cortex Thickness (mm) | 3.285 (0.791) |

## Discussion

The youngest AD patient who underwent head MRI examination was 61 years old and the oldest was 85 years old. In this study, subjects suffering from Alzheimer's disease were divided into two age groups, which were <65 years and >65 years. The age group <65 years was included in the EOAD group (early onset AD) and the age group >65 years was included in the late onset AD or LOAD group[5]. Most patients with Alzheimer's disease were at above 65 years with a total of 12 patients. The age distribution in this study was the same as a study conducted by Van Der Vlies et al.[6], in 2009 where people with Alzheimer's disease above 65 years old had a greater proportion than people with Alzheimer's disease below 65 years old.

From a total of 14 patients with Azheimer's disease who underwent head MRI examinations, it was found that the incidence rate was higher for women than men. In this study, there were 9 female patients and 5 male patients. The gender distribution in this study was similar to a study from Viña and Lloret[7] in 2010 regarding the proportion of gender in people with Alzheimer's disease. The main risk factors for the development of AD are age and gender. The incidence of Alzheimer's disease was higher in women than men, and this could not simply be attributed to a higher life expectancy in women than in men. The explanation regarding the pathological process of higher incidence of Alzheimer's disease in women must be clearly explained. Viña and Lloret[7] explained that mitochondria in young women are protected from amyloid-β toxicity, less Reactive Oxygen Species (ROS) production by mitochondria, and less apoptogenic signal release. These mitochondrial protections were caused by the presence of estrogen hormone which protects cells from amyloid-β toxicity. Furthermore, estrogen level plummets in older women, reducing mitochondrial protection.

From 14 patients with Alzheimer's disease in this study, most patients (10 patients) had non-undergraduate education. With this data, it could be concluded that most of the study subjects had a lower level of education in this study. The result in this study was similar to a study by Sharp and Gatz[8] in 2011. This study[8] is a systematic review that analyzed a total of 88 studies, where 27 studies analyzed the correlation between education background and AD, 37 studies analyzed the correlation between education and total dementia, and 24 studies analyzed the correlation between the two (AD and total dementia). In this study, 51 studies (58%) reported a significant effect of low education on dementia risk, whereas 37 studies (42%) reported no significant correlation between low education and dementia outcome.

From the head MRI examination in Alzheimer's disease patients, hippocampal volume of entorhinal cortex thickness measurement was conducted using FSPGR sequence on coronal slices using manual tracer technique. From the head MRI measurement, it was found that the mean hippocampal volume in the right, left, and total hippocampus were 1.700±0.395 cm3; 1.670±0.349 cm3; and 3.370±0.725 cm3, respectively. The mean entorhinal cortex thickness in the right, left, and total entorhinal cortex were 1.821±0.459 mm; 1.463±0.369 mm; and 3.285±0.791 mm, respectively.

The mean hippocampal volume in this study has a minimal difference in a study from Dhikav et al.[9], where the results of the hippocampal volume were 1.64±0.55 cm3 in the right hippocampus and 1.59±0.55 cm3 in the left hippocampus. In another study from Vijayakumar and Vijayakumar[10] and Leandrou et al.[11], the hippocampal volume had a fairly wide margin, especially in a study from Leandrou et al.[11] because the study population did not represent the Asian race. Another factor that might have an effect is the severity of Alzheimer's disease patients in the study population and the size of the study population. The entorhinal cortex thickness in this study has a minimal difference in a study from

Holbrook et al.[12], where the mean entorhinal cortex thickness was 1.97±0.19 mm. In another study from Velayudhan et al.[13], the mean entorhinal cortex thickness had a fairly significant difference with of the entorhinal cortex thickness in this study, which 2.6±0.53 mm.

This study has several limitations. The number of subjects in this study was not large due to the high cost of MRI examinations and this examination is not covered by the patient's health insurance. Patients with severe Alzheimer's condition or restlessness could not have an MRI examination without the aid of anesthesia. It is possible that patients with severe conditions have lower hippocampal volume and entorhinal cortex thickness.

## Conclusion

From the hippocampal volume measurements, it was found that the right, left, and total hippocampal volumes were 1.700±0.395 cm3; 1.670±0.349 cm3; and 3.370±0.725 cm3, respectively. Meanwhile, from the entorhinal cortex thickness measurements it was found that the right, left, and total entorhinal cortex thickness were 1.821±0.459 mm; 1.463±0.369 mm; and 3.285±0.791 mm, respectively.

There is a possible difference between the early and late stages of AD in the same patient, therefore the decrease in hippocampal volume and entorhinal cortex thickness could not be evaluated. Furthermore, continuous measurement of hippocampal volume and entorhinal cortex thickness might help predict AD progression and initiate early treatment. Further studies with larger cohorts are needed to examine these correlations.

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Ethical clearance

The protocol of this study was approved Institutional Ethics Committee, Faculty of Medicine, Airlangga University, Surabaya.

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# The Higher Level of Neutrophil – Lymphocyte Ratio (NLR) and Serum Syndecan-1 Based on Timeline (First, Sixth, and Twenty-Fourth Hour) in Sepsis-Induced Acute Kidney Injury

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## Abstract

This study aims to analyze the difference between the NLR and serum syndecan-1 level at the first, sixth, and twenty-fourth hours with the incidence of acute kidney injury (AKI) and non-AKI in sepsis. We observed thirty-one adult sepsis patients who admitted to the emergency room and ICU of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from March 30, 2020, to July 5, 2020 (four-month), and differentiated into two groups, AKI group (n=14) and non-AKI group (n=17). All septic patients have been given the standard treatment based on Survival Sepsis Campaign 2018. The results showed that the NLR at 1st-hours, 6th-hours, and 24th-hours in the non-AKI group were lower than those in the AKI group (1st: 15.9±2.6 vs 33.0±11.3; 6th: 14.2±1.8 vs 19.3±3.8; and 24th: 11.6±1.3 vs 19.9±3.8) ($p < 0.00$). Almost NLR at every groups based on serial hours were decreasing, except in the AKI group, the NLR at the 24th-hour group was higher than the NLR at 6-hour group, but still lower than the NLR at 1st-hours. Serum syndecan-1 levels were lower in the non-AKI group than in the AKI group, at 1st, 6th, and 24th-hours, (1st: 532.5±72.0 ng/mL vs 597.2±85.8 ng/mL; 6th: 557.6±71.6 ng/mL vs 612.0±73.9 ng/mL; and 24th: 548.4±76.6 ng/mL vs 586.4±84.6 ng/mL) ($p < 0.05$). It can be concluded that the neutrophil-lymphocyte ratio (NLR) and serum syndecan-1 levels at 1st, 6th, and 24th hours is higher in the AKI compared to non-AKI groups in sepsis. Hence, NLR and serum syndecan-1 have a potential biomarkers for sepsis-induced AKI.

***Keywords:*** *sepsis, acute kidney injury, neutrophils-lymphocytes ratio, syndecan-1, mortality*

## Introduction

Sepsis is an emergency condition which described as a systemic immune response of the host that leads to organ damage and death caused by infection. Although the technology of hemodynamic monitoring and resuscitation improved significantly, it still does not change a lot the morbidities and mortalities evidence [1,2]. The worldwide incidence of sepsis is around 707 cases per 100,000 people with an in-hospital mortality rate of 20 – 43% [3]. The incidence of Sepsis-Induced acute kidney injury (S-AKI) is around 19 million cases worldwide and about 1 in 3 cases of sepsis will become acute kidney injury (AKI) with a significantly higher mortality rate of around 44% [4].

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Neutrophils and lymphocytes have an important role in inflammation and tissue damage. The role of neutrophils and lymphocytes in AKI has been recognized as an important modulator of the immune response. Ischemic, nephrotoxic, and AKI caused by endotoxemia are associated with an increase in neutrophils influx into the kidney [5]. Increase in the neutrophil-lymphocyte ratio (NLR) has been reported to be a useful prognostic marker for several diseases, include for sepsis. The NLR is an examination that is simple, easy to do and inexpensive, so the use of the results of the NLR examination as a predictor of the incidence of AKI is promising and has the potential as a parameter to differentiated the degree of AKI in sepsis [6].

Sepsis could cause endothelial injury, of which Syndecan-1 is one of the biomarkers, leading to endothelial glycocalyx degradation, vasodilation, capillary leakage, and hypoperfusion leading to AKI [7–9]. Increased serum syndecan-1 levels in sepsis and septic shock patient is associated with higher Sequential Organ Failure Assessment (SOFA) score and Acute Physiology And Chronic Health Evaluation *II* (APACHE II) score, increased length of stay, severity, and mortality [10–12]. Nevertheless, the NLR and serum Syndecan-1 level based on timeline in S-AKI to predict the recovery or severity are unclear. Hence, the objective of this research is to analyze the difference the NLR and serum Syndecan-1 level at the first, sixth, and twenty-fourth hours with the incidence of AKI and non-AKI in sepsis, which is cheaper and more practical.

## Material and Methods

Approval for this research has been obtained from Health Research Ethics Committee Dr. Soetomo General Academic Hospital (No.1807/KEPK/I/2020).

### Samples

Thirty-two adult sepsis patients who admitted to the emergency room and ICU of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia from March 30, 2020, to July 5, 2020 (four-month), and had met the inclusion criteria and not met exclusion criteria.

The inclusion criteria were 18th – 65th year sepsis patients who meet the criteria based on The Third International Consensus Definitions for Sepsis and Septic Shock 2016 (Sepsis-3). A critically ill septic patient not eligible to make their own decisions, so the guardian and/or patient's family (at least 2 persons) stated their willingness that the patient was included in the study by signing information for consent and an informed consent approval.

The exclusion criteria were the evidence of the history of vascular disorders, chronic kidney disease, dyslipidemia or using dyslipidemia drug in the statin or non-statin class. A diagnosis of sepsis for referred hospital previously and/or one-hour time zero of sepsis diagnose had passed after the triage.

All septic patients gave the standard treatment based on sepsis standard management protocol at Dr. Soetomo General Academic Hospital. We observed and collected the data in the first twenty-four hours, such as the history of the patient, comorbid, SOFA Score, APACHE Score, physical examination, vital sign, fluid resuscitation, fluid balance, routine laboratory – radiology examination, and diagnosis. Blood sampling was taken for NLR and syndecan-1 serum at first, sixth- and twenty-fourth hours of treatment. NLR was measured by dividing the absolute neutrophil with lymphocyte count from complete blood count result. For syndecan-1 sampling, the blood was centrifuged then examined with *Cusabio Human Syndecan-1/CD138(SDC1) ELISA Kit* reagent. Thirty-one samples were included in this research. AKI was diagnosed by Kidney Disease: Improving Global Outcomes (KDIGO) criteria, using creatinine and urine productions parameter, at early sepsis were diagnosed, and evaluated in 24 h of treatment.

## Statistical Analysis

The data were analyzed with SPPS software. Normality test was performed to evaluate data

distribution. The Independent-T test and the multivariate – ANOVA were used to analyze the difference between groups and serial variables. The Chi-square and the wilcoxon-mann whittney test were applied when it was not normally distributed or the data was in ordinal scale.

## Results

In the AKI group, men are dominant compare to women (10 vs 4) and in the non-AKI group women more dominant than men (5 vs 12) (p = 0.022). Age, weight, height, and BMI were the same in both groups and there was no statistically significant difference. The SOFA score in the AKI group was higher than the non-AKI group, both at admission evaluation and at 24-hour evaluation (admission p = 0.003; 24 hours p = 0.001). There was no significant difference between the two groups for the lactate level. Mortality in the AKI group was 38.7% higher than the non-AKI group with 22.6%. In the AKI group, the highest mortality was at 7 days mortality (19.4%) and in the non-AKI group, 7-day mortality was much lower (9.7%). Mortality and survival in the two groups were statistically significant (p = 0.023) (Table 1).

**Table 1. Sample Characteristic.**

| Variable | Group | | p value |
|---|---|---|---|
| | Non-AKI (n=17) | AKI (n=14) | |
| Sex | | | 0.022* |
| Man (n) | 5 | 10 | |
| Women (n) | 12 | 4 | |
| Age (year) | 46.7±14.7 | 48.9±13.8 | 0.218 |
| Body Weight (Kg) | 60.0±8.6 | 62.5±10.5 | 0.094 |
| Body Hight (cm) | 158.3±8.1 | 160.9±7.6 | 0.098 |
| BMI | 24.1±4.0 | 24.1±3.7 | 0.293 |
| SOFA score (admission) | 4.7±2.0 | 7.1±2.1 | 0.003* |
| SOFA score (24 hour) | 4.7±1.9 | 7.9±3.3 | 0.001* |
| APACHE II score | 17.1±7.3 | 23.3±4.6 | 0.003* |
| Lactate 1st hour (mmol/L) | 2.0±1.5 | 2.7±1.6 | 0.292 |
| Lactate 6th hour (mmol/L) | 2.0±1.8 | 2.1±1.3 | 0.474 |
| Overall Mortalities (19) + | 7(22.6%) | 12 (38.7%) | 0.023+ |
| 7-day mortality (n) | 3 (9.7%) | 6 (19.4%) | |
| 28-day mortality (n) | 4 (12.9%) | 5 (16.1%) | |
| >28-day mortality (n) | - | 1 (3.2%) | |
| Survive / Discharge (12) | 10 (32.3%) | 2 (6.5%) | |

Note: * Chi-square (significant; p<0.05), + Mann-Whitney Test (significant; p<0.05)

Table 2 shows that the NLR at 1st and 6th hours in the AKI group were higher than in the non-AKI group, but the NLR at 24 hours in the non-AKI group was higher than in the AKI group (p<0.05) (Table 2). The NLR in AKI decreased at its lowest point in the 6th hour and increased again in the 24th hour, although only slightly. However, in the non-AKI case, there was a downward trend from the 6th hour and continued to fall at the 24th hour.

**Table 2. Neutrophil-lymphocyte ratio.**

| Variable | Group a | | p value |
|---|---|---|---|
| | Non-AKI (n=17) | AKI (n=14) | |
| NLR 1st hours | 15.9±2.6 | 33.0±11.3 | 0.000* |
| NLR 6th hours | 14.2±1.8 | 19.3±3.8 | 0.000* |
| NLR 24th hours | 11.6±1.3 | 19.9±3.8 | 0.000* |

Note: [a] Mean (Standard Errors), * Multivariate ANOVA test (significant; p<0.05).

Serum syndecan-1 levels were higher in the AKI group than in the non-AKI group, both at 1st, 6th, and 24th hours, and this difference was significant (p <0.05) (Table 3). The highest levels occurred at 6th hours or after initial resuscitation, in both the AKI and non-AKI groups. In the non-AKI group, the impressive serum syndecan-1 levels continued to increase and this was not found in the AKI group.

**Table 3. Serum syndecan-1 level.**

| Variable | Group a | | p value |
|---|---|---|---|
| | Non-AKI (n=17) | AKI (n=14) | |
| Syndecan-1 1st hours (ng/mL) | 532.5±72.0 | 597.2±85.8 | 0.000* |
| Syndecan-1 6th hours (ng/mL) | 557.6±71.6 | 612.0±73.9 | 0.000* |
| Syndecan-1 24th hours (ng/mL) | 548.4±76.6 | 586.4±84.6 | 0.000* |

Note: [a] Mean (Standard Errors), * Multivariate ANOVA test (significant; p<0.05).

## Discussion

Sepsis Has A Complex And unique pathophysiology, so AKI in sepsis is a syndrome that is different from another AKI. Sepsis is defined as a syndrome of organ dysfunction due to infection so that infection with AKI is the same as sepsis [4]. AKI in sepsis is associated with poor clinical conditions. Compared with other critically ill patients with AKI, septic patients with AKI had a higher risk of death in hospital (odds ratio: 1.48) and a longer hospital stay compared with AKI from other causes (37 vs 21 days) [13]. In this study, the mortality rate in sepsis with the AKI group was high (38.7%) with a survival rate of only 6.5% compared to the non-AKI group (22.6%) with a survival rate of 32.3%. This difference from the statistical test results was significantly different (p = 0.023) indicating that sepsis with AKI had a higher mortality risk than sepsis without AKI. Sepsis with AKI had the highest mortality rate before day 7 (19.4%) followed by mortality before day 28 (16.1%).

Increased NLR can occur in septic patients with AKI [6]. The increase in NLR during the first 48 hours was associated with the incidence of organ failure in critically ill male trauma patients (OR 2.06 (1.04-

4.06), p = 0.04) [14]. However, in our study, the highest NLR was found at the first hour of sepsis (p = 0.000). This phenomenon can be interpreted that when a patient is diagnosed with sepsis, there is a severe systemic inflammatory process and during the NLR evaluation at the 6th and 24th hours, where the patient has received standard sepsis management therapy (based on Survival Sepsis Campaign Guideline 2018), such as giving fluids, maintaining MAP, giving antibiotics and so on, causing a decrease in the systemic inflammatory process, even though the systemic inflammatory process is still in a dangerous phase. In another retrospective study of 13,678 AKI patients who were critically ill, it showed that an NLR higher than 12.14 was a predictor of all causes of death (HR 1.83 (1.66-2.02), p <0.0001) [14]. Although the standard limit values for NLR have not been determined [15].

Sepsis can cause endothelial injury and cause endothelial cell dysfunction which can lead to vasorelaxation or vasodilation, impaired vascular permeability and extravasation of leukocytes or neutrophils [16]. Syndecan-1 is one of the components forming the endothelial glycocalyx and is one of the main proteins that attach to the apical membrane of endothelial cells [9,17]. Serum syndecan-1 levels can be measured at admission which is independently associated with severe AKI. The accuracy level of serum syndecan-1 in non-septic conditions for the diagnosis of severe AKI is moderate (area under the ROC curve, 0.77; 95% confidence interval, 0.68-0.85) [18]. Syndecan-1 serum levels have been associated with an increased risk of death by 90 days of mortality. In addition, glycocalyx and endothelial injury on admission to the ICU was associated with an increased risk of AKI and death. Higher serum syndecan-1 levels have been associated with AKI in a study among 175 septic patients. Although serum syndecan-1 levels in the study were higher among AKI patients within 12 hours and syndecan-1 serum levels were not independently associated with AKI [18–20]. Our research showed that there was a significant difference (Multivariate ANOVA test; p = 0.000) between septic patients with AKI or without AKI, both at the 1st hour, 6th, hour and 24th hour. However, the highest Serum syndecan-1 level was obtained at 6 hours, after fluid resuscitation was given. This is in contrast to the findings of Inkinen et al. who found no significant difference in syndecan-1 serum levels associated with fluid balance during the first 24 hours [20].

## Conclusion

The conclusion of this study is the neutrophil-lymphocyte ratio (NLR) is higher in the 1st and 6th hours in AKI, however it is lesser at 24th hours in the AKI in sepsis. The serum syndecan-1 levels at 1st, 6th, and 24th hours were greater in the AKI in sepsis. Therefore, the NLR and serum Syndecan-1 level based on timeline have a potential to become biomarkers for sepsis-induced AKI. However, further research is needed, especially for direct correlation with other markers or clinical symptoms.

### Author Contributions

Lila Tri Harjana assisted in conducting the research, performed the statistical analysis, and data visualization and wrote the manuscript. Eddy Rahardjo designed and conducted all of the research. Windhu Purnomo performed the statistical analysis and data visualization. Nancy Margarita Rehatta designed all of the research. Lilik Herawati performed the statistical analysis and data visualization, and wrote the manuscript. All authors have read and approved of the final manuscript.

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# The Potential of A Balinese Traditional Medicine Kelor Leaves *(Moringa oleifera)* For Male Infertility Treatment : A Mini Review

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## Abstract

Infertility is one of the causes of reproductive welfare disorders of married couples around the world. One of the causes of infertility at the molecular level is *Oxidative Stress* (OS) because the products of *Reactive Oxygen Species* (ROS), both endogenous and exogenous, exceed the levels of antioxidants in the body. The aims of this review is to find out the potential of traditional medicinal from Bali, Indonesia with local name Kelor *(Moringa oleifera)* leaves in preventing the occurrence of potential infertility in men based on the results of research in vivo using animal trials. The method used by the writer is a literature study.The results of this literature review found that kelor/moringa had a positive effect on sexual behavior, especially an increase in libido. In addition, it has a positive effect on spermatogenesis, the quality of spermatozoa especially increases sperm motility, sperm count/volume, germ cell count, renews endogenous antioxidant enzyme activity, reduces levels of ROS, and provides a protective effect on the testes from damage. *Moringa oleifera* contains many free radical blocking molecules. Some phytochemical test results show that moringa contains powerful antioxidants, including alkaloids, flavonoids, saponins, triterpenoids/steroids, tannins.

***Keywords:*** *Moringa oleifera, male infertility, antioxidant, oxidative stress.*

## Introduction

Living things throughout their lives will be exposed to oxidative stress. Oxidative stress in the body's formed cells can form continuously, and it is part of physiological, metabolism, and biochemical reactions, in addition to being obtained from exogenous factors. Oxidative stress has reactive properties that can cause damage to lipid cell membranes, proteins, and DNA [1,2]. Under certain physiological conditions, all aerobic organisms will have a defense in maintaining a balance between oxidative stress, enzymatic, and non-enzymatic antioxidants. However, if there is an imbalance, oxidative stress can impact of DNA damage and can result in damage to body cells [1–3] . This is the basis for various diseases that can interfere with human health, including disorders of the reproductive system.

Spermatozoa have a DNA structure called Mitochondrial DNA (mtDNA). The complex systems that occur in the mitochondria such as oxidation and

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reduction reactions make mtDNA very susceptible to exposure to oxidative stress. mtDNA is 10-100 times more susceptible to oxidative stress than nuclear DNA, this is due to the proximity of the mtDNA to ETC *(Electron Transport Chain)* and the relatively low DNA repair mechanism[4,5].Damage to spermatozoa mtDNA will cause disruption of the respiration enzyme coding complex, reduce ATP production, interfere with the spermatogenesis process, increase free radicals that damage the spermatozoa plasma membrane, causing infertility[4–8].

Infertility is a disorder caused by the inability of a sexually active partner to get a pregnancy within 1 year without using contraceptives [9–12]. The frequency of infertility is about 10-15% of couples, and 40% of cases are caused by abnormalities in men [13]. The factors that cause male infertility can be classified into two, which are generally influenced by age, frequency of intercourse, and length of effort, while specific factors are divided into four categories, namely the occurrence of reproductive tract obstruction, inflammation, sexual disorders such as erectile dysfunction, and failure to ejaculate which has the potential to reduce the quality of male sperm production eg, complete blockade of spermatogenesis, low sperm count, poor morphology or function, and abnormal sperm motility[14,15].

One of the causes of infertility at the molecular level is oxidative stress (OS) because the products of reactive oxygen species (ROS), both endogenous and exogenous, exceed the level of antioxidants needed by the body. Endogenous ROS molecules are produced in mitochondria. ROS is usually physiologically produced and used to maintain cellular processes such as sperm maturation, capacity, and sperm-oocyte interaction[16]. Research focusing on improving ROS proves that this class of compounds can interfere with spermatogenesis, spermatozoa motility and spermatozoa morphological abnormalities, decreased spermatozoa concentration, DNA integrity, resulting in sperm function becoming deformed and causing infertility[16,17].

ROS can come from within or from outside the body. Research on the increased production of ROS which affects the proper functioning of the diet process has been investigated and has shown a decrease in the thickness of smooth muscle and the lumen of the dorsal artery of the penis, the number of Leydig cells to spermatogenesis [18,19]. Accumulation of fat which adversely affects the vascular system that disrupts the male reproductive system has been found to involve the molecular transcription factor HIF-1[20].

One of the treatments for mitochondrial damage is the use of antioxidant compounds as a therapy to prevent cell oxidative stress, maintain cellular respiratory activity and mitochondrial energy production [21–23]. The body needs antioxidants that serve to prevent new free radicals, protect cells in the body from attacks of free radicals, ward off free radicals and prevent chain reactions so that greater damage does not occur, and repair cells and tissues damaged by free radical attacks. Most natural sources of antioxidants come from plants that were widely used by ancient people as traditional medicine, one of which is Kelor or Moringa *(Moringa oleifera)* [24].

Moringa contains a lot of free radical blocking molecules [25]. Moringa contains 46 powerful antioxidants, these compounds can work to prevent new free radicals, prevent chain reactions, protect cells in the body from free radical attack so that they can prevent oxidative damage to most biomolecules and provide significant protection against oxidative damage[26,27]. In this review, the scientific literature aimed at evaluating the activity of Moringa leaves in preventing male infertility uses animal models as a reference for development.

## Methods

This study aims to collect data on the effectiveness of the local Kelor or Moringa *(Moringa oleifera)* plant published locally and internationally during the period between 2010 and 2020. This review discusses the potential of the Moringa plant according to scientific information obtained as medicine in

curing or preventing infertility. men on an animal trial scale through publications and theses from various University sources. The internet is also used to collect data or reports published in various international scientific journals via the PubMed search engine and Google Scholar. Medical or biomedical books were also used to help complement this review. A literature search illustrating the use of experimental (in-vivo) animal models [28]. Key words used include *Moringa oleifera*, male infertility, antioxidants, oxidative stress.

Briefly, this review summarizes scientific information about the potential of Moringa leaves as traditional medicine ingredients including, scientific name and local name (Source from ITIS Report website with taxonomic serial no: 503874), distribution, bioactive compounds, dosage of extracts, duration of exposure, estimation of parameters, and results of anti-infertility studies. The mechanism of work that may occur in this plant is also described in the discussion section.

## Result and Discussion

### Taxonomy and General Distribution

*Moringa oleifera* is a plant native to Southeast Asia and is widely grown in tropical and subtropical regions around the world including in Indonesia. In Indonesia, this plant has many local names such as Kelor (Bali), Daun Marunggai (Minang, Sumatra), Limaran (Java), and there are still many local seeds for this plant in large areas in Indonesia. In addition to Indonesia, this plant also has designations in other countries such as Moringa, Horseradish tree, drumstick, tree west Indian Ben (English) [29], Sajina (Bangladesh) [30], Mrum (Cambodia), Ben aile (Perancis) [31], 'ii h'um (Laos) [32], Meringgai, Gemunggai, Kelor (Malaysia) [32], and Malunggay (Filipina) [32].

Moringa *(M. oleifera)* has the following classification :

Kingdom : Plantae

Divisi : Tracheophyta

Class : Magnoliopsida

Order : Brassicales

Family : Moringaceae

Genus : Moringa

Species : *Moringa oleifera* Lam. (Source : ITIS Report)

The distribution of moringa plants is quite wide and almost widely found in various countries especially in countries with tropical and subtropical regions. The spread of this plant is found in Asia region, Africa, America, and Oceania [33,34]. However, the sub-district reports, the influx of this plant in Indonesia was mediated by India at the time of viewing and was widely associated with a strong influence with the influx of Hinduism and Buddhism in Indonesia [35]. In addition to being known as a traditional medicinal plant, people also hook this plant with mystical things that are believed to be repellents of fine creatures for newly built houses to be widely used as a means of religious ceremonies, especially by Hindus in Bali Province[36].

### Metabolites Compounds

A plant maintaining its survival by carrying out a series of metabolites that produce primary and secondary metabolites [37]. Various studies have stated that secondary metabolites have pharmacological bioactivity. Secondary metabolites can be in the form of phenolic compounds, phenylpropanoids, saponins, terpenoids, alkaloids, tannins, steroids, and flavonoids [38,39]. There have been many observations of flavonoid compounds in plants related to their effects as antioxidants, antibacterials, anti hyperlipidemia, or anti-hyperglycemia [20].

Based on the results of several phytochemical tests, including using samples of Moringa leaves taken in the North Denpasar, Bali, it is known that Moringa leaf extract is dominated by bioactive ingredients

such as alkaloids, flavonoids, saponins, triterpenoids / steroids and tannins[40]. The ability of antioxidants to capture DPPH free radicals with an IC value of 4.33 mg / mL [41]. The sample used from Ende shows that the ethanol extract of Moringa leaves contains flavonoids, phenolics, triterpenoids, steroids, and tannins. The ability of antioxidants to capture DPPH free radicals with an IC value of 4.33 mg / mL [42].

Parts of the Moringa, especially the leaves part have a high antioxidant content. Metabolites of Moringa leaves that have an important role in the action of antioxidants are flavonoids. The possibel of mechanism of Moringa can see in Figure 1. Some of the main bioactive phenolic compounds of the flavonoid group such as quercetin, kaempferol, and others[39]. Quercetin is a strong antioxidant with an ability 4-5 times higher than vitamin C and E as a potential antioxidant developed as a medicinal ingredient [42]. Some of the bioactive ingredients can see in Table 1.

**Table 1. Secondary metabolites, classes, and role/function of Moringa *(M.oleifera)***

| No. | Secondary metabolites | Classes | Role/Function | References |
|---|---|---|---|---|
| 1. | Alkaloid | -Morphine<br>-Quinine<br>-Ephedrine<br>-Strychnine<br>-Nicotine | Alkaloids are one of the important chemical compounds as a source of discovery of new drugs and this compound is widely developed as herbal medicine one of which is cancer because it has antiproliferative properties and male reproductive problems. | 43,44 |
| 2. | Flavonoid | -Flavones<br>-Flavonols<br>-Isoflavones<br>-Chalcones<br>-Flavonols<br>-Anthocyanins | Flavonoids are exogenous antioxidants that have been shown to prevent cell damage due to oxidative stress. The direct or indirect mechanism of the action of flavonoids is signaled to have antioxidant potential. The direct function of flavonoids is to donate hydrogen ions so that they can neutralize the adverse effects of a free radicals and can stimulate the formation of systematic antioxidants marker such as SOD, catalase (CAT), and glutathione peroxidase. | 45–48 |
| 3. | Saponin | -Triterpenoid saponin<br>-Steroid saponin<br>-Alkaloid saponin | Saponins have medical properties that depend on their chemical structure.<br>Saponin compounds have antioxidant effects by forming hydroperoxide as a secondary antioxidant to inhibit the formation of lipid peroxide. | 46,49 |
| 4. | Tannin | -Gallotannins<br>-Ellagitannins<br>-Complex tannins<br>-Condensed tannins | Tannin which functions as an antioxidant, tannin compounds are composed of polyphenol compounds which have free radical scavenging activity, but if the concentration of tannins is excessive, they can have an effect as peroxidant, tannins can degrade DNA and contribute to the formation of hydroxyl radicals | 50 |

## Animal Studies

Several studies have reported the potential of Moringa leaves as anti-infertility tested in animals (animal trials) in vivo. Recent research reported the effect of giving ethanol extract of *M.oleifera* leaves to fertility hormones and cement quality in male albino mice. The administration of the extract is carried out orally for 30 days at doses of 100, 200, and 400 mg/kg. The results stated that the administration of Moringa leaf extract at different doses was able to increase weight, the weight of sexual organs, serum testosterone, Follicle-stimulating hormone (FSH), and Luteinizing hormone (LH) when compared to the control group [51]. Similar research was also conducted by Fatoba et al. [52] which utilizes Moringa root extract against the sperm production of albino mice. The dose of treatment given is 5, 10, 15, and 20 mL orally for 10 days. The results showed that Moringa root water extract was able to support spermatogenesis and produce good sperm quality.

Moringa leaf extract (MO) is also reported to have radioprotective properties against mobile phone-induced electromagnetic exposure in mice. The experiment was conducted by dividing the test mice into four groups, namely, group I (control) given standard feed, II (200 mg/kg mo leaf extract), III (exposure to 900 RF/MW MHz Field continuously for one hour daily and for 7 days a week), and group IV (exposure to cell phone electromagnetic radiation and MO extract). The results reported the supplementation of MO can regenerate the activity of antioxidant enzymes, lower ROS levels, and increase the activity of *Proliferating cell nuclear antigens* (PCNA) that have an important role in aspects of DNA replication and processes related to replication, bypass, replication due to induction, inappropriate repair of DNA, and chromatin assembly[53]. These results confirm that MO extract has the potential as a radioprotective that can damage the quality of sperm indicated by the increase in mouse sperm parameters[54].

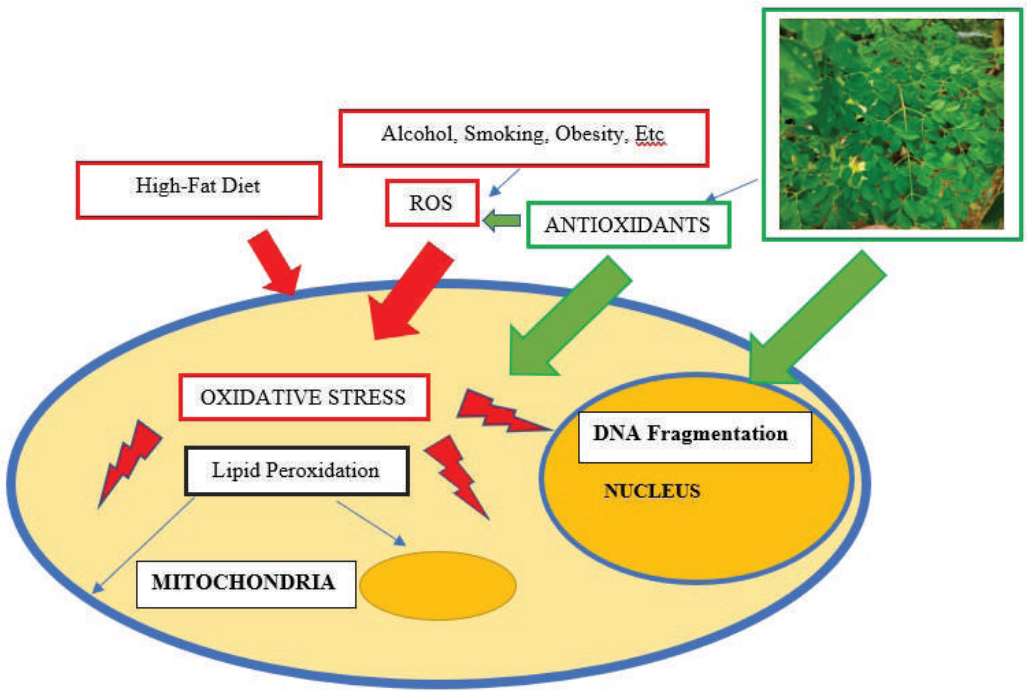


**Figure 1. Possible mechanism of antioxidant from Moringa leave in sperm cell (red : negative effect, green : positive effect) (Source adapted & modified from Benatta et al [55])**

## Conclusion

One of the causes of infertility at the molecular level is oxidative stress (OS) because the products of reactive oxygen species (ROS), both endogenous and exogenous, exceed antioxidant levels in the body. Moringa oleifera contains many free radical blocking molecules. Several phytochemical test results show that Moringa contains strong antioxidants, including alkaloids, flavonoids, quercetin, saponins, triterpenoids/steroids, tannins, zeatin, vitamin C, beta-carotene, selenium, and polyphenols. this compound can protect the body from the bad effects of free radicals. The antioxidants present in Moringa leaves work to neutralize free radicals thereby preventing oxidative damage to most biomolecules and providing significant protection against oxidative damage. The results of this literature review found that moringa had a positive effect on sexual behavior, especially an increase in libido. In addition, it has a positive effect on spermatogenesis, the quality of spermatozoa especially increases sperm motility, sperm count/ volume, germ cell count, renews endogenous antioxidant enzyme activity, reduces levels of ROS, and provides a protective effect on the testes from damage.

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# The Effect of Plyometric Training on Improving Values of Some Biokinematics Variables of High-Jump Shooting Skill with the Accuracy in Handball

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## Abstract

This study aimed to find out the effect of plyometric training on improving values of some kinematics variables of high-jump shooting skill and accuracy in Handball.

The researcher used the single group experimental design. The research sample was the players of Naft Al-Janoob Club, season 2020- 2021, they were chosen intentionally. The researcher photographed the research sample using a camera. "Sony" (250 images/sec), And he conducted the kinematic analysis using the "dart-fish" program. The implementation of the proposed training curriculum continued for (8) weeks at (24) training units and a rate of (3) training units per week. The researcher took into account the time specified for each training unit and the number of training units per week, and the general capabilities of the players, as well as the availability of devices and tools, as the researcher was ensured that the curriculum vocabulary is consistent with the scheduled exercises prepared previously for the team. the researcher concluded that there was an improvement in the values of kinematics variables while applying the plyometric training through the results of pre and posttests and for the favor of posttest, as well as the used training method that included plyometric training improved the shooting accuracy performance in high shooting in the post-test due to the use of the same exercises of the motor pathway. The researcher recommended using plyometric training to develop the values of kinematics variables of high-jump shooting skill.

***Keywords:*** *Plyometric Training , High-Jump Shooting , Accuracy in Handball*

## Introduction

The development in Handball skills, offensive and defensive, led to an improvement in the level of technical and competitive performance, specialized scientific researches helped in the training process to achieve a better physical and skill level to reach the higher levels in

Handball. Muscular strength is one of the most important physical characteristics that has been included in specialized studies, which is one of the basic elements that have affected the improvement of the level of performance of many activities, as this game relies on the characteristic of muscle strength greatly due to the dependence of its skills on the muscular strength of legs and arms, such as the skill high-jump shooting that witnesses a great development in technical and physical performance, which gives the player high self-confidence in performing the offensive as required. Studying and analyzing the variables on which the performance of this skill depends on its mechanical aspects affecting performance, in a way that contributes to the development of the physical, skill, and psychological level of the players. high-

jump shooting skill is one of the important offensive skills in handball and as a result of its effective impact in determining the outcome of the match, teams began to focus on developing the level of technical and planning performance for this skill, and through the researcher's observations, he noticed the weakness in the technical performance of this skill due to a weakness in the muscle strength on which most handball skills depend, especially offensive skills and the non-use of modern scientific training methods to develop high - jump shooting in Handball by linking the physical and mechanical side to improve the motor pathway of the skill led to a decline the level of technical performance of this skill. Therefore, the use of plyometric training according to a correct scientific method has a great role because it moves towards the correct motor pathway and thus it works to develop the physical, mechanical, and skill aspects alike. The importance of the research lies in setting up a training curriculum according to the scientific and objective basics, as well as choosing different plyometric exercises that contribute to improving the technical performance level of this skill by upgrading the values of the biomechanical variables that ultimately constitute the general form of the skill, thus reaching the best performance in Handball. Higher levels of access are one of the important matters that required a wide knowledge of the most important mechanical variables that contribute to mastering the skill. Therefore, kinematic analysis is a logical method by which the phenomenon is evaluated objectively to determine the most important areas of strength and weakness of the level of player's performance.

**Research aim**

This study aimed to find out the effect of plyometric training on improving values of some kinematics variables of high-jump shooting skill and accuracy in Handball**.**

Research Areas:

**Sample:** The players of Naft Al-Janoob Handball Club, season 2020-2021

**Time:** from 4\11\2020 to 25\2\2021

**Place:** Naft Al_Janoob stadium ,Basra ,

**Research Methodology**

The researcher used the single group experimental design for its suitability to the research type.

**Research Sample**

The research sample was 5 players chosen intentionally from Naft Al-Janoob Club, season 2020-2021, they were proficient In high-jump shooting, and the researcher identified some variables that represent the sample specifications to ensure their consistency, and the researcher conducted statistical analysis for those variables using (coefficient of variance).

**Tools and devices used:**

The researcher used a form for each player to record their body measurements, a special form for each player to measure the accuracy of high-jump shooting skill, signs, a metal tape measure, boxes and barriers ( different heights), medical balls weighing (1,2,3) 3 kg, scale, drawing scale (1m length), (Casio) electronic stopwatch, CDs, A master meter, laptop (DEEL) INSPIRON 501, a camera. "Sony" (250 images/sec) and software and applications used for kinematic analysis.

**Tests**

Accuracy test of high-jump shooting in handball

**Method of performance and recording:** when the player hears the whistle, he starts off to receive the ball from his colleague and takes the approximate steps allowed, then jumps over the floor without touching it and points the ball to the precision squares fixed in the goal (9) areas (1, 3, 7.9) representing the four corners of the goal, if hit by the tester, obtain (4) degrees and an area (8, 2) that represents above the goalkeeper's head and between his feet, and the player who succeeds in shooting gets (3) degrees and areas (6, 4) which represent the side of the goalkeeper's

arms (2) A score for the player who hits the center of area No. (5) get a score of 1, and a score of zero is given for failed shot [1].

**Physical training**

The researcher chose a set of Plyometric exercises based on ( Kelff, 2006) [2] Several exercises were selected and written in a special questionnaire to present them to experts and specialists in (sports training, tests, and measurement, handball) the following exercises were selected : (high jump from stability, wide jump from stability, medicine ball throwing).

M**ain experiment**

**Pretest**

on 3\12\2020 the researcher photographed the research sample in Naft Al-Janoob Club Stadium in Al Basra\ Iraq to extract the accuracy and values of the kinematics variables of high-jump shooting skill, the number of attempts were (30), each player had (6) attempts. The camera was placed at a height of (1.56) meters, measured from the ground to the lens of the camera, and at a distance of (7,40) meters from the place where the player's performance, to ensure the appearance of the player from the start of the movement until the stage of landing, and the camera was placed at a vertical angle on the right side of the player while performing his attempt.

**Biokinematics variables:** The researcher selected the following kinematics variables (steps speed, knee joint maximum flexion, angle of advancement, player jump speed, the maximum height of the hip joint at the moment of shooting, ball speed).

Computer analysis

By using the Dart Fish program kinematics variables that were used for this study were calculated and extracted after completing the videotaping procedures.

**Proposed experimental method**

The implementation of the proposed training curriculum continued for (8) weeks at (24) training units and a rate of (3) training units per week. the researcher took into account the time specified for each training unit and the number of training units per week, and the general capabilities of the players, as well as the availability of devices and tools, as the researcher was ensured that the curriculum vocabulary is consistent with the scheduled exercises prepared previously for the team and the researcher based when developing the contents of the training curriculum on the principles of the science of sports training, and in what follows a summary of the training curriculum vocabulary:

1- Training curriculum continued for (8) at (24) training units and a rate of (3) training units per week.

2- Training unit time was 90 minutes for each unit 5 exercises.

3- plyometric training was carried out in the main section immediately after the warm-up, that is, at the beginning of the main section, due to the players not being subjected to tired and stress at the beginning of the unit, and with an intensity that ranged between (80-100%) and the intensity was calculated by using the maximum repetition that the player could perform when applying various exercises.

4- The researcher took into account the individual differences between the players, as he used the arithmetic mean of the sample in determining the maximum repetition that the player can perform and repeat, according to his ability.

**Posttest:** on 1\2\2021 posttest were conducted for the research sample in the same order and conditions of a pre-test.

**Statistical Analysis**: the researchers used the static bag SPSS: using, arithmetic mean, standard deviation, coefficient of variance, and T-test

## Results

Pretest results of kinematics variables of high-jump shooting skill and the performance accuracy :

**Table (1): Shows the pretest arithmetic mean and standard deviation of kinematics variables of high-jump shooting skill and the performance accuracy**

| No | Variables | Measurement unit | arithmetic mean | standard deviation |
|---|---|---|---|---|
| 1 | Approach speed | Meter \second | 3.58 | 0.27 |
| 2 | knee joint maximum flexion | Degree | 125.4 | 1.87 |
| 3 | angle of rising | Degree | 86.65 | 0.84 |
| 4 | jump speed | Meter \second | 3.32 | 0.23 |
| 5 | maximum height of hip joint at the moment of shooting | Meter | 2.64 | 3.77 |
| 6 | ball speed | Meter \second | 14.70 | 1.30 |
| 7 | performance accuracy | Degree | 12.6 | 1.34 |

Table (1) shows the values pre-test of some kinematics variables and the accuracy of the performance of high-jump shooting, as the arithmetic means of the approach speed variable reached (3.58) with a deviation (0.27), while the arithmetic means of the variable of knee joint maximum flexion reached the (125.4) and with a deviation of ( 1.87), the arithmetic mean of the rising angle variable reached (86.65) with a standard deviation (0.84), the arithmetic mean of the variable jump speed was (3.32) and a standard deviation (0.23), and the arithmetic mean of the variable of the maximum height of the hip joint at the moment of shooting the ball was (264.57) With a standard deviation (3.77), the ball speed variable reached the arithmetic mean (24.7) and a standard deviation (1.30), the accuracy variable arithmetic mean reached (12.6) and a standard deviation (1.34).

**Posttest results of kinematics variables of high-jump shooting skill and the performance accuracy:**

**Table (2): Shows the posttest arithmetic mean and standard deviation of kinematics variables of high-jump shooting skill and the performance accuracy**

| No. | Variables | Measurement unit | arithmetic mean | standard deviation |
|---|---|---|---|---|
| 1 | Approach speed | Meter \second | 3.95 | 0.20 |
| 2 | knee joint maximum flexion | Degree | 122.01 | 1.42 |
| 3 | angle of advancement | Degree | 83.27 | 0.88 |
| 4 | jump speed | Meter \second | 3.84 | 0.22 |
| 5 | maximum height of hip joint at the moment of shooting | Meter | 2.78 | 4.81 |
| 6 | ball speed | Meter \second | 17.78 | 3.27 |
| 7 | performance accuracy | Degree | 16.8 | 1.79 |

Table (2) shows the values posttest of some kinematics variables and the accuracy of the performance of high-jump shooting, as the arithmetic means of the approach speed variable reached ( 3.84) with a deviation (0.20), while the arithmetic means of the variable of knee joint maximum flexion reached the (120.01) and with a deviation of (1.42), the arithmetic mean of the rising angle variable reached (83.27) with a standard deviation (0.88), the arithmetic mean of the variable jump speed was (4.48) and a standard deviation (0.22), and the arithmetic means of the variable of the maximum height of the hip joint at the moment of shooting the ball was ( 278.64) With a standard deviation (4.81), the ball speed variable reached the arithmetic mean (17.78) and a standard deviation (3.27), the accuracy variable arithmetic mean reached (16.8) and a standard deviation (1.79).

**Pre and post-test differences**

**Table ( 3 ): Shows the differences in the values of kinematics variables and the accuracy of performance between the pre and post-tests of high-jump shooting**

| No. | Variables | Pretest | | Posttest | | Calculated T value | Result |
|---|---|---|---|---|---|---|---|
| | | arithmetic mean | standard deviation | arithmetic mean | standard deviation | | |
| 1 | Approach speed | 3.58 | 0.27 | 3.95 | 0.20 | 7.4 | Sig. |
| 2 | knee joint maximum flexion | 125.4 | 1.87 | 122.01 | 1.42 | 7.79 | Sig. |
| 3 | angle of advancement | 86.65 | o.84 | 83.27 | 0.88 | 6.65 | Sig. |
| 4 | jump speed | 3.32 | 0.23 | 3.84 | 0.22 | 10.4 | Sig. |
| 5 | maximum height of hip joint at the moment of shooting | 2.64 | 3.77 | 2.78 | 4.81 | 12.45 | Sig. |
| 6 | ball speed | 14.70 | 1.30 | 17.78 | 3.27 | 4.73 | Sig. |
| 7 | performance accuracy | 12.6 | 1.34 | 16.8 | 1.79 | 10.24 | Sig. |

Table (3) shows the significant differences in the approach speed variable between the pre and posttests and for the favor of the post-test, and the researcher attributes this to the body gaining required strength as a result of the plyometric exercises that developed the approach velocity values in the post-test, as these exercises have a clear effect on the development of elasticity in the muscles which contributes to increasing the speed of motor performance during the approaching stage as well as its positive effect on the work of the nervous system, and since the motor speed depends in its work on the nervous system, which explains the reason for the existence of a positive relationship between plyometric exercises and movement speed [(3)].

knee joint maximum flexion variable shows that there is a significant difference between the two tests and the researcher attributes this to the plyometric exercises that had an effective effect in stimulating the muscle that led to the development of explosive strength in the material and second muscles of the legs, which led to the development and improvement of the knee joint angle in the posttest as well as the

similarity of the exercises used with the kinetic path of high-jump shooting skill also the improvement of the angle of the knee joint enables the player to invest the strength gained from the approximate steps to obtain a suitable height for the movement of the center of the body during the rise stage (the angle of advancement), which requires that the body is in a vertical position on the line of influence of the force the vertical position qualifies it to achieve better. Either the value of this variable in the pre-test we notice an increase in the angle of the knee joint and this leads to the position of the body being downward, so the reaction of the ground is less than the weight of the body and thus the strength is low and this is due to the weakness in muscle strength of the two legs.

As for the angle of the rising variable, Table (3) shows that there are significant differences between the two tests in this variable, as the process of coordination between the speed and the angle of rising is a very important factor, as that angle must be proportional to the horizontal velocity to preserve the movement momentum gained during the approximate steps. And based on the law of thrust in activities that require speed in their performance, such as high-jump shooting skill (the rise should be done in a short period so that the final movement amount is greater than the amount of the first movement and thus the effect of the force used is greater to obtain a better result [4].

This is found in the value of the angle of rising in the posttest, the researcher emphasized the importance of bending legs joints during the performance of the plyometric exercises and also emphasized the increase in the extension in these joints during the jump, which means increasing the resistance of the two legs by increasing its extension when conducting the post-jump exercises pretest, the player tries to stand up at a greater angle to compensate for the decrease in the approximate speed due to the weakness of the legs muscle strength, and it is known that the higher values of the angle of advancement, the higher values of the center of gravity of the body [5].

For Jump speed variable table (3) shows that there are significant differences in the values of the player's flight speed variable between pre and posttests and for the favor of the post-test. The researcher thinks that the development of the muscle strength of the two legs enhances its angular velocity, which affects the increase in the linear velocity of the center of gravity of the body considering that the angular velocity has a direct relationship with the peripheral velocity, thus achieves an increase in the player's flight speed, as the kinematic speed increases through muscle strength and this is what is included in the training exercises, and the development of the values of flight speed is one of the most important factors that help to achieve better altitude, either the value of the flight speed in the pretest, it was less because this variable depends on the amount of linear momentum the player possesses during the approach stage, which was relatively weak due to the weakness in the player's muscular strength [6].

table 3 also shows the differences between pre and post-test of maximum height of hip joint at the moment of shooting variable and for the favor of posttest, The researcher attributes it to the use of experimental method led to the development of performance in the posttest as a result of the Plyometric training, especially the deep jumping exercises, which have the effect of stimulating the agonist's muscle groups, which led to the improvement of performance and in a shorter time, and as a result, strength developed in the material and second muscles of the two legs and in the vertical direction as there is an increase in strength, leads to an increase in the recruitment of the motor units involved in muscular work. (The force resulting from muscle contraction is related to the number of motor units involved in this contraction and the ability of the nervous system to recruit the largest number of motor units participating in the muscular contraction increases as a result of strength training, thus increasing the resulting muscle strength [7].

While the value of this variable in the pretest was relatively low for not enhancing the muscle loading for

the increased stretching loads that were applied in the training curriculum as the increased loading works to develop the adequacy of the stretch-shortening loop in the muscle, in the eccentric extension phase of muscle contraction the largest amount of elastic energy is stored inside the muscle and this energy is used in the next central contraction phase that increases the strength of the agonist's muscle.

The ball speed variable table (3) shows that there are differences in the values of the ball velocity variable between the pre and posttests and for the favor of the post-test, as the plyometric exercises used in the training curriculum have affected the development of strength and performance at the same time and as long as the exercises included movement paths similar to the movement paths of high-jump shooting and the use of different medical weights balls and positions similar to the straightened arm positions require high efficiency in the muscle power working in performance, which includes the muscles of the arms, shoulder, and trunk, allowing the player to achieve better performance by performing the maximum muscle contraction in the shortest possible time as (medical ball exercises lead to the development of strength the muscles that surround the shoulder joint and the elbows also improve the flexibility of the shoulder joint [8].

This means an increase in the velocity of the shooting arm, therefore, the greater velocity of the shooting arm, the greater force acting on the shooting the ball. Therefore, the plyometric exercises, especially the jumping exercises, which enhanced the values of the hip joint height at the moment the ball is shot, and the jumping force achieved in the posttest was a result of the plyometric exercises that achieved for the player a good vertical displacement and good vertical force that helped the player shot the ball towards the goal with a sharp starting angle, as for the ball velocity variable in the pretest was less than it was in the post-test due to the weakness of the arm muscles and the low value of the variable height of the center of gravity of the body at the moment of shooting.

As for the performance accuracy variable, it appears from Table (3) that there are significant differences in the accuracy variable between the pre and post-tests and for post-test favor. The ball is towards the desired place, as indicates that accuracy is related to the adequacy of the nervous-muscular system [9].

The researcher also attributes that the difference in the accuracy variable between the two tests and the development of performance in the posttest is due to the type of the plyometric exercises that contributed to the development of kinematics variables that ultimately form the final form of performance as the production of additional strength for the agonist's muscles in performance allows the player to lengthen the jump time thus directing the ball in the areas of accuracy specified in the test. On the contrary, the value of the precision variable in the pretest was less than in the posttest due to the non-use of plyometric exercises according to a correct scientific method that helps in developing the special physical and mechanical capabilities that enable the player better shooting performance than high-jump.

## Conclusions

This study shows that there is an evolution in the values of kinematics variables while applying the plyometric training through the results of pre and posttests and for the favor of posttest, as well as the used training method that included plyometric training improved the shooting accuracy performance from high shooting in the post-test due to the use of exercises similar to the movement motion high-jump shooting.

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# The Impact of Local Attitudes on the Development of Health Tourism

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## Abstract

**Introduction:** Marketing techniques such as positive imaging of health tourism destinations can be a powerful tool in attracting users of health tourism services. The presence of satisfied citizens who create a stable identity for the city and strongly defend the city's brand can guarantee the success of branding programs.The aim of this study was to identify the effect of local attitudes of citizens about development of health tourism in their city and offer suggestions to improve attitudes and align the interests of citizens as stakeholders in this industry.

**Methods:** we have used aqualitative approach based on conventional content analysis by semi-structured interviews. Content analysis was used as a systematic method to describe a specific phenomenon in- depth, which leads to revision, valid conclusions from information, and the production of new knowledge and insights, and to examine people's experiences and attitudes toward the specific subject.

**Results:** The results of the analysis of interview data revealed three main themes include hygiene concerns, economic concerns, and safety concerns.

**Conclusion:** According to the concerns of citizens extracted from this study, it is suggested that policy makers in the field of health tourism, along with other relevant organs and organizations, take steps to plan to reduce this concerns. In regard to provide,health services tourism attractionsby supporting citizens in creating a positive image of destination.

***Keywords:*** *Health tourism, place marketing, citizens acceptance, conventional content analysis*

## Introduction

Familiarity with medical science and transfer of medical knowledge in the regions of the world has caused the creation of branches of the tourism industry as health tourism.[1] The World Tourism Organization (UNWTO) defines health tourism as the use of services that improve or enhance a person's health and morale (using mineral water, weather or medical interventions) and last outside the person's residence which is more than 24 hours".[2] As the definition shows, health tourism can be divideinto two parts including medical tourism and wellness tourism.[3]Travel to treat physical ailments or performing some type of surgery under medical supervision in hospitals and medical centers is call medical tourism.[4] In this type of health tourism, the patient may need to use facilities and services after treatment.[5]The health tourism industry market(In both sections) is known as one of the most profit-making and competitive

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industries worldwide.[2] Currently, due to the low cost and high-income industry, increasing competition has been created among different countries, especially developing Asian countries, to attract health tourists.[5] According to the 20-year national vision, Iran should become one of the main poles of medical tourism in the region by 2025.[6] In fact, the aim of this effort is to share Iran in global markets in order to make progress and profit. Despite the many advantages, due to the inefficiency of information system regarding Iran's medical tourism capabilities and lack of necessary infrastructures, Iran's medical tourism industry has no favorable status.[7]The use of marketing techniques in this area can be a powerful tool in attracting users of health tourism services.[8]One of these marketing techniques is branding, which is defineas "actions increase the emotional level associated with a product or service, thereby increases its value to customers and other stakeholders".[9] The American Marketing Association (1960) defines a brand as "A name, word, symbol, or design or combination that aims to introduce the products or services of a salesperson or group of salespeople to customers and to differentiate their products".A brand adds dimensions to a product or service to differentiate that product or service from others.[2] These distinctions can have a logical function, either tangible or even intangible.[10] However, according to the definition of marketing dictionary, branding includes all the steps involved in creating and maintaining a unique name (brand).[9] In fact, branding is the art of aligning what you want people to think about you with what people really think about your company, and vice versa.[11]As companies need to manage the brand of their organization and goods, citiesand places also need to pay special attention for branding in order to design, shape or change the mental images of their audiences and create information shortcuts for tourists and investors in order to gain fame and credits.[12] Just like the goods and services that organizations provide, cities also have capabilities that, by identifying them, can differentiate themselves and outperform their competitors.[12] It is necessary to have a marketing perspective in this area.[13]The city branding has received serious attention since 1990s with the views of some scientists like Porter and the attention of economists and policymakers to local and regional development. Factors influencing local development are accelerating the spread of marketing thought in urban areas.[14] In this regard, urban branding can be considered as a subset of place marketing.[15]

City branding is a comprehensive and long-term strategy along with the city development and economic development strategy of the city including a series of integrated strategies, processes and activities that will ultimately promote the city and increase its competitiveness.[10,16,17]Places are a complex package of goods, services, feelings and perceptions of customers and all their combinations, and the city brand can be a complex combination of inferences and mental perceptions of the audience about a city and its citizens, living and business space and its tourist attractions.[18]Researchers have identified the existence of satisfied citizens who create a stable identity for the city and vigorously defend the city brand to ensure the success of branding programs.[12] In this study, using the qualitative method of conventional content analysis, the effective factors on creating satisfaction and acceptance by the citizens of a health tourism destination were identified and introduced.

## Methods

This research has been done with a qualitative approach based on conventional content analysis and using semi-structured interviews. Content analysis is used as a systematic method to describe a specific phenomenon in- depthwhich leads to revision, valid conclusions from information, and the production of new knowledge and insights, and to examine people's experiences and attitudes toward the specific subject. Content analysis focuses on the life experience, interpretations, and meanings that individuals have encountered.[19]

Thirty-nine participants with maximum varietywere selected through purposive sampling gradually until the information saturation was

reached among the citizens of Mashhad. The data were collected through face-to-face interviews in the form of in-depth semi-structured interviews. The interviews were conducted with the main questions and the opinions of the research group.The interview guide included questions such as "What do you know about health tourism?" They were also asked to talk about their experiences with health tourists. Exploration questions were used to obtain more in-depth information.

The interviews were done separately. The place of the interview was in their homes, parks and educational and religious spaces according to the participants'' request. The duration of each interview was determined by agreement of the participants. The mean interview time was 50 minutes. With the informed consent of the participants, the interviews were recorded on a tape recorder. To determine trustworthiness, the data used four criteria proposed by Schwandt, Lincoln & Guba.[19]

For data credibility, close interaction with participants, engagement and long fieldwork and field notes were used. To ensure data conformability, comments and defaults were checked to prevent their impact on data analysis and interpretation. By providing a detailed description of the participants, the research process, the measures taken and the research limitation, an attempt was made to ensure the transferability of the research findings. Dependability of the research was obtained with the help of similar results between the researcher and two experts involved in the data analysis process. The data analysis was performed simultaneously with sampling.

After typing the text, all the interviews and notes were entered into MAXQDA software version 10 to facilitate data organization. The text of each interview was read and reviewed many times. For data analysis, the proposed Zhang & Wildemuth method was used, which has eight steps of data preparation, defining the unit of analysis, designing categories and coding, testing the coding on a part of the text, encoding all the text, checking the coding stability, concluding the coded data and reporting the method of work and findings.[20] For this purpose, after preparing the text, according to the research question, semantic units were identified and appropriate codes were written for each semantic unit. After six interviews, the initial codes were categorized and named based on conceptual similarity (subtheme) and after reviewing, modifying and confirming by the research team, data collection and analysis continued. The subthemes were compared and placed in the main categories. Finally, the methodology and findings were reported. All extracted codes and themes were reviewed and approved by researchers.

## Results

Using the content analysis method, three main themes were extracted from the findings including health concerns, economic concerns and security concerns. Table 1 shows the themes and sub-themes extracted from the interviews.

**Table 1: Main themes and related sub-themes**

| | |
|---|---|
| hygiene concerns | Transmission of non-endemic diseases to the city |
| | Reduce the quality of service to citizens |
| | Increase the waiting time to receive services |
| | Increase the cost of services in high quality clinics |
| Economicconcerns | Income generation for citizens with non-health related businesses |
| | Reduce the quality and quantity of products needed by local citizens |
| safety concerns | Unfamiliarity of tourists with local laws and culture |
| | Abuse of local conditions by some tourists |
| | Decrease the quality and amount of services provided to citizens due to increased insecurity following the presence of tourists in the city |

**Hygiene concerns**

The results of this study showed that factors such as the possibility of transmitting non-native diseases to the people of a city by patients who have traveled for treatment orreducing the quality of services provided for native patients because of medical tourists' presencecan affect negatively on their opinion about medical tourism. It is also worrying to increase the waiting time to receive services and increase the cost of services in high quality clinics for native patients due tomore attention paid by health service providers to health tourists.

**Economic concerns**

Incomegeneration for citizen of a destination of medical tourism with non-health related businesses can be a factor affect positively on people's attitudes about medical tourism development in their area. On the other hand, it has reverse effect when people Concludethat it can reduce the quality and quantity of products needed by them especially when there is no balance between these two attitudes.

**Safety concerns**

According to answers in an optimistic state, peoplebelieve that unfamiliarity of tourists with local laws and culturecan be harmful for their life and in a pessimistic state, abuse of local conditions by some tourists make their location unsecured and it may cause to decrease the quality and amount of services provided to citizens due to increased insecurity following the presence of tourists in their city.

## Discussion and Conclusion

Identifying the concerns and factors affect the satisfaction and acceptance of citizens in a health tourism destination, along with paying attention to the main product, which provide health services for health tourists, and trying to address these concerns and eliminate the factors causing concern among citizens can be a factor in creating an advantage for a health tourism destination.[21] The results of the present study indicated that the citizens of a health tourism destination are not passive towards the development of this industry in their place of residence and taking into account the individual and social interests perceived by themselves and relatives, they will have a reaction on this issue and its consequences.

The main issue and concern of the citizens of Mashhad in this study was related to health and hygiene factors, problems and complications that can cause the presence of foreign and non-local patients for the people of the city and how to manage this issue by the authorities. Many of the interviewees were concerned about the spread of non-endemic diseases due to the presence of foreign patients in the city, and also reduced the quality of health services provided to the citizens of Mashhad, especially in public hospitals such as Imam Reza and Ghaem hospitals. In addition to reducing the quality, increasing the waiting time to receive services in these centers and even private centers caused dissatisfaction of citizens with the expansion of health tourism in the city.

In addition to these issues, revenue generation due to the presence of tourists for the city in areas other than treatment was a factor affecting satisfaction and also reducing the quality and amount of these services was a factor affecting dissatisfaction. The third main concern of the interviewees regarding health tourists was related to the presence of their companions and their unfamiliarity with the laws and sometimes the Iranian culture andthe occurrence of norm-breaking behaviors in the city. Involvement of security forces in the city with issues related to tourists, which reduces the quality and amount of services provided to citizens, as well as the irregular behavior of some tourists, which causes insecurity in the city, were also significantly emphasized by the interviewees.

According to the concerns of citizens extracted from this study, it is suggested that the trustees and policy makers in the field of health tourism, along with other relevant organs and organizations, take steps to plan and coordinate for reducing the concerns

of citizens in this regard to providing health services and attract the support of citizens in creating a positive image of this health tourism destination.

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# The Effect of Feeding *Punicum Mombasa* on The Production of Total Gas, Methane and Digestion Factor in Vitro

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## Abstract

This study was conducted in the Animal Production Department of the College of Agriculture - Baghdad University. This study included the addition of *Ponicom Mombasa* as a form of green roughage to the ration of concentrated ratio of 25, 50 and 75% of coarse fodder to study it is effect on the total gas production, methane gas and digestibility. The results indicated a significant decrease in the production of total gas and methane gas by 75% of coarse fodder and the rates during the period of 12, 24 and 48 hours. It also gave the best effect in the characteristic of the dry matter digestibility, the digestion of organic matter and the energy exchanged.

***Keywords****: Gas production, methane production, Punicum Mombasa, digestibility*

## Introduction

Different types of gases are produced inside the animal rumen as a result of fermentation process of different organic materials, which include hydrogen gas, hydrogen sulfide, methane and carbon dioxide. The formation of methane gas is an effective mechanism to reduce the amount of carbon dioxide and to get rid of hydrogen gas formed in the rumen, and the amount of gas produced in ruminants varies depending on several factors, including: animal type, breed, rumen pH, the percentage of acetic acid, propionic, the composition of the feed, the amount of concentrated feed provided to the animal [6]. The production of methane gas in ruminants is similar to that of compost [12], and methane is 23 times hotter than carbon dioxide [6].

The laboratory gas production gives an indication of the quality of rumen fermentation, and it is one of the quick and inexpensive methods for estimating the nutritional value of the feed [5]. Therefore, it was important to conserve the feed energy by reducing the formation of methane gas [7]. In addition, the microbial fermentation inside the rumen is a major source for the production of methane gas, which is called greenhouse gas [10]. Therefore, reducing the high emissions of these gases from the digestive system of ruminants is related to determining the quantities of these gases produced in the animal rumen when feeding on concentrated and coarse feed [12,7]. The aim of this study is to investigate the effect of feeding green fodder from the punicum plant on the total gas production and methane in Vitro and to measure the digestibility after different incubation periods.

## Materials and Methods

### Experimental Design and Treatments

This study was conducted in the Nutrition Laboratory in the Faculty of Agriculture of Baghdad University, to determine the effect of feeding *Punicum Mombasa* with concentrated feed on total gas production and methane in Vitro and digestibility of dry matter, organic matter and metabolized energy (Tables 1 and 2).

**Table (1): components of the concentrated diet used in the experiment**

| ingredient | Barley | corn | Wheat bran | Soybean meal | limestone | Mineral salts | Total |
|---|---|---|---|---|---|---|---|
| % | 35 | 35 | 20 | 8 | 1 | 1 | 100% |

**Table (2): Chemical composition of the concentrated and coarse diet components included in the composition of the diets used in the study.**

| Items Ingredient | DM % | OM % | ASH % | CP % | CF % | EE % | NFE % | ME |
|---|---|---|---|---|---|---|---|---|
| Concentrate | 90.45 | 86.01 | 4.44 | 11.53 | 7.38 | 2.09 | 64.82 | 10.47 |
| Straw | 91.83 | 84.98 | 6.85 | 9.98 | 2.83 | 4.87 | 67.30 | 11.27 |
| Punicum | 39.65 | 88.20 | 10.15 | 16.95 | 18.80 | 2.35 | 51.75 | 10.11 |

**Table (3): Chemical composition and metabolized energy of experimental diets**

(MJ / kg dry substance).

| Items Ingredient | DM % | OM % | ASH % | CP % | CF % | EE % | NFE % | ME |
|---|---|---|---|---|---|---|---|---|
| T1 | 92.94 | 82.81 | 10.13 | 12.37 | 20.56 | 1.65 | 48.23 | 9.78 |
| T2 | 91.12 | 81.65 | 9.47 | 11.06 | 19.41 | 1.81 | 49.37 | 9.76 |
| T3 | 92.15 | 82.71 | 9.44 | 11.31 | 20.02 | 1.22 | 50.16 | 9.74 |
| T4 | 91.16 | 81.58 | 9.58 | 10.25 | 20.10 | 1.62 | 49.61 | 9.68 |

**T1:Control**

**T2: Concentrated fodder with coarse feed ( P. Mombasa 25% + 75% straw)**

**T3: Concentrated fodder with coarse feed (P. Mombasa 50% + 50% straw)**

**T4: Concentrated fodder with coarse feed ( P. Mombasa 75% + 25% straw)**

**Estimate total gas and methane production in the laboratory**

The total gas production in the laboratory was estimated according to the method [13] by taking 4 replicates for each sample, as 200 mg of experimental feed materials were weighed and 20 ml of industrial saliva and 10 ml of filtered rumen liquid were added and placed in 100 ml glass syringes, then gas was added. Carbon dioxide was added to each syringe and the syringes were closed with the plunger while pushing the piston to completely remove the air. Planck work for each period of cuddling (4 replications). The injection was withdrawn to calculate the total gas production and then 4 ml of 4% NaOH was added to only 2 samples to calculate the methane gas production

according to the method [9]. Then, the metabolized energy (ME) (mJ / kg dry matter) and the laboratory digestibility factor of the organic matter% (IVOMD) were calculated using the following equations:

Eq1:ME (MJ/ kgDM) = 1.06 + 0.157GV + 0.084CP + 0.22CF −0.081A(Ash)

Eq2:

IVOMD(%)= 14.88 +0.889 GV + 0.45CP +0.651 x A(ASH)

A= Ash, ME= Metabolized Energy, GV= Total gas production (ml), CP= Crude protein%, CF= Crude fiber%, IVDMD= digestibility of Dry matter. IVOMD= digestibility of organic matter

Measurement of digestibility of dry matter and organic matter (%)

Both the digestibility of dry matter and the digestibility of organic matter were estimated according to the method (14).

**Chemical Analysis**

The forage samples were analyzed for dry matter, organic matter, ash, ether extract, crude protein, and crude fiber according to A.O.A.C [1].

**Data Analysis**

The data were analysed using analysis of variance (ANOVA), and means were separated by Duncan test at significant level $p < 0.05$ using (SAS 9.4, Cary, NC, USA, 2009). Correlation analysis was carried out to indicate the strength of relationship among the parameters when the first-order interaction was found to be significant.

## Results and Discussion

**The effect of adding *Punicum Mombasa* on total gas and methane production in the laboratory**

The results (Table 3) indicated that there was a significant decrease ($p<0.05$) in total gas production and methane gas at T4 (75% Punicum + 25% straw) which the total gas amount reached 25.55 ml / 200 mg dry matter and methane amount 2.03 ml / 200 mg dry material. While, the total gas production recorded 27.12, 30.20 and 35.70 ml / 200 mg dry substance, and 2.20, 2.14 and 3.55 ml / 200 mg dry material of methane gas produced for the treatments T3, T2 and T1, respectively, after 12 hours. The same response was observed after 24 hours of treatments, that the total gas volume reached 27.42, 29.24, 32.22, 37.87 ml / 200 mg dry substance, and 2.47, 3.25, 3.28 and 3.75 ml / 200 mg dry material of methane gas at treatments T4, T3, T2 and T1 respectively. This result was in agreement with [14, 16] who indicated that the increase the amount of green fodder in the transactions led to a decrease the total production of gas and methane gas due to the fact that the green fodder (*Punicum Mombasa*) which led to the provision of digested energy in the animal's rumen, which result in reducing the energy spent for the production of total gas and methane gas by reducing the time of fermentation of foodstuffs.

Whereas, the same significant difference was observed in the total gas and methane production which recorded 31.50, 35.53, 36.42, 39.05 ml / 200 mg dry matter, 3.78, 3.90, 4.72 and 4.99 ml / 200 mg dry matter at treatments T4, T3, T2, and T1, respectively, after 24 hours. The significant increase in the total amount of gas and methane produced is related to the fermentation processes that occur in the rumen of the animal after this period which in agreement with [16, 17, 18]. On the other hand, there was no significant differences appear after 72 hours on total production of gas and methane gas which recorded 35.65, 37.31, 39.57, 40.10 ml / 200 mg dry matter, 4.55, 4.67, 4.95 and 4.92 ml / 200 mg dry material at T4, T3, T2 and T1, respectively. This results may be due to the reduction in the fermentation obtained in the treated feed after more than two days according to [17, 18].

**Table (4): Effect of adding *P. Mombasa* on total gas and methane production in the laboratory (ml / 200 mg dry matter)**

| Studied Characters | Total gas | | | | Volume of methan | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **12** | **24** | **48** | **72** | **12** | **24** | **48** | **72** |
| T1 | 0.24 ±35.70 a | 0.30 ±37.87 a | 0.20 ±39.05 a | 0.42 ±40.10 a | 0.84 ±3.55 a | 0.08 ±3.75 a | 0.40 ±4.99 a | 0.07 ±4.92 a |
| T2 | 0.34 ±30.20 b | 0.44 ±32.22 b | 0.46 ±36.92 b | 0.56 ±39.57 b | 0.04 ±2.14 c | 0.17 ±3.28 a | 0.17 ±4.72 a | 0.13 ±4.95 a |
| T3 | 0.41 ±27.12 c | 0.54 ±29.24 c | 0.37 ±35.53 b | 0.12 ±37.33 a | 0.09 ±2.20 b | 0.13 ±3.25 b | 0.04 ±3.90 b | 0.04 ±4.67 a |
| T4 | 0.41 ±25.55 d | 0.32 ±27.42 d | 0.40 ±31.50 c | 0.47 ±35.65 a | 0.15 ±2.03 c | 0.40 ±2.47 c | 0.13 ±3.78 b | 0.06 ±4.55 a |
| | * | * | * | N.S | * | * | * | N.S |

**T1:Control**

**T2: Concentrated fodder with coarse feed ( P. Mombasa 25% + 75% straw)**

**T3: Concentrated fodder with coarse feed (P. Mombasa 50% + 50% straw)**

**T4: Concentrated fodder with coarse feed ( P. Mombasa 75% + 25% straw)**

**Effect of adding *P. Mombasa* on in vitro digestibility of dry matter, organic matter, and alternating energy**

The result (Table. 5) showed that the treatment T4 was the best treatment in terms of the digestibility of dry matter (66.23%) as compared with (62.31, 63.41 and 60.18%) under T3, T2 and T1, respectively. Similar of the digestibility of dry matter, treatment T4 result in high value of digestibility of organic matter (64.08%) as compared with T3, T2 and T1 which recorded (63.31, .4861 and 60.07%) respectively. This results are in agreement with [3, 4], they found that using green fodder decreasing total gas and methane production.

The higher alternating energy at T4 (9.23 mJ / kg) was associated high with digestibility of dry matter and organic matter opposite the low values under T3, T2 and T1, (9.54, 9.61 and 9.82 MJ / kg) respectively. The difference in the digestibility of dry matter and organic matter were associated with the reduction in the energy exchange according to [16, 17, 18].

**Table (5): Effect of adding *P. Mombasa* on in vitro digestibility of dry matter, organic matter, and alternating energy**

| Studied Characters<br>Treatment | IVDMD | IVOMD | ME(MJ/kgDM) |
|---|---|---|---|
| T1 | 0.87 ±60.18<br>a | 0.73 ±60.07<br>a | 0.13 ±9.82<br>a |
| T2 | 0.67 ±63.41<br>a | 0.66 ±61.48<br>a | 0.10 ±9.61<br>a |
| T3 | 0.53 ±62.31<br>a | 0.43 ±63.31<br>ab | 0.08 ±9.54<br>a |
| T4 | 0.30 ±66.23<br>b | 0.77±64.08<br>b | 0.04 ±9.23<br>b |
| | * | * | * |

| | |
|---|---|
| **T1:Control** | **T2: Concentrated fodder with coarse feed ( P. Mombasa 25% + 75% straw)** |
| **T3: Concentrated fodder with coarse feed (P. Mombasa 50% + 50% straw)** | **T4: Concentrated fodder with coarse feed ( P. Mombasa 75% + 25% straw)** |

## Conclusion

Considering the high nutritional value of the *P. Mombasa* plant, its use in animal nutrition affects the productive characteristics as well as the amount of energy spent to produce the total gas and methane gas, as it leads to a reduction in the energy spent for its production and thus has a significant effect on the exchange energy that the animal benefits from in improving its productive qualities. The reduction in total gas and methane production was associated with increasing the feed level of *P. Mombasa*.

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**Ethical Clearance**: Not required

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# Galactooligosaccharide (GOS) Fortified Formula Feeding in Premature Infants

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## Abstracts

**Background:** Nutritional problems are one of the serious problems in low birth weight or preterm infants. This causes medical and nutrition management of premature infants to be more individual.

**Objective:** To evaluate the outcome of the premature infants with Galactooligosaccharides (GOS) fortified formula feeding

**Methods:** This was prospective, open label cohort study that conducted during March- December 2019 in Neonatology Unit at the one of the main referral hospitals in East Java, Indonesia. The population of the study was very low birth weight infants (1,000 g - ≤1,500g) who needed formula feeding. Weight, body length, head circumference, fecal models and the incidence of diarrhea, colic, regurgitation, and vomiting was observed. Patients were observed for 28 days or adjusted according to length of stay.
**Results:** Totally, 20 infants were included. Mean birth weight was 1236.2±148.5 grams. Mean total volume Galactooligosaccharides fortified formula at the start of recruitment was 209.4±46.1 ml and at the end of observation was 267.9±41.2 ml. There were change in amount and consistency of feces before and after intervention. No patient experienced adverse events (diarrhea, colic, regurgitation or vomiting) while consuming Galactooligosaccharides fortified formula.

**Conclusion:** Galactooligosaccharides fortified formula in premature infant formula did not have a detrimental effect on premature infants and did not cause intolerance

***Keywords:*** *Formula feeding; Oligosaccharides supplementation; low birth weight infant; premature*

## Introduction

Nutritional problems are one of several problems in low birth weight (LBW) or preterm infants. This is closely related to various conditions or complications in organ systems of the body such as the airway, central nervous system, gastrointestinal tract, liver, kidneys, and others. In general, nutrition is an absolute necessity for optimal survival and growth or prevention of complications. In addition, conditions in premature infants can vary widely. This is influenced by many factors such as gestational age and birth weight. This condition causes the medical and nutrition management of premature infants to be more

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individual. Specific nutritional problems in premature infants are low nutrient reserves, immaturity of organ function, unpredictable potential for rapid growth, and a high risk of morbidity.[1] The main objective of nutritional support in premature infants is to achieve optimal growth and development. Oligosaccharides are one of the main components in human milk, which contain about 10 g / l neutral oligosaccharides and 1 g / l acidic oligosaccharides.[2] The concentration of these oligosaccharides changes with the lactation period, with the largest oligosaccharide concentrations found in colostrum. The oligosaccharide composition of breast milk is very complex and it has been reported that there are more than 100 oligosaccharide-like structures present in the human milk.

Breastfeeding and formula feeding may have different effects on the development of microorganisms in the digestive tract. Studies show that the gastrointestinal tract of exclusive breastfed infants is dominated by *Bifidobacteria* and *Lactobacilli* compared to formula-fed infants.[3] This predominance of bacteria is believed to reduce the risk of diseases related to the digestive tract. Oligosaccharides in human milk are believed to contribute to the development of *Bifidobacteria* and *Lactobacilli* in infants, thereby human milk is said to have a bifidogenic effect. Furthemore, the composition and structure of oligosaccharides in human milk is very complex. Prebiotic compounds that have been used in many studies for various infant formula products are GOS and FOS. Clinical studies that have been carried out prove that the addition of GOS or FOS to formula milk produces a bifidogenic effect, which stimulates the growth of *Bifidobacteria* and *Lactobacilli*.[4] Studies have shown that giving fortified formula with a mixture of GOS / FOS with a concentration of 0.4 g / 100 ml or 0.8 g / 100 ml for 28 days showed increased fecal *Bifidocateria* and *Lactobacilli*. The increase in *Bifidobacteria* depends on the dose of oligosaccharides. Long-chain (5-60 monomer) inulin mixtures with GOS (2-7 monomers) at a ratio of 10-90% have been added to European infant milk for more than 5 years.[5] Clinical studies show that this prebiotic fortified formula is significantly affect the composition of microorganisms in the feces similar to the composition of microorganisms in the stool of breastfed infants, improve stool consistency, reduce intestinal permeability, and reduce the risk of gastrointestinal infections, respiratory tract and skin diseases in infants. Fortification of formula milk with a mixture of GOS and FOS also increases calcium absorption. Giving weaning foods fortified with oligofructose at a dose of 4.5 g / day for 6 weeks showed an increase in the number of *Bifidobacteria* in the feces and decreased clostridia and softened the stool and reduced the risk of gastrointestinal diseases.

## Materials and Methods

This study is a prospective, open label cohort study conducted during March-December 2019. The study was conducted in the Neonatology Unit at the one of the main referral hospital in East Java, Indonesia. The aim of the study was to evaluate the outcome of the premature infants that give Galactooligosaccharides (GOS) fortified formula.

The population of the study was infants with very low birth weight (1,000 g - ≤1,500g) who needed formula feeding. Before performing intervention, parents were given information for consent and signed an informed consent to use formula milk for their infants according to standard operating procedures (SOP) hospital. Inclusion criteria were infants with hemodynamic stable conditions, infants with severe infections or infants with mothers who died during childbirth, so they could not get their own mothers' milk, infants with mothers with absolute contraindications to breastfeeding (mothers with HIV or mothers in chemotherapy medication), or infants with insufficient enteral nutrition of the human milk. The exclusion criteria were infants with congenital abnormalities and infants who their nutrition needs had fulfilled by the exclusive breastfeeding. The dropout criteria were arising of serious side effects, failure to follow up, and parents proposing the use

only exclusive breastfeeding. The sample size was 20 patients with an estimated number of screenings of 40 patients**.**

Infants that included in inclusion criteria would obtain the intervention of GOS fortified formula feeding. GOS fortified formula volume is adjusted to the doctor's recommendation. Outcomes that observed were weight, body length, head circumference, the incidence of diarrhea, colic, regurgitation, vomiting, and fecal models (with *Amsterdam Stool scale criteria Chart*). These outcomes would be observed at baseline (measurement before intervention, at the early of observation) and at end line (measurement after intervention, at the end of observation) Patients were observed for 28 days or adjusted according to length of stay. If after 28 days of observation, the patient is still hospitalized, then the intervention will be continued until the patient is discharged. Patients still receive medication regarding to the patient's condition. All of medication will be written in the *Case Report Form* (CRF).

## Findings

**Table 1. Infants characteristic data**

| Variable | Patient (n=20) | |
|---|---|---|
| | n | Percentage(%) |
| Gender | | |
| Boy | 11 | 55 |
| Girl | 9 | 45 |
| Mode of Delivery | | |
| Sectio caesarean | 13 | 65 |
| Vaginal | 7 | 35 |
| Feeding | | |
| Formula feeding | 0 | 0 |
| Mixed feeding | 20 | 100 |

**Table 2. Infant anthropometric characteristic data at baseline and end line**

| Variable | Baseline | | Endline | | Δ Baseline and Endline |
|---|---|---|---|---|---|
| | Mean±SD | Median (Min-Max) | Mean±SD | Median (Min-Max) | Mean±SD |
| Weight (gr) | 1345,4 ± 121,7 | 1398,5 (1100-1466) | 1585,0 ± 123,4 | 1579,5 (1372-1815) | 240,3 ± 170,4 |
| Length (cm) | 39,9 ± 3,4 | 40,3 (34-45,4) | 40,4 ± 3,3 | 41 (35-45,5) | 0,5 ± 0,6 |
| Head circumference (cm) | 28,1 ± 1,5 | 28 (26-31,5) | 28,5 ± 1,3 | 28,5 (26,8-31,5) | 0,5 ± 0,5 |

A total of twenty infants were included in this study. Infants were dominated by the eleven male infants (55%). Sectio Caesarean was dominated the mode of delivery in 13 infants (65%). All infants consumed formula milk and human milk and none of them consumed formula milk only (table 1). Data at the early of recruitment (baseline) showed the mean of body weight was 1345.4 ± 121.7 grams, mean of body length was 39.9 ± 3.4 cm and the head circumference of 28.1 ± 1.5 cm. At the end of the observation, the mean body weight of the infants increased to 1585.0 ± 123.4 grams, the lenght of the body increased to 40.4 ± 3.3 cm and the head circumference to 28.5 ± 1.3 cm (Table 2).

**Table 3. Total of GOS fortified formula consumption at baseline and end line**

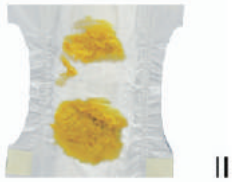
| Variable | Baseline | | Endline | | Δ Baseline and Endline |
|---|---|---|---|---|---|
| | Mean±SD (ml) | Median (Min-Max) (ml) | Mean±SD (ml) | Median (Min-Max) (ml) | Mean±SD (ml) |
| GOS fortified formula volume | | | | | |
| Infant with partial feeding (human milk and formula feeding) | 209,4 ± 46,1 | 228 (60-264) | 267,9 ± 41,2 | 264 (216-360) | 58,5 ± 66,6 |

The longest time of intervention was 28 days with the mean of total volume consumption was 229.1 ± 23.1 ml. Infants with the shortest intervention time was 2 days with the mean volume consumption was 246 ± 25.5 ml. The highest and the lowest of GOS fortified formula volume consuming were 276 ± 48.7 ml and 194.8 ± 83.6 ml, respectively. Table 3 shows the data on the total of all infant volume consumed of GOS fortified milk, both at the beginning and at the end of recruitment. The mean total volume at the beginning of recruitment was 209.4 ± 46.1 ml and at the end of observation was 267.9 ± 41.2 ml. During the study and observation period, no patient experienced any adverse events (diarrhea, colic, regurgitation or vomiting) while consuming GOS fortified formula.

**Table 4. The infant feces model at baseline and end line**

| Variable | Baseline (n=20) | | Endline (n=20) | |
|---|---|---|---|---|
| | n | Percentage (%) | n | Percentage (%) |
| **Amount** | | | | |
| 1: smear | 0 | 0 | 0 | 0 |
| 2: up to 25% | 13 | 65 | 6 | 30 |
| 3. 25-50% | 7 | 35 | 14 | 70 |
| 4. >50% | 0 | 0 | 0 | 0 |
| Consistency | | | | |
| A. Watery | 1 | 5 | 0 | 0 |
| B. Soft | 10 | 50 | 8 | 40 |

***Cont...*** **Table 4. The infant feces model at baseline and end line**

| | | | | |
|---|---|---|---|---|
| C. Formed | 8 | 40 | 12 | 60 |
| D. Hard | 1 | 5 | 0 | 0 |
| Color | | | | |
|  I | 1 | 5 | 0 | 0 |
|  II | 16 | 80 | 16 | 80 |
|  III | 3 | 15 | 4 | 20 |
|  IV | 0 | 0 | 0 | 0 |
|  V | 0 | 0 | 0 | 0 |
|  VI | 0 | 0 | 0 | 0 |

Observation of feces was using the patient's stool model refers to the *Amsterdam Stool Chart* (Figure 1). Observation of the feces included volume, consistency and color of the feces. At the beginning of recruitment (baseline) the amount of feces was dominated by number 2 in the Amsterdam Stool Chart model (up to 25%), but at the end of fecal observations there was an increment of the feces volume (dominated by number

3 of the feces amount in the Amsterdam Stool Chart model). Observation on feces contingency, at the baseline, consistency was dominated soft consistency and at the end of observation was dominated by the formed consistency. Regarding color at both the start and end of recruitment, the majority of infants dominated by number 2 of the feces color on the Amsterdam Stool Chart model (Table 4).

## Discussion

Nutritional problems are one of several serious problems in preterm neonates. This is closely related to various conditions or complications in various systems or organs of the body such as the airway, central nervous system, gastrointestinal tract, liver, kidneys, and others. On the other hand, nutrition is an absolute necessity for optimal survival and development or prevention of complications. Meanwhile, nutrition also can lead to complications. Every premature infant has a different condition that influenced by many factors. Two of them are gestational age and birth weight. Medical management and nutrition of premature infants are crucial. Premature infants have some specific nutritional problems such as low nutrient reserves, immaturity of organ function, low potential for rapid growth, and a high risk of morbidity.[1] The main objective of nutritional support for preterm infants is the achievement of optimal growth and development.

Oligosaccharides are the third largest component in human milk. It is known that the main function of oligosaccharides is as a prebiotic.[1] Prebiotic is a term used for foodstuffs that can be a source of energy and nutrients for certain species in the human intestine, especially the Bifidobacterium and Lactobacillus. [2.3] The composition of the intestinal microbiota which is rich in both types of bacteria can increase the acidity of the feces and the short-chain fatty acids. As a result, the consistency of the stool becomes soft and the frequency of bowel movements becomes more frequent. [3.4] Oligosaccharides also have a function in intestinal protection because of anti-adhesion function that can resemble ligands where certain pathogens attach. It also has the effect of modifying the glycosylated activity of intestinal epithelial cells.[5] The protective function of these oligosaccharides is associated with a reduced risk of intestinal infection in infants who are exclusively breastfed.[1.5] Because of its advantages, researchers propose an idea to produce synthetic oligosaccharides and added to infant formula so that infants who are not breastfed can obtain the benefits.[6]

The composition of the intestinal microbiota is necessary as a defense against pathogenic bacteria.[1] The Bifidobacterium and Lactobacillus groups are the dominant microbiota in the intestines of breastfed infants, whereas in formula-fed infants there are more variations, consisting of Bacteroides or Clostridium group, Staphylococcus, and Enterobacteriaceae. [1,2] Factors that affect the diversity of the microbiota composition in the infant's gut, including gestation, mode of delivery, environment, drug consumption, and type of infant nutrition.[3] Many studies show that oligosaccharides can provide benefits as prebiotics and have bifidogenic effects such as those found in human milk.

To be classified as a prebiotic, a food ingredient must be acid-resistant and cannot be hydrolyzed or absorbed in the upper tract, can be fermented by the intestinal microflora, and selectively increases the growth and activity of one or more commensal bacteria in the colon.[5,6] The expected functional effects of prebiotics are to affect the production and determination of stool, increase the bioavailability of several minerals such as calcium and magnesium. These substances an immunomodulatory that can reduce the risk of diseases such as infectious diarrhea, metabolic syndrome, obesity, and inflammatory bowel disease, and colon cancer. [7–9] Several types of prebiotics that have been accurate are inulin, fructooligosaccharide (FOS), galactooligosaccharide (GOS), polydextrose (PDX), and lactulose. [6,8]

Ziegler et al found intolerant effects in the form of gas, vomiting and diarrhea, especially in the group who received the prebiotic mixture. Compared to the control group, all categories of the intervention group experienced more frequent adverse effects.[17] In this study we did not find any subjects who experienced intolerant effects in the form of diarrhea, colic, regurgitation and vomiting. The two clinical trials above also assessed the effect of GOS fortification in formula milk on stool characteristics (feces amount, consistency and color). At the end of the study, it was found that there was no statistically significant difference between all the intervention groups and the control group, but the consistency of the feces in the subjects who received the prebiotic combination was found to be softer than the control group. However, in this study, there was no breastfeeding only group as a comparison.[10] In another study, it was found that the highest number of defecations occurred in subjects who received GOS and other prebiotic combinations at the start of administration (30 days), but in subsequent observations (> 30 days) the number of defecations was not different meaningful with other groups.[11]

In this study, we also monitored the effect of GOS fortified formula feeding on the feces characteristics of premature infants, included amount, consistency and color of the feces, and intolerance. Parameters of feces characteristics were observed before and after the intervention. The consistency of the subject was changed from hard consistency to be softer consistency after the intervention. The effect on feces color did not have a significant change after intervention.

Through the results of this and another study about GOS fortified formula feeding in preterm infants, GOS as supplementation for a premature infant does not interfere with the growth process and does not cause significant differences in stool characteristics, both consistency and color of stool. Also includes the absence of any intolerant effects during the consumption of premature infant milk with partial GOS supplementation. Although in the comparison process, we found only a few studies related to GOS as a single supplementation, because most studies used a mixture of two or three other prebiotics.[12] However, despite different methods, this study and other research propose the widely used of prebiotics. The effect of adding GOS fortification on growth rate and feces characteristics was not different. In this study, the feces characteristics of the patients who got GOS fortified formula also showed the same results as the systematic review and other meta-analyzes of several prebiotics either alone or in combination.[8, 13,14, 15] In these studies, the composition of the gut microbiota also showed a predominance of Bifidobacterium (bifidogenic effect) and a reduction in the number of pathogenic bacteria. The study of Watson et al, FOS, GOS, inulin, maltodextrin, PDX and lactulose, showed that GOS had the best ability to increase the amount of Lactobacillus and Bifidobacterium compared to other types of prebiotics[16]

## Conclusion

Nutritional support is the important problems in preterm infant for optimal survival, growth and prevention of complications. Galactooligosaccharides (GOS)is one of human milk component that affect the composition of microorganisms in the feces and can reduce the risk of gastrointestinal infections. In our study, we conclude that GOS fortified formula did not have a detrimental effect which is shown by no one of participant experience an adverse event. GOS fortified formula might be safe for the nutritional support choices in premature infant. Research with a larger number of infants could be conducted to carry out continuing research in GOS fortified formula feeding for preterm infants

**Conflict of Interest**: Author declares that there is no conflict of interest

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# Prevalence of *Cryptosporidium spp.* among Patients with Diarrhea at Wasit Province/ Iraq

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## Abstract

*Cryptosporidium* is an important obligate enteric protozon parasite that infects humans and wild range of animals with morbidity and mortality especially amongimmuno-suppressed individuals[1]This study carried out in al-kut city /Iraq in two major hospital Al- kut hospital for gynecology ,obstetric and pediatric and al-Karamma teaching hospital which included collection of stool samples from diarrheal patients from October / 2020 – January / 2021 , (109) stool samples taken from patients with different ages to both gender examined by microscopic examination(Modified ziehl nelson) and multiplex PCR technique, the results showed the percentage of *cryptosporidium spp.* infection by using M.Z.N stain was 44(40.4%) while by using PCR technique was 38(39.6%) and 4(4.2%) for C.parvum and C.hominis respectively .

The rate of infection is significantly associated with residence when using M.Z.N stain and PCR for *C.parvum*, also significant associate among those who >5 years old. water source for drinking were significant associated factors, other factors (gender, education level) Showed no significant association with infection, the present study aimed to detection of *Cryptosporidium spp***.** and possible association between the infection and some risk factors by using microscopic examination and detecting DNA of *Cryptosporidium* parasite by multiplex PCR technique.

***Keywords****: Cryptosporidium, M.Z.N stain, multiplex PCR Cryptosporidiosis.*

## Introduction

*Cryptosporidium* is a common parasite that infects intestinal epithelial cells in humans and a variety of animals worldwide and is an emerging infectious disease of importance to public health around the world [2]This disease burden varies between and within countries/regions, diarrhea is a common clinical symptom of *cryptosporidiosis* in infected hosts, varying depending on their health status, In humans, immune-competent individuals usually experience self-limiting diarrhea; however, immune-compromised individuals, particularly those with HIV infection suffer from intractable diarrhea[3, 4] The Cryptosporidium is the second-highest priority Organisms/Biological Agents at the National Institutes of Health (NIH), and was found this parasite is the fifth leading cause of death from diarrhea in children under the age of five years, About 84.4% of deaths due to cryptosporidium infection are in this age[4, 5]. yearly 1 million deaths caused by *cryptosporidiosis* resulted in over 50,000 deaths caused by the Apicomplexan phylum that is" phenotypically and genotypically diverse" species of Cryptosporidium which are parasitized and located" intracellular and extracytoplasmic on the microvillus of the excretory system and epithelial respiratory tract" of humans, fish, amphibians, birds, and mammals[6]And in the humans caused worldwide diarrhea disease that similar to cholera disease which considered by the Centers for Disease Control and Prevention (CDC)

as an emerging pathogen, this disease is mostly not noticed and asymptomatic but might be presented with mild to severe diarrhea and fever which is self-limiting and life-threatening in immunocompromised individuals like AIDS[7, 8]. Currently, more than 30 species of the *cryptosporidium parasite* have been identified[9]. A person can acquire cryptosporidium through several modes, such as preservation through the oral-fecal through the consumption of contaminated water and food[10]. In addition, people can become infected through personal contact, and contaminated food, raw meat, unpasteurized milk, and contaminated juices and drinks have a role in transmitting the parasite[11] Finally, this parasite was isolated from mucous secretions from the nose, and the patients were diagnosed with an infection, at first, it was learned that it was a respiratory disease[12]

To determine the rate of infection in wasit province and correlation with risk factors this study was conducted.

## Material and Methods

### Samples collection

109 Stool diarrheal samples were collected from different ages to both gender from October 2020 to the January 2021 and they were attending to the Al-kut hospital for gynecology ,obstetric and pediatric and Al-Karama teaching hospital in Al-kut city. (Organization 1992)

### Microscope examination

All stool samples were examined microscopically stained by modified Ziehl- Neelsen method[13] The stool sample was spread on the slide. The slides left in the open air to dry for a while 10 minutes without using a flame, the dried smear was fixed with absolute methanol for 1minutes, Carbol-Fuchsine solution was added to the slide covering the whole smear for 15 minutes, the slide was washed gently with tap water using a dropper, after this, decolorizer by acid alcohol for 30 seconds and the slide was washed off with clean water again, the methylene blue was added for 2minutes and washed again, and left to dry, the smear was examined microscopically, using the 40x and 100x (oil immersion lens) objectives and scanned thoroughly for parasite identification In this technique, the oocysts appear as pink to red, spherical to ovoid bodies on a blue or purple background[14]

### DNA extraction and multiplex PCR

Genomic DNA was extracted from frozen fecal samples, Stool DNA extraction Kit from (Geneaid, Taiwan) according to the manufacturer's instructions, all samples were treated with thermal shock for5 cycles and boiling in a water bath for each for 5 min), then incubated at 56˚C for 10 min, and extend for 1 hr at 95˚C. DNA was extracted and amplified by multiplex PCR targeting heat shock protein 70 (hsp70) gene using two sets of primers for detection *Cryptosporidium parvum* and *Cryptosporidium hominis* were designed in this study using NCBI-Genbank (AF221534.1 and KR296809.1) and primer 3 plus design, these primers were provided from Scientific Researcher. Co. Ltd, Iraq as the following table(1) The products amplified were by 1.5% agarose gel electrophoresis and ethidium bromide

**Table (1): Primer Sequences**

| Primers | Sequence 5'-3' | | Product size |
|---|---|---|---|
| C. parvum hsp70 gene | F | ATGGTGAGCAATCCTCTGCC | 618bp |
| | R | CAGTTTGGTTGTGCTCGAGC | |
| C. hominis hsp70 gene | F | TGAGGGTGAGAGAGCCATGA | 524bp |
| | R | GCATACCACCCTCAGCAGAG | |

**Statistical analysis** Data obtained were entered into a computer database, Statistical package for social science(SPSS)software was used for statistical analysis, data were recorded as number and percentages, Percentages were compared using the chi-squared test, P≤ 0.05 was considered significant[15]

## Results

**Prevalence of *cryptosporidium spp.* by microscopic examination** The current study includes examination of 109 patient stool samples with diarrhea examined by modified Ziehl–Neelsen for *Cryptosporidium spp.* by using light microscope (table 2). Showed the percentage of infected patients which were 44 (40.4%) .

**Prevalence of *cryptosporidium spp.* by molecular technique** this study include examination 96 patient stool samples by using multiplex PCR showed the percentage of infected patients which were 42 (43.75%) were positive for cryptosporidiosis as (table 2)

**Table (2):Prevalence of the *Cryptosporidium spp*. according to the microscopic examination and multiplex PCR results**

| Methods Screening Test | Total | Results | |
|---|---|---|---|
| | | No | % |
| modified ziehl- neelsen stain | 109 | 44 | 40.4% |
| PCR technique | 96 | 42 | 43.75 |

**Prevalence of the *Cryptosporidium spp.* infection according to socio -demographic characteristic**

By using M.Z.N stain, the distribution of *Cryptosporidium spp.* according to gender, The highest infected patients found 19 (55.9%) in females and lowest infected patients found 32 (51.6%) in males , while by using PCR technique the distribution of *C.parvum* infection according to gender the highest percentage was found in females 14(41.2%), and lowest percentage found in males 24(38.7), and the highest rate of infection for *C. hominis* was found in males 3(4.8%) and lowest was found in females 1(2.9%)as showed in (Table 3).while according to residence by using M.Z.N stain showed in table(3) the highest infected patients found 33 (64.7%) in rural area and lowest infected patients found 18(40%) in urban area, and by using PCR technique the distribution of *C.parvum* as prevalence of the *Cryptosporidium spp.* infection according to according to residence the highest infected patients found in rural area27(52.9%) and lowest infected patients found in urban area11(24.4%). And The distribution of *C. homini* according to residence the highest infected patients found in urban area3(6.7%) and lowest infected patients found in rural area 1(2%). As well as Prevalence of the *Cryptosporidium spp*. infection according to water source by using M.Z.N stain the highest percentage found in participant used bottled water which was 13(54%) and lowest percentage found in tap water which was 38(52.8%) While by using PCR technique the distribution of *C.parvum* according to water source the highest infected patients found in participants whose used bottle water were 14(58.3%) and lowest infected patients found in participants whose used tap water were 24(33.3%). And the distribution of *C. hominis* according to water source the highest infected patients

found in participants whose used tap water were 3 (4.2%) and lowest infected patients found in participants whose used bottle water were 1(4.2%).shown in (table 3)

**Table (3): Prevalence of the *Cryptosporidium spp*. infection according to socio -demographic characteristic**

| Methods / Socio-demo characteristic | Number | Microscope | | PCR technique | | | |
|---|---|---|---|---|---|---|---|
| | | | | *C.parvum* | | *C.hominis* | |
| | | + | % | + | % | + | % |
| Gender \ Male | 62 | 32 | 51.6 | 24 | 38.7 | 3 | 4.8 |
| Female | 34 | 19 | 55.9 | 14 | 41.2 | 1 | 2.9 |
| Total | 96 | 51 | 53.1 | 38 | 39.6 | 4 | 4.2 |
| P value | | 0.688 | | 0.813 | | 0.656 | |
| Residence \ Urban | 45 | 18 | 40 | 11 | 24.4 | 3 | 6.7 |
| Rural | 51 | 33 | 64.7 | 27 | 52.9 | 1 | 2.0 |
| Total | 96 | 51 | 53.1 | 38 | 39.6 | 4 | 4.2 |
| P value | | 0.015* | | 0.004* | | 0.250 | |
| Water source\ bottle | 24 | 13 | 54.2 | 14 | 58.3 | 1 | 4.2 |
| water Tap | 27 | 38 | 52.8 | 24 | 33.3 | 3 | 4.2 |
| Total | 96 | 51 | 53.1 | 38 | 39.6 | 4 | 4.2 |
| P value | | 0.906 | | 0.03* | | 1.000 | |

*Significant using Chi-squared test at 0.05 level of significance

**Prevalence of the *Cryptosporidium spp*. infection according to age group**

By using M.Z.N stain the highest percentage found age group (11-15) which was15(60%) and lowest percentage found in age group (16-20) yearswhich was 3(20%).while by using PCR technique the distribution of *C.parvum* according to age group the highest infected patients found in age group (≤5 years) 19.(57.6%) and lowest infected patients found in age group >20 years 2 (13.3%) there were statistical significant between *C. parvum* and age group and the distribution of *C. hominis* according to age group the highest infected patients found in age group ≤5 years 3( 9.1%) and lowest infected patients found in age group 20>years1 (6.7%) as shown in (table 4)

**Table (4) prevalence of *cryptosporidium spp*. according to age groups.**

| Methods / Age | number | microscope | | PCR technique | | | |
|---|---|---|---|---|---|---|---|
| | | | | C.parvum | | C.hominis | |
| | | + | % | + | % | + | % |
| 5≤ | 33 | 17 | 51.5 | 19 | 57.6 | 3 | 9.1 |
| 6-10 | 22 | 11 | 50 | 8 | 36.4 | 0 | 0 |
| 11-15 | 15 | 9 | 60 | 7 | 46.7 | 0 | 0 |
| 16-20 | 11 | 3 | 27.3 | 2 | 18.2 | 0 | 0 |
| 20> | 15 | 11 | 37.3 | 2 | 13.3 | 1 | 6.7 |
| Total | 96 | 51 | 53.1 | 38 | 39.6. | 4 | 4.2 |
| P value | | 0.213 | | 0.023* | | 0.364 | |

*Significant using Chi-squared test at 0.05 level of significance

**Prevalence of the *Cryptosporidium spp*. infection according to educational level**

By using M.Z.N stain as shown in(table 5) the highest percentage found in uneducated participant which was31(50.8%) and lowest percentage have education level (higher) which was4(80%) , while by using PCR technique the distribution of *C.parvum* according to educational level the highest infected patients found in uneducated participant which was 25(41%) and lowest infected patients found in participants whose higher educated level 2 (40%). And the distribution of *C.hominis* according to educational level the highest infected patients found in uneducated participant which was 4(6.6%)

**Table(5) prevalence of *cryptosporidium spp*. according to Educational Level**

| Methods / Education. L | number | microscope | | PCR technique | | | |
|---|---|---|---|---|---|---|---|
| | | | | C.parvum | | C.hominis | |
| | | + | % | + | % | + | % |
| Uneducated | 61 | 31 | 50.8 | 25 | 41 | 4 | 6.6 |
| Primary | 21 | 13 | 61.9 | 7 | 33.3 | 0 | 0 |
| Secondary | 9 | 3 | 33.3 | 4 | 44.4 | 0 | 0 |
| Higher | 5 | 4 | 80 | 2 | 40 | 0 | 0 |
| Total | 96 | 51 | 53.1 | 38 | 39.6 | 4 | 4.2 |
| P value | | 0.302 | | 0.923 | | 0.495 | |

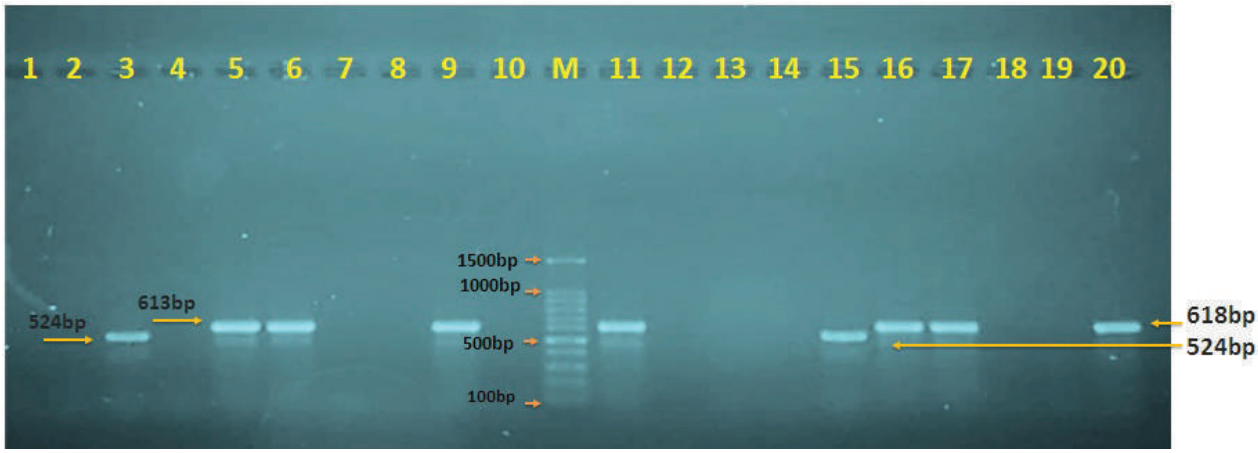


**Figure (1): Agarose gel electrophoresis image that showed the Multiplex PCR product analysis of hsp70 gene in C. parvum and C. hominis from Human samples. Where , the Lane (M): DNA marker ladder (1500-100bp) and the Lane (1-20) were showed some positive hsp70 gene in C. parvum at 613bp and C. hominis at 524bp PCR product size.**

## Discussion

The current study included the examination of 109 diarrheal patient stool samples with examined by M. Z.N stain for *cryptosporidium* species and showed 44 (40.4%) , The positive rate in this study was agreement with [8] in Al-Najaf City were recorded 29 (58%) samples positive for *cryptosporidiosis* by using M. Z.N stain in patient with diarrhea and abdominal discomfort , While our results was disagreement with [16]at Al-Dewanyia, city- Iraq that records *cryptosporidiosis* rate 4 (0.625%)in children with diarrhea by using M.Z.N stain , also [7]in Turkey which recorded 6(3.5%)in immunocompromised patient with diarrhea using M.Z.N stain. The prevalence of *cryptosporidium spp*. infection in diarrheal patient varies substantially among countries and different geographic regions in the same country could be due to differences in climatic and sociodemographic factors and differences in the number of participants examined and type of populations studied(e.g. urban or rural), location, This may explain the variation in positivity[4]

The results of extracted genomic DNA from 96 stool samples were 38(39.6%) positive for *C.parvum* agreement with [17]in Tehran which recorded 32 (1.2%) in children with diarrheal samples using nested PCR and disagreement with [18]at Al-Rifai City/Thi-Qar-Iraq that recorded 20 (10.4%) positive rate from patients with different ages to both sexes using conventional PCR , increase numbers of *C. parvum* infection 38 positive samples at al- kut city may be due to associated with contaminated drinking water supplied to these peoples with taking into account that the lowest infected dose for *C.parvum*, ranging from approximately 10 to 1,000 for healthy humans[19]Regarding the prevalence of *Cryptosporidium hominis*, This is the first study in Al-Kut city that recorded positive cases of *C. hominis* infection in diarrheal patients were 4(4.2%), the result was in agreement with [20]in Iran which recorded infection rate for *C. hominis* 4 (25%) in Immunocompromised Patients and Children by using PCR-RFLP assay. While,

disagreement with [21]which recorded 17 (81%) among symptomatic children in Egypt by using nested PCR. There are two important species of *Cryptosporidium* that are responsible for human infection (*C. parvum and C.hominis*), it is a very important issue to determine the species of this protozoan in clinical cases even though the treatment could be identical, This differences in the percentage of *C. hominis* infection may due to the variation in the sample size for each study [22] The current study showed a higher percentage of positivity among females than males although there was no statistically significant association between positivity and gender of *cryptosporidiosis* patients these results were in agreement with the findings of [23]in Jordan which record no statistically significant association between cryptosporidiosis and gender among Hemodialysis Patients in Jordan by using M.Z.N stain also [24]in Pakistan registered no significant difference between cryptosporidium spp. and gender among children by using M.Z.N stain and disagreed with the result by [25]in Nigeria which recorded statistical significant between *cryptosporidiosis* and gender by using M.Z.N stain the variation in the rate of infection between males and females may be because females are more movement and active and their contact with the external environment factors, It may lead to a lack of attention to personal hygiene and wash hands which increase the chances of being infection[26]

In the current study distribution of the *Cryptosporidiosis* infection according to residence by using M.Z.N stain, there was statistically significant association between positivity of *cryptosporidiosis* and residence p=(0.015) this results agreement with [27]in Beni-Suef, Egypt were recorded P-value= 0.03 in diarrheic Immunocompetent Patients and disagreement with [28]in Erbil City-Iraq were recorded no statistically significant association between positivity of *cryptosporidiosis* and residence among children from regular visitors of Raparin Hospital using M.Z.N stain. A strong significant association was found between *C.parvum* infected and residence by using multiplex PCR p=0.004 the current study agreement with [29]in Mid-Euphrates Area which recorded significant association between *C.parvum* infection among children and those who living in rural area by using PCR technique. And disagreement with [30]at Wasit Province were recorded no statistical significant between *C.parvum* and residence P-value= 0.07 among diarrhea patients by using multiplex PCR in a rural community can be subject to a higher degree of environmental exposure to potential sources of infection (e.g. contaminated water, farm animals and wildlife) and presence of grazing such as cows and sheep in rural areas compared with urban areas[31] while regarding the prevalence of *cryptosporidiosis* infection and association with water source the current study shown that the highly infection appear in participant who used bottle water in compared among who used tap water when used M.Z.N stain and there was statistically significant association recorded by using PCR technical method between *C.parvum* and water source p=0.03. This result disagreement with [32]in Taiz which recorded high infection occur among participant who used tap water from children of different ages by using M.Z.N stain also [23] in Turkey were recorded results that disagreement and no statistical significant between *C.parvum* and water source p=0.33 among Immunosuppressive and Immunocompetent Cases with Diarrhea by using PCR. These variation in results may be due to The oocysts are very resistant to chlorine, chloramines, and chlorine dioxide, which are commonly used in methods of water system disinfection, and keep vital for infection in the environment for a long time [33].

Regarding the prevalence of *cryptosporidiosis* infection and association with age groups the results in table(4 ) shown that the highly infection appear in age group> 5 years old and there was statistically significant association recorded by using PCR technical method between *C.parvum* and those who had been≥ 5 years old p=0.023 This result disagreement with [34]in Sulimania city-Iraq which

record high infection appear in age group under 5 years old by using M.Z.N stain among children in Sulaimani Pediatric Teaching Hospital . While the result of the current study was agreement with that registered by [35]in Korea which record high infection rate occur in old age group (50–69)years old in a small rural village occupied predominantly by aged people in Hwasun-gun, Chollanam by using M.Z.N stain ,and agreement with [21]in Egypt which recorded there was statistically significant association between *C.parvum* and age group p= 0.045 among Symptomatic Egyptian children using nested polymerase chain reaction , also disagreement with [36]in sharjah which recorded no statistically significant between *C.parvum* and age group p= 0.544 among asymptomatic healthy expatriate workers in sharjah by using real-time PCR (qPCR). Children have more activities, inside or outside the home, and lack knowledge about personal health habits and practices, so they are more exposed to the causative agents than other age groups, the current study showed the prevalence of *cryptosporidiosis* infection and association with educational level a higher percentage of positivity among educated participant than among who have no educated although there was no statistically significant association between infection and education level this result disagreement with [25]in Nigeria which recorded statistical significant between *cryptosporidiosis* and education level p=0.156 among Human Immunodeficiency Virus Seropositive Patients within Kaduna- Nigeria by using M.Z.N stain and [19]in Qatar that recorded statistically significant association between educational level and parasitic infection p= <0.001 among immigrants in Qatar with a special focus on food handlers and housemaids by using quantitative PCR (qPCR) .The reason for the wide spread of Cryptosporidiosis infection among uneducated people may be regarded to fact that infected people specially children who do not have an education about the specific personal hygiene [19]

## Conclusions

Our study showed that the prevalence rate of *cryptosporidium spp.* was found to be higher in the rural areas and the most commonly infected in age group was >5 years old, according to the gender; the highest infection recorded in female comparison with male and there was statistically significant associated between infection and residence. This is, to the best of our knowledge, the first report from Al-Kut city to document the present *Cryptosporidium hominis* in diarrheal patients in Al-Kut city.

**Conflict of Interest:** This is to certify that I Dr. May Naji Al-khanaq the author of the Prevalence of Cryptosporidium spp. among patients with diarrhea at wasit province/ Iraq. Certify that there is no conflict of interest regarding this manuscript.(NIL)

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**Ethical Clearance**: Taken From Institutional Ethical Committee

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# International Outsourcing of Quality Healthcare Through Tele-Medicine: Binding Arbitration Makes Tele-Medical Dream a Reality

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## Abstract

The development of communication technologies has a dramatic influence on culture. The internet, cell phones and e-mail are different domains, and if health care professionals want to join this space, they will be careful to do so. Telemedicine has medical-legal implications for aspects of identification, licensing, insurance, protection, privacy and confidentiality, as well as other risks related to online healthcare communication. The International Advisory Group of the World Health Organization (WHO), which met in Geneva in 1997, identified telemedicine as providing healthcare services, where distance is a critical factor, to health care providers, who use the information and communications technologies to exchange relevant information for the diagnosis, treatment and prevention of diseases and injuries, and to continue to do so.

In the context of the COVID-19 Pandemic Lockdown, the Indian Government has authorized telemedicine legal status. The Governing board, established by the Government at a meeting of the Indian Medical Council with the Ministry of Health and Family Welfare's approval, published a notice dated 25 March 2020 ('Amendment') modifying Guidelines of the Indian Medical Council 2002 on telemedicine in India. The amendment introduced 'Telemedicine consultation' to the law.

A basic knowledge of how medical negligence compensation is calculated and adjudicated in the judicial courts of India. The paper concludes with an assessment of the rules. This paper will seek to determine whether binding arbitration is the best possible solution to resolving malpractice disputes, or whether traditional litigation, while costly, is the safest choice. To do this, the paper will examine both the advantages and the disadvantages associated with using arbitration as opposed to litigation.

***Keywords:*** *Outsourcing Healthcare, Tele-Medicine, Tele Medicine Overview, Telemedicine Guidelines, Arbitration; Civil liability, Medical Negligence.*

## Introduction

The development of communication technologies has a dramatic influence on culture. The internet, cell phones and e-mail are different domains, and if health care professionals want to join this space, they will be careful to do so. Telemedicine has medical-legal implications for aspects of identification, licensing, insurance, protection, privacy and confidentiality, as well as other risks related to online healthcare communication. The International Advisory Group of the World Health Organization (WHO), which met in

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Geneva in 1997, identified telemedicine as providing healthcare services, where distance is a critical factor, to health care providers, who use the information and communications technologies to exchange relevant information for the diagnosis, treatment and prevention of diseases and injuries, and to continue to do so.

Telemedicine in India was formally launched on March 30th, 2000, when Bill Clinton— the then President of the United states— commissioned the first telemedicine unit in the village of Aragonda in Southern India, about 200 km from the tertiary care centre in Chennai while he was witnessing a live cardiac teleconsultation[1]

In the context of the COVID-19 Pandemic Lockdown, the Indian Government has authorised telemedicine legal status. The Governing board, established by the Government at a meeting of the Indian Medical Council with the Ministry of Health and Family Welfare's approval, published a notice dated 25 March 2020 ('Amendment') modifying Guidelines of the Indian Medical Council 2002 on telemedicine in India. The amendment introduced 'Telemedicine consultation' to the law.[2]

The demand for telemedicine is rising exponentially. With advances in technology, telemedicine devices are more usable and affordable. Telemedicine has been developed to treat people with minimal access to healthcare services in remote areas. However, it is now a comfort device in the area of healthcare[3]. The increasing use of mobile phones is a significant factor responsible for developments in this field. The availability of mobile apps lets patients monitor their health status. This proactive approach enables them to make use of creative means of accessing health services such as telemedicine.

Digital consultation is a process of receiving a medical opinion without meeting a doctor in person, which is the only distinction from a conventional medical consultation. Digital consultations often allow a physician to engage in discussion with another physician to consult various electronic medical records. The primary objective of telemedicine and remote consultation is to provide adequate healthcare services in India. It includes promoting access to care for wealthy and underprivileged populations, offering easier, cheaper and effective patient interaction, expert follow-up, and monitoring.

In most cases, telemedicine is beneficial. This provides access to healthcare services in remote areas for persons with mobility issues and people with disabilities. This may also overcome geographic barriers to the delivery of healthcare services. It can provide an incentive for the patient and caregiver to minimize health care costs and save time[4]. With telemedicine's advent, a medical practitioner or hospital can consult with a range of specialties, irrespective of where they are located. Telemedicine helps patients communicate more often with their health care providers in a relaxed manner, leading to a greater relationship between the doctor and the patient. Patient check-ups are expected to be higher and could improve outcomes. Telemedicine, in general, has the potential to deliver affordable health care to people.

Mediation and arbitration in medical negligence cases have brought to light the different forms of remedies that victims or patients can seek, in addition to compensation, as well as the limited time it takes.[5] The flexibility of alternative dispute resolution measures allows for the variety of remedies, including (a) admission of negligence on the part of the doctor, (b) institution of training programs to prevent avoidable faults, and (c) emergency training to hospital staff, which by their own admission have provided great satisfaction to the victims.

**What is Arbitration?**

Arbitration is a more formal and binding form of ADR. Parties are typically represented by attorneys who argue the case before an arbiter or arbitration panel. The arbiter then issues a decision. The main distinction of arbitration is that the arbiter's decision is typically binding. It is popular therefore among parties

who fear the capricious nature of jury verdicts and is seen as a means of risk management. One form of arbitration that is gaining popularity in the healthcare field is the pretreatment arbitration agreement. This is an agreement that patients sign as a condition of being seen by a healthcare provider stating that should a dispute arise, it will be handled through arbitration. Physicians may include such clauses in their initial contracts with new patients and so protect themselves from litigation. Several legal challenges have been raised to these clauses, but in every case, such clauses have been deemed legal and binding[6]. As such, pretreatment arbitration clauses are used by clearly on the rise, whether in agreements between physician and patient, physician and malpractice insurance provider, or patient and insurance company or HMO. Even entire states are starting to require arbitration[7].

The binding nature of arbitration can hurt both the plaintiff and defendant alike, however. The overwhelming majority of times that a physician is sued, there is no negligence involved, as the outcomes of trial litigation have confirmed repeatedly. Physicians may therefore find it advantageous to go to jury trial to clear their names and prove there was no negligence[7]. Binding arbitration means the physicians forego this right and must take their case to an arbiter. Although arbiters award much more modest awards than juries, they are also more likely to award some type of award to the plaintiff whether there is negligence or not[8]. The propensity of arbiters to force compromise is one criticism of arbitration. Other critiques are that it is too rigid and adversarial, only one step removed from an actual trial. Costs are higher than mediation and the process is more acrimonious because lawyers are involved[7]. Satisfaction rates among both parties are lower than mediation and, similar to jury trials, the only form of redress is monetary. Still, there are definite time and cost savings compared with litigation, and the fact that it is binding means many potential lawsuits are diverted from the courthouse.

Arbitration also has some unique strengths. Arbiters can be selected for their unique scientific background. This makes arbitration a particularly good choice for disputes over specific issues of scientific fact. Rather than leaving the matter to a jury that is unlikely to comprehend the issue—or to a negotiation when there is a great discrepancy between the understanding of the scientific issues at play—arbitration has a unique advantage of having a skilled and knowledgeable arbiter as a decider of fact. As a binding decision, arbitration effectively only goes to trial when one of the parties appeals the decision. Even this is expedited, however. The decision of an arbiter can only be overturned for procedural error, bias, or fraud.

Arbitration of Medical Malpractice Claims in a Managed Care System.

In recent years, arbitration has arisen as a proposed solution to many of the problems associated with the litigation of medical malpractice claims. Therefore, we should examine what it is about arbitration that has precipitated this change, and why many HMOs and managed care plans choose to include arbitration clauses in their provider contacts today.

**The Strengths of Arbitration**

One of the primary benefits of arbitration is that it benefits both providers and patients by limiting the resources that are required to resolve disputes, in terms of both time and money. In litigation, where disputes are resolved by laypersons, jurors must first be educated in the basics necessary to understand the medical issues. This alone can take weeks of expensive trial time under the most ideal conditions. However, even this understates the problem. Jurors not only have to listen and learn, they must also weigh the testimony of different experts who may, in some cases, have divergent opinions about even the most basic of matters, complicating things immensely.

Arbitration, on the other hand, bypasses this learning curve completely. An experienced and knowledgeable arbitrator will not need to be educated about the science, but rather, only about the facts. The

savings in terms of time and money are huge.

The benefits of this speed and efficiency are twofold. Not only does this savings of time mean that complex cases can be resolved more quickly, it also means that small disputes, which under a litigation regime may never have been heard, may now also be resolved providing a benefit to the public at large. Further, as stated above, a reduction in the cost of litigating disputes should, in theory, lead to reduced costs to insurers and lower rates for the insured.

A secondary benefit of arbitration is that it preserves existing relationships. As noted earlier, a significant drawback to the use of litigation in a managed care environment is that litigation tends to polarize parties and pit them against one another, a crucial concern when an entire block of employees may vote to stay or leave a provider at the same time. The collaborative forum provided by arbitration has become important for other reasons as well. As miracle cures are developed for common illnesses, expectations that doctors can treat even the most difficult medical conditions increase. Therefore, doctors may now seek arbitration not only as a cost-cutting device, but also as an appropriate forum to explain to angry patients why they, or their loved ones could not be cured.

A final benefit of arbitration is that it allows qualified fact finders to make decisions based on information given by less biased or even court-appointed independent experts. This factor is of great significance when, as has been noted, the use of jurors in litigation of technical issues often results in long and costly jury trials.

**B. The Weakness of Arbitration**

Despite the fact that arbitration is generally faster, cheaper and more consistent than litigation, there are also some troubling problems associated with it. One problem with arbitration is that it fails to develop legal precedent in a rapidly changing legal area. This can lead to the squandering of resources through the re-arbitration of previously decided disputes, inconsistent decisions, and other, equally troubling problems. Despite the fact that arbitration allows only for abbreviated process and limited discovery, as a general rule courts have enforced arbitration agreement in provider contracts, rejecting arguments by consumers that they are contracts of adhesion. What effect enforcement of these contracts will ultimately have on poor, disadvantaged, or disempowered groups has yet to be fully determined, but some commentators have expressed concern that the informality of alternative dispute resolution processes too risky for these groups.

## Conclusion

Looking at the numbers, India's telemedicine sector's size seems very promising, and with an upward trajectory, one with enormous potential[9]. A recent study by the McKinsey Global Institute estimated that implementing telemedicine technology could save around $4-5 billion annually and replaces half of India's in-person emergency consultations. The Global Market Insight report estimates that the global telemedicine industry will cross a value of $130.5 billion by 2025, with India's market expected to rise to 2.4 per cent CAGR. Telemedicine services can prove to be an effective way to bridge the gap between urban and access to quality care facilities in rural areas. The current Government's strong focus on digitization has served as a catalyst to boost business expansion.

Even so, this industry's fate and future remain uncertain in the absence of consistent rules and regulations on telemedicine services in India. In order to eliminate any possible danger, manufacturing companies can ensure that they comply with all applicable regulations in place and are contractually covered. Additionally, the impact of the Covid-19 has led to an urgent need of availability of alternative platforms of consultation in health-sector for non-chronic and less serious medical problems, keeping in view the risk posed to both the patients as well as the

doctors by physical consultations in hospitals.

The Guidelines mention that 'in the interim period, the principles mentioned in the guidelines need to be followed'. While the term interim has not been defined, this suggests the encouragement to the doctors to use the sector during the lockdown phase. In such situations, the Telemedicine Guidelines is a much-needed initiative.

Several nations around the world have promoted alternative dispute resolution. They have been pragmatic to use administrative ADR wherein administrative bodies dedicated towards. ADR facilitates early settlement of disputes. It may also mean that an important relationship can be repaired and maintained, something which may be at risk in adversarial litigation. This legalistic approach often overlooks other avenues of settlement opportunity, which may better address a client's underlying interests and needs.[10] It must become such a well established part of it that when considering the proper management of litigation it forms as intrinsic and as instinctive a part of our lexicon and of our thought processes, as standard considerations like what, if any, expert evidence is required. Mediation and arbitration in medical negligence cases have brought to light the different forms of remedies that victims or patients can seek, in addition to compensation, as well as the limited time it takes. The flexibility of alternative dispute resolution measures allows for the variety of remedies, including (a) admission of negligence on the part of the doctor, (b) institution of training programs to prevent avoidable faults, and (c) emergency training to hospital staff, which by their own admission have provided great satisfaction to the victims.[11]

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# Psychosocial Impact and Protective Factor of COVID-19 Confirmed Patient During Isolation Enactment: A Systematic Review

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## Abstract

**Background:** An individual who is positively confirmed COVID-19 is required to undergo a long-term isolation. As a consequence, this condition can cause physical and psychosocial impacts. This review article aims to identify psychosocial impacts COVID-19 patients and protective factors the patients during isolation enactment. **Method:** This article is a systematic review by doing article identification from four data bases (ScienceDirect, ProQuest, EBSCO and PubMed) published between January to October 2020. The articles were selected using PRISMA based on assigned inclusion and exclusion, keywords on psychological impact, COVID-19, and confirmed patient. **Results:** Based on the 28 article analysis divides psychosocial domain into four aspects; they are emotional impact, social, and behavior. Protective factor was divided into four aspects, they are social support (family, social, medical team, and government support), personal ability (religious, cognitive, affective, psychomotor aspects), positive belief (internal and external), and material asset (facility and financial). **Conclusion:** Psychosocial impacts of confirmed COVID-19 patients during isolation enactment can be minimized through psychologist intervention by increasing patients' protective factors. Based on the finding, the suggestion was the part of nursing in isolation COVID-19 important to increase health condition of mental patients undergo isolation.

***Keywords:*** *confirmed patient, COVID-19, isolation*, *protective factor, psychosocial impact*

## Introduction

Coronavirus disease (COVID-19) is caused by the acute respiratory syndrome of coronavirus 2 (SARS-Cov-2) [1]. SARS-CoV, MERS-CoV, and 2019-nCoV can cause disease in human [2,3]. COVID-19 is almost the same as SARS and MERS in characteristics, but spreads to human faster [4]. COVID-19 infection from human to human is infected through direct contact and splat (droplet) when someone coughs or sneezes [5]. Infection can be happened easily to vulnerable group [6]. COVID-19 was found first in Wuhan, China [7] and spreads to almost the entire world [8].

Confirmed cases of COVID-19 in the world up to August 2020, reach 19,936,210 cases with death cases of 732,499 [9], the highest death cases in the age >85 years old with 10%-27% [10]. In Indonesia as of May

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22, 2020, a total of 20,796 people have tested positive for COVID-19 and the mortality rate has reached 6.4 % [11]. The effects of COVID-19 are very similar to all countries by considering the consequences of mental health or psychologically [12]. The Psychology impact of COVID-19, such as fear and Anxiety are caused by its high infection risk, high morbidity and mortality, and the vaccine has not been found [13]. Anxiety symptoms increased substantially compared to before the COVID-19 pandemic among vulnerable persons with a pre-existing medical condition [14]. Research in China shows that there are 53.8% major psychological impact, 16.5% reports major depression symptoms, 28.8% reports major Anxiety symptoms, and 8.1% reports high level stress to the society during the pandemic [15].

Society Health Status is generally divided into 4, They are suspect case, probable case, confirmed case, and definition of contacts [9]. A person is stated positive COVID-19 through swab test (RT-PCR) [6]. A patient needs treatment in the hospital if the patient has severe or medium sick with difficulty or comorbid, suffer disease, certain condition, or serious [16]. During isolation time, the patients only get medicine based on the symptoms, supportive treatment and complication prevention because the medicine of the target has not been found yet [17].

Isolation treatment prohibits patients to meet their family and communications done through social media [6]. Based on those conditions the patient encountered many psychosocial impacts during the isolation. Thus, it is important to identify those impacts as a base to give intervention based on the patients' symptoms. Intervention given also needs consideration to the protective factor of the patients. Patients who a protective factor, family support, friends, and medical personnel can minimize the depression and Anxiety risk during isolation [18]. Psychosocial support [7], mental health [17], and adaptive coping source can prevent major depression symptoms that experienced by the patients [7]. Nurse can also gives intervention by supporting the patient to maximize protective factor that had as an effort to minimize psychological impact during isolation time [19]. Psychosocial impact and protective factors are important to discuss because of the most articles only explained specific psychological impact [19]. It has not comprehensively explained the psychosocial impact and protective factors of the COVID-19 confirmed patient during isolation undergoing. Based on the problem that has explained, this article aims to identify psychological impact and protective factors that had by the patient during isolation time, so it can used as a base to give psychological intervention.

## Methods

This systematic review study discerned psychological impacts and protective factors of confirmed COVID-19 patients during isolation enactment. We employed PRISMA checklist to select article that has been found from database [20].

### Literature Searching Strategy and Selection Process

The strategy used to search for relevant article in this study was based on PRISMA guidelines (Figure.1). The article discussed about psychosocial impacts and protective factor of the confirmed COVID-19 patients who undergo the isolation and it can be developed a systematic process. Literature searching was done within the widely reputable publication databases such as ScienceDirect, ProQuest, EbscoEBSCO, and PubMed. Besides, we also used Boolean and keywords combination of "psychological impact AND COVID-19 AND confirmed patient".

The boundaries of the review question were defined through the development of inclusion and exclusion criteria using the PICOS (P = Population, I = Intervention or Issue of interest, C = Comparators, O = Out comes, S = Study type) format. The population were confirmed COVID-19 patients who undergo isolation. Issue of interest was a psychosocial impact and had not comparators. Out comes related to the psychosocial aspect and protective factor COVID-19

patients who undergo isolation. And study type the literature was not limited on research articles, but all kinds of articles, included letter of editor, review, and research articles. The articles written in English and published in the data based during January 2020 to October 2020.

### Assessment of study quality and risk of bias

Study Quality Valuation and Refraction Risk from each article used JBI Critical Appraisal Tools that was suitable in this systematic review. Based on the appraisal using The JBI Appraisal Tools got we collected 28 articles that are suitable with the systematic review. Appraisal on JBI was divided on answering choice 'yes', 'no', 'unclear', or 'not applicable' by 1 value given for 'yes' and '0' for another answer. The research that fulfilled 60% appraisal critical criteria is worthy and got into this systematic review. We exclude a low-quality study to avoid refraction in based on the validity and review recommendation.

## Results

Our systematic review obtained 1983 articles considering of 642 articles from ScienceDirect, 731 articles from ProQuest, 14 articles from Ebsco and 596 articles from PubMed. We identified 97 articles for text filtration completely to evaluate its worthiness. Then, we found 28 articles that are suitable with the inclusion criteria that have been determined and worthy to be reviewed. Literature filtration process is explained on Figure 1.

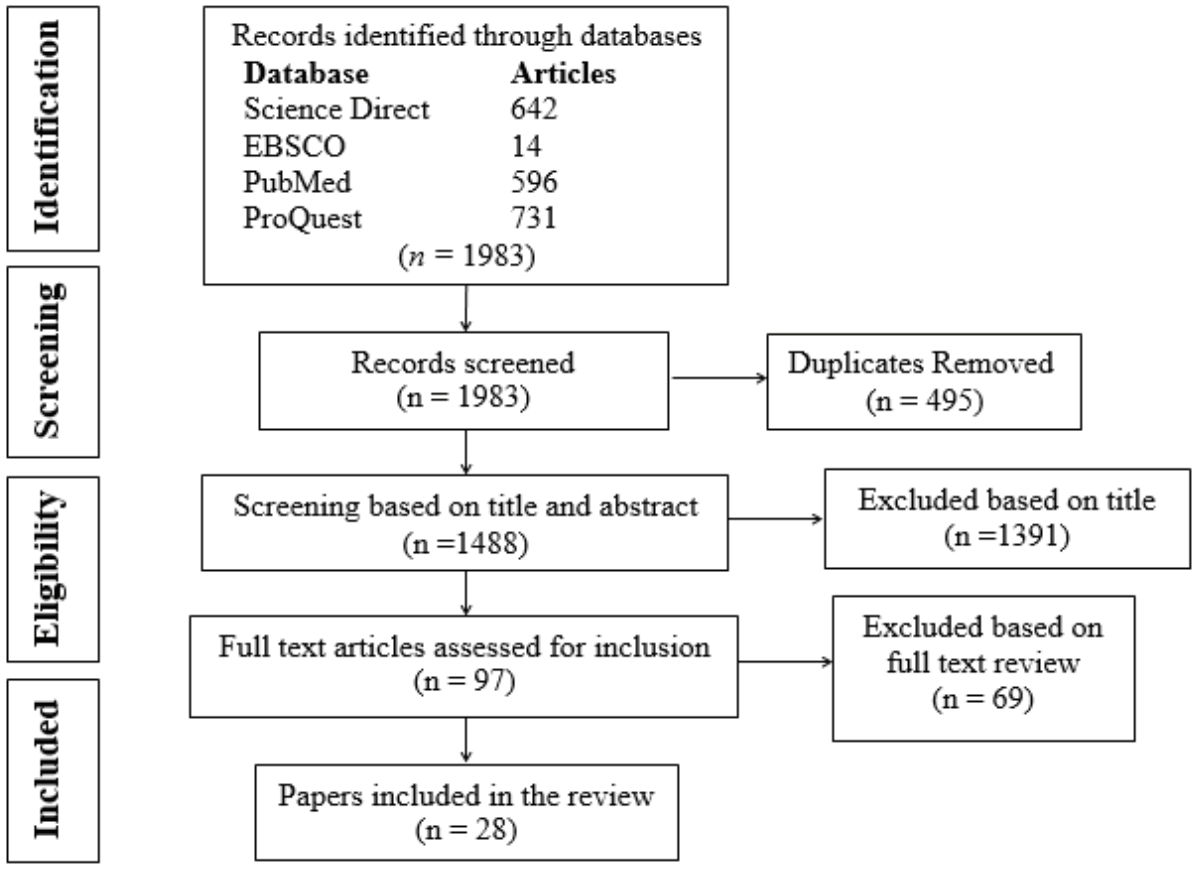


**Figure 1. Reporting items for study flow diagram**

Table 1 shows the summary of systematic review. There were 20 research articles (71.4%) and 8 non-research articles (28.6%) consisting of 4 review articles and 4 letters of editor. Most of the articles (57.1%) were written by Chinese scholars and the rest, 4 USA articles, 3 Indian articles, 3 Ecuador articles, 1 Algerian article, and 1 Jordan article.

**Table 1. Summary of Article Included in the Study (n=28)**

| Authors and years | Study design | Psychosocial Impact | | | Protective Factor | | | |
|---|---|---|---|---|---|---|---|---|
| | | Emotional | Social | Behavior | Social Support | Personal Ability | Positive believe | Material Asset |
| (Rongfeng et al., 2020) | Cross Sectional | √ | √ | √ | √ | | | |
| (Madani et al., 2020) | Cross Sectional | √ | | √ | √ | | | |
| (Xie et al., 2020) | Case Control | √ | | √ | √ | | | |
| (Bruns et al., 2020) | Clinical Practice Report | √ | √ | √ | √ | √ | | |
| (Dai et al., 2020) | Cross Sectional | √ | | √ | √ | | | |
| (Guo et al., 2020) | Mix Methode | √ | √ | | √ | √ | | |
| (Sun et al., 2020) | Qualitativ | √ | √ | √ | √ | √ | √ | |
| (Chen et al., 2020) | Cross Sectional | √ | √ | | | | √ | |
| (Chen et al., 2020) | Qualitativ | √ | √ | √ | √ | √ | | √ |
| (Werner et al., 2020) | Literature Review | √ | | | √ | | | √ |
| (Yang et al., 2020) | Cohort Study | √ | | √ | √ | | | |
| (Diaz & Baweja., 2020) | Letter of Editor | √ | | | | | | |
| (Liu et al., 2020) | Letter of editor | √ | √ | √ | √ | | | |
| (Paz et al., 2020) | Letter Of editor | √ | | | | | | |
| (Zhang et al., 2020) | Cross Sectional | √ | | | √ | √ | √ | |
| (Mo et al., 2020) | Article Review | √ | √ | √ | √ | | | |
| (Paz et al., 2020) | Cross Sectional | √ | | | | √ | | |

***Cont...*** **Table 1. Summary of Article Included in the Study (n=28)**

| Authors and years | Study design | Psychosocial Impact | | | Protective Factor | | | |
|---|---|---|---|---|---|---|---|---|
| | | Emotional | Social | Behavior | Social Support | Personal Ability | Positive believe | Material Asset |
| Samrah et al., 2020) | Cross Sectional | √ | √ | | √ | √ | √ | |
| (Ma et al., 2020) | Cross Sectional | √ | √ | | | √ | | |
| (Liu et al., 2020) | Cross Sectional | √ | √ | | | | | |
| (Hu et al., 2020) | Cross Sectional | √ | √ | | | | | |
| (Sahoo et al., 2020) | Cross Sectional | √ | √ | √ | √ | √ | √ | |
| (Mi et al., 2020) | Letter of editor | √ | | | | | | |
| (Mazza et al., 2020) | Cross Sectional | √ | √ | √ | | | | |
| (Upadhyay et al., 2020) | Descriptive Research | √ | | | | | | √ |
| (Dubey et al., 2020) | Clinical Research & Review | √ | √ | √ | √ | √ | | |
| (Zhong et al., 2020) | Case Control | √ | | | | √ | | |
| (Garcia et al., 2020) | Case Report | √ | | √ | √ | | | √ |

The confirmed COVID-19 patients who are discussed are varied, from symptoms patients and asymptomatic patients. The isolation undergone by the patients are quarantined at home, hospital, and independently isolated. Psychosocial impacts experienced by the confirmed COVID-19 patients during isolation were divided into three impacts explained in Figure 2.

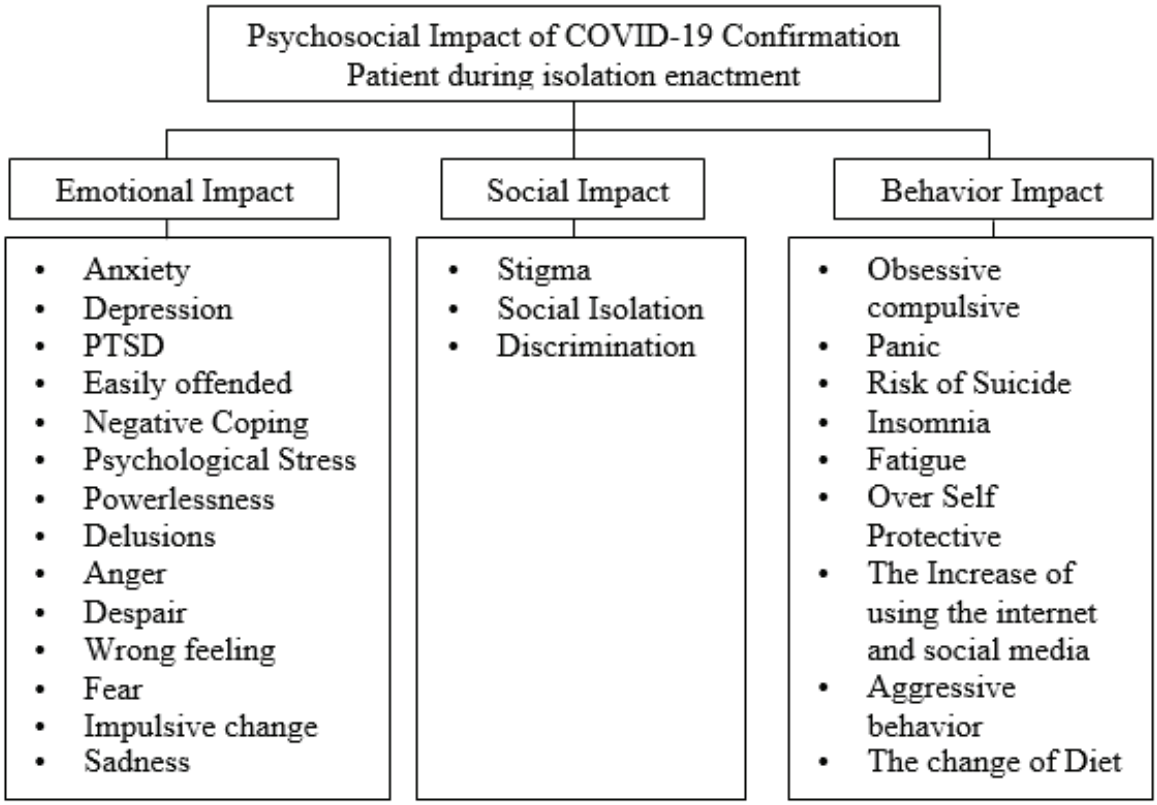


**Figure 2. COVID-19 Confirmed Patients' Psychosocial Impact Analysis**

Based on the analysis 20 articles (71.4%) informed that the protective factors among the patients during isolation enactment can minimize the psychological impacts. Protective factors in among confirmed COVID-19 patients during isolation at home or in the hospital were divided into four factors explained in Figure 3.

## Discussion

### Psychological Impacts on Confirmed COVID-19 Patients Undergoing Isolation

Confirmed COVID-19 patients must undergo a long term isolation during treatment and medication. During the isolation patients experience some physical and psychosocial impacts. Based on the article review analysis psychological impacts can worsen patient condition during isolation enactment. These were divided into emotional, social, and behaviour impact.

#### *Emotional Impact*

Based on the analysis, emotional impact consists of Anxiety, depression, PTSD, offended, negative coping, psychologically stress, helpless, delusion, anger, hopeless, guilty, mood changing, sadness and feared. Fear during COVID-19 included on being discomfort thinking about the pandemic, clammy hands, being afraid of losing life, nervousness and anxiety when watching news, insomnia and palpitating [21]. Prior to being hospitalized, early symptoms experienced by the patients are insomnia, aggressive behaviour, delusion, and major Anxiety [22]. Mental health problems of confirmed COVID-19 patients are depression and Anxiety happened from minor to major [23,24]. Psychology impacts like Anxiety and depression are affected by negative stigma in the society [25]. Anxiety happened because of different point of view and uncertainty of pandemic development [26]. Based on gender aspect, female patients tend to experience

Anxiety because of contacts with colleagues who are positively confirmed COVID-19 [27]. Felling distress appeared when understanding that this disease recovery tends to be difficult, and even it ends on with death. Female patients also tend to experience depression and Anxiety with family members who are positive COVID-19 [28]. Patients who experiences sleeping disturbance is easier to experience Anxiety and when the sleeping quality is worsen it will be risky into depression [26]. Patients will tend to experience the Anxiety after isolation so mental health readiness of the patients and family when go home to be taken care of [29].

Depression happened when having a family member who is COVID-19 diagnosed [28,30]. A patient with a family member who is infected may be more depressed, maybe because of excessive psychological pressure, guilty about disease infection to another family member, and societal stigma [18,29]. Depression is much happened to the female patients because of worrying about the health service process and uncertain disease medication [31]. Depression is also triggered by unsatisfying patients with the service and bad communication with the medical personnel. Minimum relation and communication between medical team and patient because of time limitation to interact and workload can trigger stress to the patients [18]. Patients in feeling depression tend to have low life quality [30]. Depression is often followed by insomnia, Anxiety [30,32], and negative coping until ends to PTSD [33].

PTSD and depression can cause the increase of inflammation factor of COVID-19 patients [28,34]. PTSD symptoms mostly happened the isolation enactment in the hospital. However, it can turn to be worse if patient have physical symptoms like sleeping difficulties [35]. Besides PTSD, a major mental disorder problem experienced by the patients is acute psychotic disorder. Stress burden felt by COVID-19 patients can be risky to experience acute psychotic disorder if it is not given suitable intervention [22]. Thus, beside giving medication to handle physical symptoms, the patients are also given sedative to mood stabilizer [22]. Giving psychotropic must consider the side effect to influencing patients' physic decreasing function [29,34]. COVID-19 patient should be cared by psychiatric because can influence the neurologist function and, worsen brain nervous that cause mood disorder [36]. Virus can replicate in cell system, influence immunity and mental, mood disorder, exhausting symptoms, appetite deficiency, social interaction decreasing, and lost interest [37].

***Social Impact***

Based on the analysis, social impact consisted of stigma, social isolation and discrimination. The patients' early response after being diagnosed with COVID-19 was fear, denial and stigma from society [18]. Community stigma and Anxiety cause of disease uncertainty that impacts on patients. Depression is a psychological problem sever and decreases patients physical condition [38]. Patients have stigma from society can increase morbidity and mortality [38,39]. Social stigma and discrimination decrease the patients' psychological condition [35]. Patients with mental disorder history are risk because they feel guilty afraid of transmitting to family and other people and have social stigma from society [29].

Self-isolation or isolation in the hospital can COVID-19 patient feel fear, worry and isolation because they have to be separated from family members [40]. Individuals feel isolated, so that Anxiety and anger cause of social networking facilities and activities [39]. Even though isolation is carried at home and does not adapt to the environment, and patients feel limited to social interaction [40]. While undergoing isolation, the patient worried about discrimination from society [23]. Discrimination and stigma from society because of the rapid transmission of the virus and people's fear [34]. Social stigma, isolation and discrimination can cause Psychological condition decrease [41].

***Behaviour Impact***

Based on the analysis, behaviour impact consisted

of compulsive obsession, panic, insomnia or sleeping disorder, exhausted, behaviour changing, aggressive, dietary habit changing, to suicide trial. Suicide urge of patient triggered by stress, negative emotion, social isolation, helplessness, hopelessness, guilt, mood change, economical burden, joblessness, and family harassment [37]. Those aspects become burdens they create financial loss of the patient [39]. Male tends to experience it because man is the responsible person in the family cannot give financial guarantee during quarantine or isolation [42]. Economic difficulty also becomes a burden for the patient [41]. Pandemic information can increase Anxiety too in society, so it needs the ability to filter the information, the government also try to keep an eye on pandemic hoax spreading [37].

Many changing of emotional conditions emotional that felt can make the patient experiences some changing as; sleeping habit, dietary, and behaviour disorder [18,24]. On panic condition, the patient becomes uncooperative so troublesome medical team to do an intervention [41]. Medical team must be able to consider the impact of social media usage to the patient relates to mental emotional condition [43]. Beside that, medical staff hoped to give psychological intervention because wherever isolation places still experience a psychological problem [40].

**Protective Factor of Confirmed COVID-19 Patients Who Undergo Isolation**

A protective factor is a condition in an individual; family, community, and society can possibly catch stressor through effective way to decrease mental problem risk [44] Based on the article analysis result, a protective factor by confirmed COVID-19 patient during isolation time consisted of social support, personal ability, positive belief and material asset.

***Social Support***

Based on the analysis, social support consists of family support, medical team, and government. Social support may decrease of psychological stress responses to negative life events or trauma historical [45]. Good social support relates to the risk of decreasing psychology disorder to the patient [33]. Social support that is needed by the patient in light COVID-19 can increase self-confidence, increase defence so it can help to lighten the psychology problem [27]. Family support, friends, and medical team help recover the spirit and do not feel alone [18]. Appropriate support, self-ability, and intervention can increase the physical and psychological recovery of the patient [18,38]. Patients get support from friends, family, and medical team who tend to have not depression. If it is impossible to meet family members, so they can sending positive messages and motivating [11]. Support can be expressed online via the phone or social media [31].

Medical team have an obligation to do intervention such as education about information and medication procedure, screening, follow up, prevention, disease prognosis, and the mortality development to minimize Anxiety [43]. Intervention can be given to the patient via online media as an effort to minimize infection risk [46]. Intervention conveying about the transparent and open information between medical team and the patient can decrease fear, Anxiety, discrimination that felt by the patient [26]. Intervention of mental health and psychosocial support can decrease depression and stigma that the patient feels.

Medical personnel, team, and society also need support from, the government in the management effort of the psychosocial problem of COVID-19 patients. The government is hoped establishes rules to minimize negative stigma in society to the COVID-19 patient and develop a research program [34]. Health promotion such as; healthy life-style, regular exercise and COVID-19 information minimum 1 hour every day can minimize psychological health effect [47].

***Personal ability***

Based on the analysis, personal ability consists of religious, psychomotor, affective, and cognitive aspects. The ability of social media usage wisely and effectively depends on knowledge level or patient

cognitive ability [46]. Social media usage, mostly Facebook and YouTube, is the second coping method that used generally [31]. The patients also needs beside the knowledge of social media, the adaptive of second coping strategy [38]. Adaptive coping and support system that individual had during isolation helped the patients not be burdened [19]. Using an adaptive coping strategy during isolation is very important for the patient to adapted to the new environment [40]. The other that, the patients' ability to adapt to the problem also minimizes Anxiety and burden that felt during isolation [27]. Patient uses religious aspect as well that trusted to face and to response problems [40]. Thus, religious aspect is often used by the nurses to give psychological intervention for the confirmed patient [40]. An individual resilient can protect from depression and Anxiety symptoms, against negative emotion to prevent stress and increase mental health [27]. Nurses often encourage the patient to do light exercise, take regular rest during isolation, so self-awareness doing those is necessary for patients' health [47].

***Positive belief***

Based the analysis, positive belief consists of internal and external factors. Internal factors sourced from the inside of the patient, and external factor sourced from patient belief in God and others. Internal factor is such as thankful, positive thinking, and optimism in life. Patients create thankful for life and problems that must be accepted and faced are lessons to be braver and stronger [18]. Self-ability of the patient as like God's belief and good spiritual level can minimize the risk of mental health problems during isolation [40]. Positive thinking can decrease the burden or psychological stress that faced the patient [27]. Positive thinking is needed not only for self but also for family, others (society or medical personnel). Positive thinking is a positive belief as well to medical personnel as persons who can help recovery and minimize depression symptoms [31]. The important thing for the patients to increase their psychological welfare is optimistic during treatment time [19]. Besides that, patients also keep increasing self-belief that they can be recovered [27].

***Material asset***

Based on the analysis, material asset consists of the financial and facility available. COVID-19 patient feels more burdened because of the family economic and the unavailable of facilities during isolation. Monetize burden, joblessness, and family harassment can become related reasons for increasing suicide intention in COVID-19 patients [37]. As if the man bears Indian customs, economics, and when he is confirmed COVID-19 then undergo on isolation, the Anxiety of family needs will appear [42]. Based on that matter, financial sufficiency can decrease stress level on the patient [37]. Relating to the facilities, the patient needs good facilities to do social relations and support the environment during isolation. Treatment from the staff and comfort living environment minimize the burden or psychosocial problems of the patient during isolation [19]. Effort that can be done to increase mental health is creating a supporting environment, easy access to health treatment services and clear policy relates to health service [38].

This is systematic review limitation relates to original article source research that discusses about special psychosocial impacts impacts-on confirmed COVID-19 patients during isolation enactment. We analyzed 4 Letter of Editor 4 articles (14.2%). Besides that, there are 3 articles (10.7%) focus on both confirmed COVID-19 patients and all pandemic impacted groups.

## Conclusion

COVID-19 patients have experienced many psychosocial impacts during isolation enactment. Psychosocial impacts during isolation were divided into emotional impact, social impact, and behaviour impact. Aside from the many psychosocial affects, during isolation enactment, patients can survive because they have protective factors. Protective factors of COVID-19 patients were divided into social support, personal ability, positive belief, and

material asset. Each protective factor in the patients can dig and push to be more increasing can be use to minimize psychological problems during isolation enactment. Based on the identification results, many psychological affects that are experienced by the patients should be given appropriate intervention using recommended methods.

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**Ethical Clearance**

Due to the characteristic of this research design is a systematic review (use of secondary data) the ethical evaluation was not required. However, statement of research ethics in the journal articles would serve as a consideration of the use of the publication for this review.

**Declaration of competing interest**

The authors declare, There is no conflict of interest.

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**Authorship**

3- All authors conceived the idea, concept and design of the study. Mira Wahyu Kusumawati writing, drafting and revising the article. Setyawati Soeharto and Heni Dwi Windarwati revising the article. All authors discussed the results and contributed to the final manuscript final approval of the version to be submitted.

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# Factors Related to Exclusive Breastfeeding in East Java – Indonesia

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## Abstract

Exclusive Breast Feeding (EBF) is very crucial for the growth and development of toddlers in the future, both physically and mentally. The study aims to analyze factors related to EBF in East Java, Indonesia. The analysis material for the study was the 2017 Indonesian Nutritional Status Monitoring, a cross-sectional survey. In East Java, the study obtained 3977 toddlers using the multi-stage cluster random sampling process. The study used binary Logistic Regression tests to interpret the data. The results show for toddlers living in cities have a 0.951 times lower probability of experiencing EBF than toddlers residing in rural areas. Toddler age (in months) is one of the determinants of EBF. All categories of maternal marital status are more likely than mothers who are never married to having an EBF toddler. All maternal education levels have a better chance than mothers who have no education to have EBF children. Except for mothers in the senior high school category, which has no significant relationship with the incidence of EBF in children under five in East Java, Indonesia. The study also found toddler's age associated with the incidence of EBF in children under five in East Java, Indonesia. Finally, the toddler boy has a probability of 0.943 times compared to the toddler girl to experience EBF in East Java in Indonesia. The study concluded that six variables were related to the incidence of EBF in East Java - Indonesia, namely residence, mother's age, mother's marital, mother's education, toddler's age, and toddler's gender.

***Keywords:*** *breastfeeding, exclusive breastfeeding, toddler's nutrition, public health.*

## Background

Exclusive breastfeeding is the gold standard in infant nutrition[1] Exclusive breastfeeding means that the infant receives only breast milk without other liquids or solids are given with exception of oral rehydration solution or drops of vitamins, minerals or medicine[2]. Exclusive breastfeeding is one of essential factor for infant development and survival[3]. Globally, only 23 countries currently have met the 2030 global goal for exclusive breastfeeding at six months[4]. CDC survey showed that most infants in United States born in 2017 started breastfeeding (84.1%), only 58.3% of infants were breastfeeding at 6 months[5]. In Indonesia, Exclusive breastfeeding coverage in 2017 was 35.7%. This results was higher than in 2016 which was 29.5%[6].

Breastfeeding offers benefits to both mother and child. Breastfeeding is fundamental importance for the survival and quality of life of the baby during its first years of life. For mother, breastfeeding can

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reduce many health diseases such as bleeding, infection, depression, stress and anxiety, cancer, endometriosis, diabetes, osteoporosis, blood pressure and cardiovascular diseases, metabolic syndrome, rheumatic arthritis, Alzheimer disease and multiple sclerosis[7]. Being breastfed is associated with general ability of children[8]. Infants should be exclusively breastfed for the first six months of life for optimal growth, development and health[2]. Evidence on the importance of breastfeeding continues to increase. A series of systematic reviews have shown the effect of breastfeeding in decreasing child infections and dental malocclusion and increasing intelligence. Breastfeeding decreases the risk of breast cancer for mother. Improving breastfeeding rates globally can prevent over 800 000 deaths in children under 5 years of age and 20.000 deaths from breast cancer annually[9].

Exclusive breastfeeding can affect the illness frequency of infant[10]. Breastmilk has many immunological properties that are likely to protect against infection in infancy[11]. Children who did not receive exclusive breastfeeding had a chance to get several health problems, such as stunting, acute respiratory tract infection (ARI) and diarrhoea. Research stated that the risk of stunting of children who did not receive exclusive breastfeeding was 2.451 times higher than children who received exclusive breastfeeding[12]. Relation was found between the lack of exclusive breastfeeding and occurrence of acute respiratory tract infection and diarrhoea[13]. Another research showed that there was an increased risk of chest infection and diarrhoea in infants with exclusive breastfeeding for less than 4 months or 2 – 4 months compared with infants who were fed according to the pre 2001 WHO policy (exclusive breastfeeding for 4 – 6 months, introduced solid but no formula before 6 months). Infection may occurred due to contamination of bottles, teats, milk, and food in infants who were not exclusively breastfed[11].

Based on East Java Health Profile, exclusive breastfeeding coverage in East Java Province in 2017 was 74.3%. Districts with lowest percentage of exclusive breastfeeding coverage were Bangkalan (55.2%) and Sampang (59.6%). The highest percentage of exclusive breastfeeding coverage was Bojonegoro (88.2%)[14].

The Indonesia government recommends infant aged 0-6 months to only be given breast milk (exclusive breastfeeding). Empirically, infant aged 0-6 months in Indonesia are not only given breast milk. A lot of infant at that age have received formula milk, sugar water, and occasionally given bananas. Various reasons and backgrounds were obtained regarding the intake pattern such as close pregnancies and cultural intake patterns[15]. Based on the background description, the study aims to analyse factors related to EBF in East Java in Indonesia.

## Materials and Methods

### Data Source

The study used secondary data from the 2017 Nutritional Status Monitoring Survey. The Directorate of Nutrition of the Ministry of Health of Indonesia performed the national scale survey using a multi-stage cluster random sampling[16]. The participants in this sample were all toddlers in East Java, Indonesia, ranging in age from 7 to 60 months. The study employed toddlers as the study's analysis unit. The sample size for this study was 3977 toddlers.

### Variables

The dependent variable in the sample was selective breastfeeding (EBF). EBF exclusively breastfeeds for the first six months, without the use of all other dietary additives [17]. The study employed seven variables as independent variables. The seven were the type of residence, mother's age, mother's marital status, mother's education level, mother's employment status, toddler's age, and toddler's gender.

### Data Analysis

The study used the Chi-Square test to test

dichotomous variables while employed the T-test to test constant variables. The research used the statistical test to see whether the dependent variable (EBF status) and the independent variables have a statistically meaningful relationship. Moreover, the study used the binary logistic regression test to evaluate multivariable data at the end of the process.

### Ethical Approval

The national ethics committee has granted the 2017 Nutritional Status Monitoring Survey an ethical license (number: LB.02.01 / 2 / KE.244 / 2017). In this survey, the researcher used informed consent to gather data, which considers elements of the data collection process and voluntariness and secrecy.

## Results and Discussion

The analysis results found that the percentage of toddlers who experienced EBF in East Java was 35.8%. This achievement figure is slightly above the national average of 35.7%[16].

Table 1 describes the EBF statistics and variables related to East Java, Indonesia. Based on the residence type, the toddler who lives in rural areas occupied the two categories of EBF in East Java. Meanwhile, based on mother's age, mothers who had EBF babies had an average age slightly older than mothers who had non-EBF babies.

**Table 1. Descriptive Statistics of EBF and Variables Related in East Java, Indonesia (n=3977)**

| Variables | Exclusive Breastfeeding | | p-value |
|---|---|---|---|
| | No (n=2558) | Yes (n=1419) | |
| Type of residence | | | 0.000* |
| ● Urban | 17.7% | 16.6% | |
| ● Rural | 82.3% | 83.4% | |
| Mother's Age (in years; mean) | (29.85) | (30.04) | 0.000* |
| Mother's Marital status | | | 0.000* |
| ● Never married | 0.3% | 0.2% | |
| ● Married | 98.8% | 99.1% | |
| ● Divorce/Widowed | 0.9% | 0.7% | |
| Mother's Education level | | | 0.000* |
| ● No Education | 1.1% | 1.1% | |
| ● Primary | 22.1% | 22.8% | |
| ● Junior High School | 25.4% | 27.5% | |
| ● Senior High School | 43.9% | 40.5% | |
| ● College | 7.5% | 8.0% | |
| Mother's Employment Status | | | 0.000* |

***Cont...*** **Table 1. Descriptive Statistics of EBF and Variables Related in East Java, Indonesia (n=3977)**

| | | | |
|---|---|---|---|
| ● Unemployed | 70.7% | 70.2% | |
| ● Employed | 29.3% | 29.8% | |
| Toddler's Age (in months; mean) | (14.43) | (14.68) | 0.000* |
| Toddler's Gender | | | 0.000* |
| ● Boy | 51.5% | 50.0% | |
| ● Girl | 48.5% | 50.0% | |

Note: Chi-Square used for dichotomous variables and T-test used for continuous variables. *Significant at the 95% level.

According to the mother's marital status, married mothers dominate both categories of EBF in East Java. Meanwhile, based on the mother's education level, the mother who passed senior high school ruled both EBF categories. Moreover, based on the mother's employment status, the unemployed mother represented all EBF types.

Regarding toddler's age, toddlers with EBF have an average age slightly older than toddlers without EBF. Finally, based on the toddler's gender, the EBF boys and girls category has a balanced proportion.

Table 2 shows the binary logistic regression test results to determine factors related to EBF in East Java, Indonesia. In this binary logistic regression test, the study used "Non-EBF" as a reference.

**Table 2. Binary Logistic Regression of EBF in East Java, Indonesia (n=3977)**

| Predictors | p-value | Exclusive Breast Feeding | | |
|---|---|---|---|---|
| | | AOR | 95% CI | |
| | | | LB | UB |
| Type of Residence: Urban | ***0.000 | 0.951 | 0.940 | 0.961 |
| Type of Residence: Rural | - | - | - | - |
| Mother's Age | ***0.000 | 1.004 | 1.003 | 1.005 |
| Mother's Marital Status: never married | - | - | - | - |
| Mother's Marital Status: married | ***0.000 | 1.544 | 1.403 | 1.700 |
| Mother's Marital Status: divorced/widowed | *0.027 | 1.128 | 1.014 | 1.255 |
| Mother's education: no education | - | - | - | - |
| Mother's Education: primary school | ***0.000 | 1.120 | 1.076 | 1.166 |
| Mother's Education: junior high school | ***0.000 | 1.171 | 1.125 | 1.219 |
| Mother's Education: senior high school | 0.604 | 1.011 | .971 | 1.052 |
| Mother's Education: college | ***0.000 | 1.167 | 1.119 | 1.217 |
| Mother's Employment Status: Unemployed | - | - | - | - |
| Mother's Employment Status: Employed | 0.422 | 0.996 | 0.987 | 10.006 |
| Toddler's Age | ***0.000 | 1.013 | 1.012 | 1.014 |
| Toddler's gender: boy | ***0.000 | 0.943 | 0.935 | 0.950 |
| Toddler's gender: girl | - | - | - | - |

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; AOR: adjusted odds ratio; LB: lower bound; UB: upper bound.

Table 2 shows that children who live in cities are 0.951 times less likely than children who live in rural areas to experience EBF (AOR 0.951; 95% CI 0.940-0.961). The situation means that toddlers in rural areas in East Java have a higher chance of experiencing EBF. This finding is different from previous studies, which found that toddlers in urban have a higher likelihood of experiencing EBF[18].

According to the study's findings, maternal age (in years) is one of the determinants of EBF in East Java, Indonesia. The condition is consistent with the results of another European multiregional review, which showed that younger, less trained mothers of toddlers were more likely to abandon breastfeeding before six months, indicating that the mother not achieved EBF. The level of schooling, parity, and social status of mothers under the age of five are all markers of high-risk under-five children who do not have EBF, according to these results[18–20].

The study results found that all categories of maternal marital status were more likely than mothers who were never married to have EBF under five in East Java, Indonesia. In Vietnam, a study on EBF performed another report with a slightly different theme. The findings of this study revealed that working together as a parenting team would help EBF succeed. The husband's function was also identified as a predictor of EBF performance in this analysis[21]. According to the findings of a study conducted in Canada, parental collaboration affects awareness, attitudes, and behaviors of exclusive breastfeeding and the length of breastfeeding[22].

Table 2 reveals that all maternal education levels have a higher risk of having EBF children than mothers with no education. Except for mothers who have completed senior high school, there is no connection between the incidences of EBF in children under the age of five in East Java, Indonesia. Another study reached the same conclusion in another analysis conducted in Eastern Indonesia, which studied data from the Indonesian Family Life Survey East 2012. With a smaller sample size (1138 toddlers), the report discovered that maternal schooling positively impacted success rates[23].

The findings of this analysis are consistent with those of many other studies. Better schooling led favourably and played an essential role in the breastfeeding process and the success rate of EBF, according to the findings of a survey of postnatal mothers in Nigeria and China[24,25]. Another study conducted in the United States used the factor self-efficacy score as an indicator of the relationship between maternal schooling and the performance of EBF delivery. An adult with a high level of education has an advantage in self-efficacy and has a positive relationship with the EBF practice[26,27].

Better education provides a better understanding of everything a baby needs. Several studies found that education is often a positive factor in health outcomes[28–30]. On the other hand, poor education is a barrier to achieving better performance in the health sector[31–33].

The study also discovered the toddler's age (in months) was linked to the occurrence of EBF in children under the age of five in East Java, Indonesia. The findings of this study backed up previous research that showed that a toddler's age was a factor in reaching EBF [34–36].

Finally, Tabel 2 informs toddler boy has chance about 0.943 times compare to toddler girl to experience EBF in East Java in Indonesia. Two previous studies in Malawi and Somalia with a related theme also identified toddler's gender as a determinant of EBF[34,37].

## Conclusions

The study concluded six variables related to the EBF incidence in East Java, Indonesia. The six variables are the type of residence, mother's age, mother's marital status, mother's education level, toddler's age, and toddler's gender.

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# Production and Purification of aflatoxin b1 from Local Isolate of *Aspergillus flavus*

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## Abstract

**Background and Objective:** Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the Aspergillus genus. Food and feed contamination by aflatoxin (AF)$B_1$ has adverse economic and health consequences. Unfortunately, these contaminants can never be completely removed, and on that account, many studies have been carried out to explore an effective process of their detoxification to a threshold level. **Results**: A thermostable enzyme purified from the boiled supernatant was designated as Horseradish aflatoxin-degrading enzyme (HADE). An overall 9.55-fold purification of HADE with a recovery of 39.92% and an activity of $3.85 \times 10^3$ U·mg$^{-1}$ was obtained using chromatography on DEAE-Sepharose. peroxidase had an estimated molecular mass of 34 kDa and exhibited the highest activity at 25 °C and pH 7.0. peroxidase is the major protein involved in $AFB_1$ detoxification. **Conclusion**: PTLC It is reliable as it is a good and reliable method for separating compounds and toxins. It is necessary to study the other compounds that the *Aspergillus fungus* secretes, as they are likely to cause harms more dangerous than aflatoxin B1.

***Key words****: aflatoxin b1, aflatoxins separation, Aspergillus genus , purification by pTLC*

## Introduction

Aflatoxins (AFs) are toxic secondary metabolites of *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* [1], and have been found in many crops resulting in serious threat to human and animal health [2]. Although the known AFs have about twenty different forms, four main AFs are commonly found in contaminated food including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) [1]. These, AFB1 is considered as the most toxic and many studies indicated that it has genotoxicity, carcinogenicity, embryotoxicity, teratogenicity, immunotoxicity, acute toxicity and chronic toxicity [3]. Therefore, safe and effective strategies are required to remove or degrade AFB1 in food. Many protocols had been conducted for AFB1 removal, mainly including chemical, physical, and biological methods. Using alkaline solution or oxidant to treat contaminated food [4], such as ammonia and ozone, are common chemical methods to degrade AFB1 in food, which have higher degradation efficiency, lower cost and easier operation, but these chemical reagents may cause secondary pollution and have an impact on composition of products. Radiation, mainly including plasma, ultraviolet and electron beam irradiation, is a widely studied physical method for AFB1 degradation, which have been proved capable of AFB1 decomposition and reduced toxicity of its degradation product than native AFB1, but high cost and damage to other components in food limit its wide application [5]. Some cationic clays and microbial cells [6], such as smectite, probiotic bacteria and Saccharomyces cerevisiae, can effectively remove AFB1 through physical adsorption, but

longer treating period and reverse recovery of toxicity result in lower efficiency of physical adsorption [7]. Biological degradation, normally including bacterial fermentation or enzyme catalysis, is another promising method to remove AFB1, which have advantages of mild operation condition, high degradation efficiency and much less toxic products than AFB1. However, for all mentioned methods except radiation, extra substances need to be added into the sample for AFB1 removal, which might result in secondary pollution and extra separation cost, especially for liquid sample. In addition, fast and convenient regeneration of these adsorbents or microorganism also remains significant technical challenges to be addressed. Aflatoxins are resistant to high temperature, strong acid and extremely difficult to be decomposed. At present, there are physical, chemical and biological methods for the elimination of aflatoxins. However, physical and chemical methods are usually limited in practical applications, such as destroying the nutrient substance, incomplete detoxification and high cost of the method[8,9] . The biological method has the advantages of high detoxification efficiency, no damage to food quality and mild reaction conditions [10]. Aims of this study :- production aflatoxins from secondary metabolic of *aspergillus flaves* and Purification aflatoxin b1 and measure by HPLC .

## Materials and Methods

This study was accomplished in Genetic Engineering and Biotechnology Institute for Postgraduate Studies/University of Baghdad, during the period from 25/ 10 / 2019 to 1/5/ 2020.

### Collection of A. flavus

Ten of commercial feed were randomly sampled from farmed of common crops The fungi of aspergillus fleavas was isolate form the corn crops and culture in PDA media according [11].

### Culture Medium and Inoculum

Thirty-nine grams of PDA added to one liter of distilled water, the medium was autoclaved at 121 °C and 1.5 kg /cm$^2$ for 15 minutes, after sterilization period, the medium was cooled out to 45 °C, then tetracycline antibiotic (250mg /l) was added to prevent bacterial growth. The medium was distributed in Petri dishes (diameter 10 cm) and left to be solid, then stored in the refrigerator until use. A loop full of *A. flavus* spores taken and then diluted to $10^{-4}$ with 10 ml of water, then from which 100 µl was withdrawn and inoculated on the Potato Dextrose Agar (PDA) plate by spread plate method and incubated at 25±2°C for 7 days. The inoculum prepared by inoculation universal tubes (1.5 by 15 cm) of PDA with fungal spores. The slanted test tubes were incubator at 25±2°C for 17 days, spore suspensions were prepared by added 5 ml of normal saline to each slant culture of *A. flavus* and shaking it, adjusted to approximately $10^6$ spores /ml by using a hemcytometer [12].

### Produce AFB1 from *Aspergillus flavus*

After the step of PCR diagnose the next step Production aflatoxins by culture in broth media yeast extract dextrose (YED) ,The toxigenic strains of fungus *A. flavus* used for production AFB1 was cultured in sterile medium which Yeast Extract sucrose Broth medium with Peptone (YEB +P) under static conditions , was added, and inoculated by 10 ml of spore suspension ($10^6$ spores/ ml) and incubated at 25± 2°C for 21 days.

### Extraction of AFs from Liquid Culture

After the incubation period, the AFB1 was extracted from culture and filtered through normal filter paper and then through Whatman no.1 filter paper to separate biomass. To the supernatant was added 100 ml of methanol and acetone (70:30) and diluted in water to each Erlenmeyer flasks and shaken for 15 minutes in the orbital shaker. The mixture extracts and then concentrated by the rotary evaporator near dryness. High-performance liquid chromatography (HPLC) was used for quantitative estimation of AFB1 [13, 14] (AOAC, 2005; Yousefi *et al.,* 2016). AFB1 extract from yeast extract broth medium was used as the stock solution of aflatoxins .

Separated aflatoxins ($AF_S$) in PTLC :

Aflatoxins it is important to be able to separate a mixture into its chemical components in order to isolate one compound or to assess the purity of the mixture. Thin layer chromatography (TLC) is one of the easiest and most versatile methods of doing this because of its low cost, simplicity, quick development time, high sensitivity, and good reproducibility. TLC is used by many industries and fields of research, including pharmaceutical production, clinical analysis, industrial chemistry, environmental toxicology, food chemistry, water, inorganic, and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis. In its simplest form, glass plates are coated with a uniform layer of silica gel (SiO2).steps separated AFs The dissolved the mixture of AFs. is placed on the plate, and the plate is inserted into a screw-top jar containing the developing solvent and a piece of filter paper. When the solvent has risen to near the top of the plate, the plate is removed, dried, and visualized using UV ligh (Santiago & Strobel, 2013).depended in The chromatography shows a characteristic profile with no interference in the retention factor (Rf) of the aflatoxins ($AFB_1$:0.7, $AFB_2$:0.6, $AFG_1$:0.5, $AFG_2$:0.35) and good separation indicating the good specificity and selectivity of the method. We cut solid phase in cleaning extracts for the aflatoxin analysis [15].

**Quantitation of AFB1 using HPLC**

The High-Performance Liquid Chromatography (HPLC) analysis of AFB1 carried out in the Department of Chemistry / Ministry of Science and Technology, Iraq. The AFB1 standard obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The AFB1 was concentrated and loaded on the matrix; elution and carried out with chloroform: methanol (11.76:0.24), at a flow rate of 5 ml/min. The extract was concentrated to the final volume of 5 ml and the amount of AFB1 in the samples was determined using UV spectrophotometer at 365 nm. The purity of AFB1 in the fraction confirmed by High performance liquid chromatography (HPLC– Shimadzu- LC-2010A) with UV detector at 365 nm as per the instructions given in Supelco instruction manual. The stationary phase was C18 Polaris column. A sample of 20 µl was injected, deionized water: acetonitrile: methanol (50:40:10) was used as mobile phase at a flow rate of 1 ml/min.

## Results and Discussion

**Morphological in PDA petri dish**

Ten samples of corn from local market contaminated with fungi colonies Plates were then incubated at 28 C for 3 days and the fungal colonies were isolated on PDA plates the colonies of this isolate on the surface of PDA medium were contained yellow-green spores on the upper surface and reddish-gold. A. flavus cultures were identified by observing the color of mycelia on culture plate having typical lime green or yellow green color of mycelia as described by Fakruddin *et al*., [16]

**Microscopic Characteristics**

The *A. flavus* Isolates To ascertain their precise identification, the microscopic characteristics (conidiophores, vesicles, mentula, phialides, and conidia) of these isolates were also examined show in Figure 1 Microscopic examination showed that, hyphae are hyaline and septate, and the conidia produce thick mycelial mats while the conidiophores are rough and colorless, phialides are arranged in one and two rows. The conidiophores were uncolored, thick walled, and coarsely roughened or pitted and were vesicle bearing. Their diameter ranged between 800 and 1200 µm. The vesicles were sub globose in some isolates and globose in others and were also variable in diameter, ranging between 1800 and 2000 µm. The cells were either uniseriate or biseriate or both. For biseriate cells, the phialides were borne on the metals, and, in uniseriate cells, they were attached directly to the vesicles. The metulae covered nearly the entire surface of the vesicles and radiated from the vesicles in all directions. The conidia were globose with thin walls, which were slightly roughened.

All previous morphological features are related to Aspergillus flavus and agree with Rodrigus et al.[17]. microscopy and culture remain commonly used and essential tools [18]. Some changes of Diagnose Procedure can enhance the value of these traditional tools, and give first diagnose by microscopic examinations; by recognizing atypical variants of common aspergilli can improve the laboratory's contribution to rapid diagnosis.

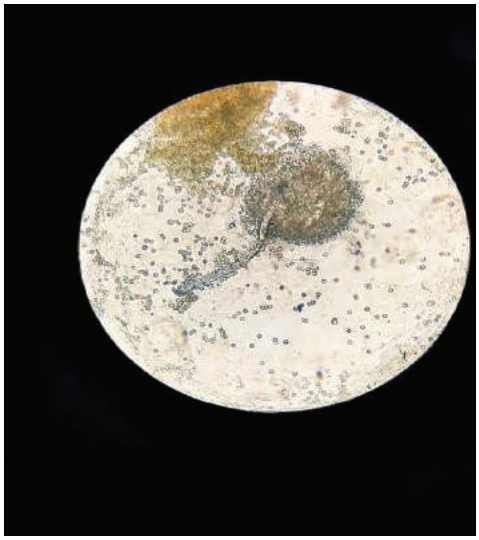

**Figure (1 ) : Microscopic characteristics of A. flavus isolates 40× objective of the Biological Light Microscope.**

**Plate the fungi *Aspergillus flavus* in media (YES+P)**

The result show *A. flavus* in media yeast extract with peptone ,high media Consumed over the days matched by an increase in fungal size ,that mean increase in product of metabolic that contain AFs . that agree with Payne and Hagler, [19], increased mycelial growth is generally associated with increased toxin production .Require an efficient extraction step.

**The detection and quantification of aflatoxins .**

Require an efficient extraction step. Aflatoxins are generally soluble in polar protic solvents such as chloroform. Thus, the extraction of aflatoxins [20]. This study results shown many of secondary metabolic produce from *A. flavus* when emigrations in Preparative thin layer chromatography (PTLC) (figure. 2),

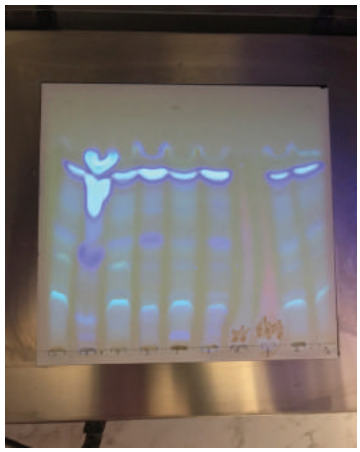

**Figure (2 ): Retention factor (Rf) of the aflatoxins in PTLC with different aflatoxins**

**(AFB1,AFB2 and another secondary metabolic of fungi ).**

Depended on the chromatography shows a characteristic profile with no interference in the retention factor (Rf) was of the aflatoxins . Develop plate 50 minutes or until aflatoxins reach Rf 0.4-0.7. remove from tank, evaporate solvent at room temperature and view under long wave UV lamp in a viewing chamber. Observe pattern of 9 florescent spots (Figure.2). All strain of *A. flavus* product AFB1 except 3,4 figure (2): . The results of retention factor (Rf) were of the aflatoxins ($AFB_1$:0.7, $AFB_2$:0.6, $AFG_1$:0.5, $AFG_2$:0.35) and good separation indicating the good specificity and selectivity of the method. The results agree with Castro and Vargas, [21] who found chromatogram shows retention factor (Rf) of the aflatoxins (AFB1:0.7, AFB2:0.6, AFG1:0.5, AFG2:0.35).

$$RF = \frac{spot\ distance}{SOLVENDISTANCE}$$

**Table (1): The retention factor (RF) volume of the aflatoxin in PTLC.**

| No.of spot | Distance spot | Distance solvent | Rf | Type of spot depended on RF |
|---|---|---|---|---|
| spot1 | 12 cm | 17 cm | 0.7 | AFB1 |
| Spot2 | 11 cm | 17 cm | 0.6 | AFB2 |
| Spot3 | 8.5 cm | 17 cm | 0.5 | AFG1 |
| Spot4 | 6 cm | 17 cm | 0.35 | AFG2 |

Cut spot of the aflatoxins (AFB1) depended on RF factor in PTLC after that dissolved measurer in UV spectrophotometer for chose strain that product high concentration of aflatoxin B1. AFB1 have absorption maxima at 362 nm [23]. AFB1 measured by a UV-detector at 360 ml [22]. A spot that cut from PTLC dissolved in chloroform solvent and measurer UV spectrophotometer AFB1 have absorption maxima at 362 nm [24]. The results shown the outweigh strain 9 to produce AFB1 comparing with another isolated strains (table 2) , this strain (strain 9) which used to produce $AFB_1$ for test of cytogenetic in this study.

**Table 2: Results measurer UV spectrophotometer of AFB1 362 nm .**

| Strain no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| O.D 360 | 0.065 | 0.008 | 0.0 | 0.0 | 0.029 | 0.045 | 0.006 | 0.055 | 0.17 | 0.018 |

The study find the strain 9 have high production of aflatoxin b1 and mycelial wight.that agree with [20] .because increased mycelial growth is generally associated with increased toxin production, mycelial growth may increase the $AFB_1$ production capacity. That result associated aflatoxin b1 production as secondary metabolic so when increase mycelial of fungi that mean increase the product secondary metabolic and aflatoxin b1 just secondary metabolic from *A. flavus* .

· Production AFB1 from strain 9 .

The results as shown in figure.(3) production of aflatoxin B1 as a bar line. Show retention factor (Rf) (in strain 9 only) of the aflatoxins in PTLC and show different aflatoxins (AFB1, AFB2 and another secondary metabolic of fungi) after that cut line we cut only (RF 0.7) as shown in (figure 3) and dissolved in solvent and measure concentration by HPLC as shown in (Figure 4).

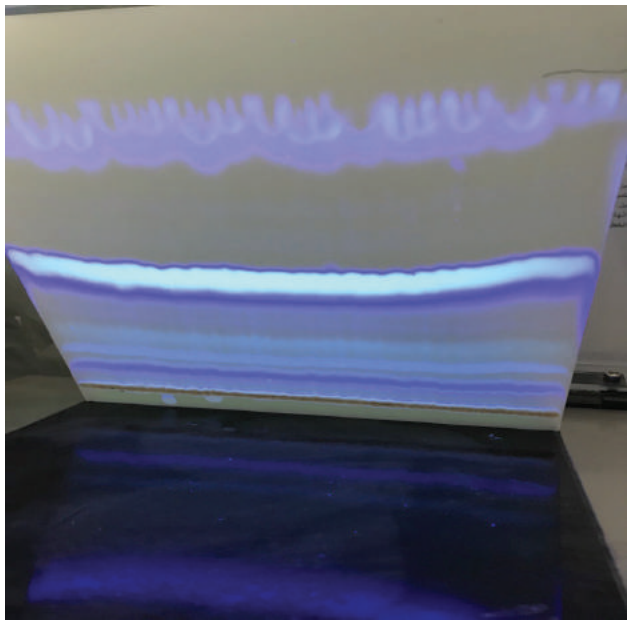

**Figure (3 ) production aflatoxin b1 from strain 9 in PTLC.**

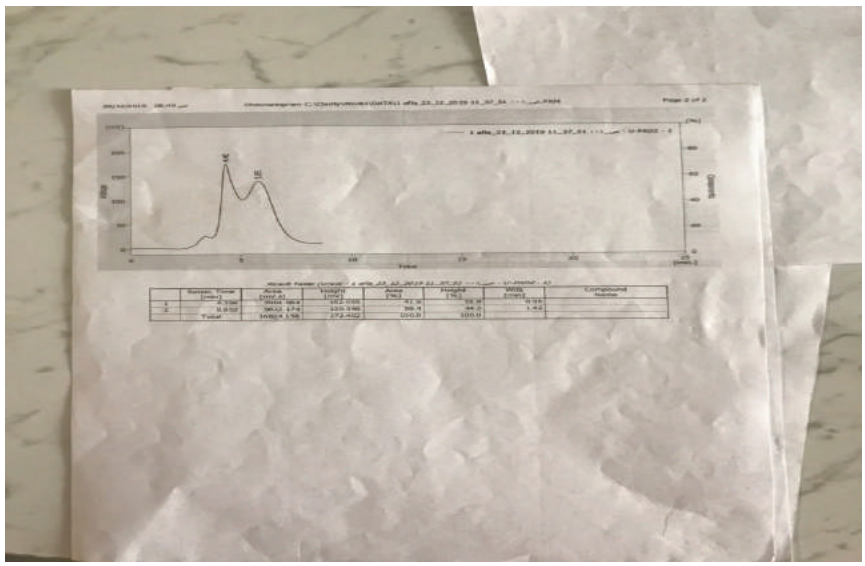

**Figure (4 ) : HPLC measure stander of aflatoxin b1 .**

$$con.sample = \frac{C.st \times Are.sam}{Are.st.} \times \frac{D.F}{Vulm}$$

$$con.sample = \frac{0.125 \times 7001}{9733} \times \frac{10}{0.1}$$

$$con.sample = 8.99 \ ppb$$

## Conclusion

The tests that detect mycotoxins in the market must be increased and reduced before reaching the final consumer, as they are in such high proportions that they can cause cancer. In cases of necessity, the necessary methods must be followed to reduce or reduce the harmful effect of aflatoxin, especially aflatoxin B1. PTLC It is reliable as it is a good and reliable method for separating compounds and toxins. It is necessary to study the other compounds that the

*Aspergillus* fungus secretes, as they are likely to cause harms more dangerous than aflatoxin B1.

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**Conflict of Interest**: Nil

The authors declare that they have no financial conflict of interest.

**Source of Funding**: Nil.

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

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# Comparative Anatomical Study of Kidney in adult Male Squirrel (*Sciurus anomalus) and* Mice *(Mus musculus)*

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## Abstract

The current study aimed to investigate the anatomical, morphological, morphometrical data of the kidney in two types of rodent, the squirrel and mice. A total number of 80 kidneys were obtaining from two species which include 40 specimens from squirrels and same number of specimens from mice.

Current study showed that the kidneys in two species lay in the abdominal cavity lying retroperitoneal against upper dorsal wall rear of abdominal cavity on either side of vertebral column.

The left kidney in squirrel was longer and thicker than right kidney while in mice was reversible and in both animal the left kidney more distant from the median plane than the right but the left kidney in squirrel was larger than right kidney the value.

Shape of kidneys in squirrel appeared semi bean like structure but in mice the shape was typical bean like structure, and in both animals the kidneys have smooth surface. Both kidneys of squirrel were by relatively solid texture in contrast with mice kidney.

The biometrical measurements of the kidney revealed that the mean weight in squirrel was significantly larger than that of mice. Whereas all micro measurements recorded showed a significant differences between squirrel and mice where the means in squirrel appeared higher than those of mice.

Renal artery which arises from abdominal aorta divided to the right branches, the right artery appeared shorter than the left artery, because the left kidney being more distant from the median plane than the right one. The renal artery in mice divided into 3 branches called anterior renal artery, intermediate renal artery and posterior renal artery but in squirrel divided to 2 branches anterior and posterior renal artery. in both animals the 2nd order of artery branches intermediate renal artery divided in mice in 4 inerlober branches but in squirrel the interlober divided into 6 branches

***Keywords:*** *Anatomical of Kidney, adult male squirrel (Sciurus anomalus) , mice (Mus musculus)*

## Introduction

The urinary system of mammals consists of the paired kidneys, ureters, urinary bladder, and urethra [(1)]. Urinary system that contributes to the maintenance of homeostasis by complex process that involves filtration, absorption and secretion .It's also regulates fluid and electrolyte balance of the body and the site of production of renin, a substance

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that regulate blood pressure and erythropoietin that stimulate the production of erythrocyte. Additional functions include excretion of waste products from the processing of food, drugs, and harmful substances; and secretion of certain hormones and its function in osmoregulation to act as thermoregulation of body [2;3;4].

The kidney is a bean-shaped structure with a convex and a concave border. A recessed area on the concave border is the renal hilum, where the renal artery enters the kidney and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perirenal fat, renal fascia, and pararenal fat [5;6].

Laboratory animals similar to rats and mice they have shorter generation interval and they yield quick return on low investment. Kidneys play an important role in maintaining homeostasis and more concentrated urine [7].

The physiological adaption of small rodents to arid conditions is achieved mainly through concentrating ability of their kidneys [8].

The conservation of water by the kidney is of crucial importance for the kangaroo rat, which does not drink and can obtain water only from catabolism while, other desert rodents obtain water from their diet [9].

Because of the rareness of studies that deals with the comparison between the urinary system of squirrel and mice, this study was conducted to investigate the morphological features and morphometric measurements of kidney in these two animals.

## Materials and Methods

A total number of 80 kidneys were obtaining from two species of apparently adult healthy male rodent animals which include 40 specimens from squirrels (*Sciurus anomalus*) and same number of specimens from mice (*Mus musculus*). All squirrels included in this study are obtained from local market (Alghazzel market) in Baghdad city and the mice were brought from (animal house in College Veterinary Medicine of Baghdad University).

The animals were weight alive by using digital electrical balance and the animals are euthanized by intramuscular injection of a mixture of ketamine and xylazine at dose ketamine 25 mg/kg of body weight and xylazine 10-12.5 mg/kg [10;11] .

A vertical midline Incision was done from the xiphoid process down to the inguinal region under the skin, abdominal muscle to the abdominal cavity and the viscera were retracted and the kidneys were exposed, location, shape, color and boundaries and relationship of kidneys were recorded then the kidneys were released from their fat covering connective tissue and gently removed.

Gross anatomical measurements include the following parameters:

a- Length of kidney / mm from cranial pole to caudal pole. By digital vernier

b- Width of kidney / mm. digital vernier

c- Thickness of kidney from middle /mm. digital vernier

d- Volume of kidney /ml. using water displacement measured using the immersion method

e- Weight of kidney / gm by electrical sensitive balance.

f- Blood supply: by using resin

Resin injection procedure:

Catheter (hospital and homecare medical device co. /made in china) used for squirrel CH/FR: 6 but in mice CH/FR:4 was inserted aortic arch reaching

into the renal artery of fresh kidney and made knot it around the the tube with the vessel will restrict and avoid the slippage during perfusion act as valve after complet injection is close the knot. The resin was prepared after mixed (Powder and liquid 1 to 4), and it is consisted of 20% monomethyl-methacrylate powder and 80% polymethyl-methacrylate liquid and adding the suitable dyes (Red, blue and yellow ink or oil painting dyes). The mixture is taken in a separate syringe and connected to the tube. The resin is perfused with constant injection pressure to avoid blockage or rupture of small capillaries after that of inject the renal blood vessels by mixture of self-cure denture material set. To differentiate the blood vessels samples are incubated at room temperature for 48-72 hours for polymerization (Duracryl Plus Company / Czech Republic)

After that, the samples of cast were macerated by using (KOH) [12;13;14;15].

## Statistical Analysis

Computer package (Sigma plot V12.0 / SYSTAT software) was used to conduct the histomorphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed using Duncan's test with significant level set on $P < 0.05$.

## Results

Present study investigated anatomical, morphological, morphometrical data of the kidney comparing between two types of rodent species, one of them was called squirrel (*Sciurus anomalus*) and the second called mice (*mus musculus*).

Gross anatomical and morphometrical study revealed that the male squirrel (*sciurus anomalus*) mean body weight was significantly higher than mice (*mus musculus*) where they measured (200.29±20.2, 31.17±2.3gm.) respectively (Table 1).

**Table 1: showing weight of both animals / grams**

| Animal | weight of body (g) | P-value |
|---|---|---|
| Squirrel | 200.29±20.2 | <0.0001 |
| Mice | 31.17±2.3 | |

Current study showed that the kidneys lay on the upper lumbar region in the abdominal cavity lying retroperitoneal against upper dorsal wall rear of abdominal cavity just above the waistline on either side of vertebral column (fig.1A,B).

In both animals male squirrel and mice the kidneys localization on each side of vertebral column asymmetrical in location, the right kidney is located more cranial than the left kidney and the right kidney settle, nearest of median plane in squirrel and mice and the cranial pole of the right kidney covered partially by the right lobe of liver and made the renal impression caudally but the left kidney related with spleen and parietal layer of stomach, the descending colon, the body of the pancreas and jejunum, while the cranial extremity of both kidneys related with the adrenal gland with each one.

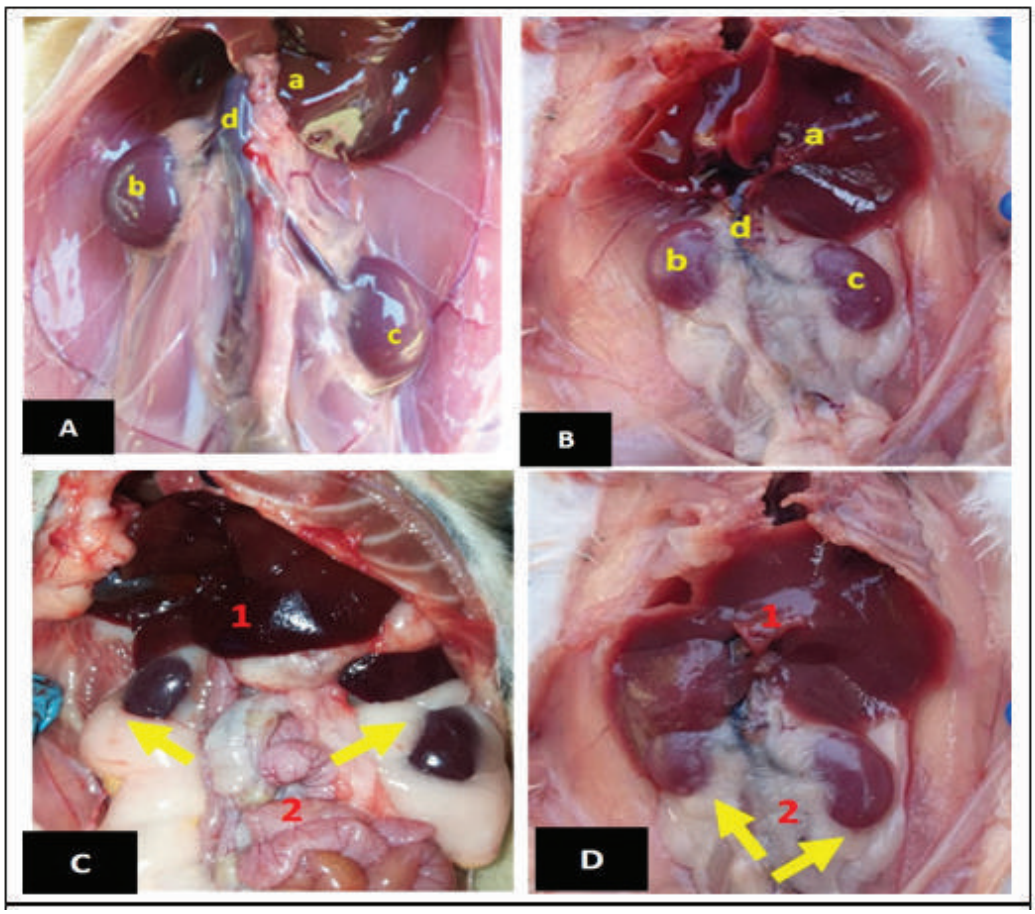


**Figure: (1)**

**A: The location of squirrel kidney a. liver b. right kidney c. left kidney d. aorta and caudal venacava.**

**B: The location of mice kidney a.liver b.right kidney c.left kidney d.aorta and caudal venacava.**

**C: Adipose tissue surrounding kidney (yellow arrow) (liver 1) (intestine 2) in squirrel.**

**D: Adipose tissue surrounding kidney (yellow arrow) (liver 1) (intestine 2) in mice.**

The left kidney in squirrel was longer and thicker than right kidney while in mice was reversible and in animals, the left kidney more distant from the median plane than the right but the left kidney in squirrel was larger than right kidney the value.

In mice (*mus musculus*), our study showed that the size of the right kidney slightly was larger than left kidney with non-significant differences.

Our study in squirrels showed there was a large amount of adipose tissue depositions completely surrounds of both kidney (fatty layer like cushion) But in mice there was a little or light quantity of the adipose tissue which occupy the renal sinus and surrounded the renal vessels and origin of ureter (fig.1,c,d) respectively.

The study revealed that the kidneys surface of both animals male squirrel and mice don't have capsular vein. The study revealed the kidneys surface of both animals don't have capsular vein

Shape of kidneys in squirrel appeared semi bean like structure, but not highly typical (fig.2 A,B) but in mice the shape was typical bean like structure, clearer

margin and in both animals the kidneys have smooth surface without any lobulation or fissures. Both kidneys of squirrel was characterized by relatively solid in contrast with mice kidney in texture.

In both animals, the kidney appeared with rounded cranial and caudal pole, dorsal and ventral surface and had medial and lateral borders, the lateral border was convex while, the medial border was concave, at the middle of medial border at deep concave region occupied by hilum, represent the site of entrance of renal artery and exist renal vein and ureter exit from the kidney. The study revealed that the color of kidneys were reddish-brown in both animal squirrel and mice.

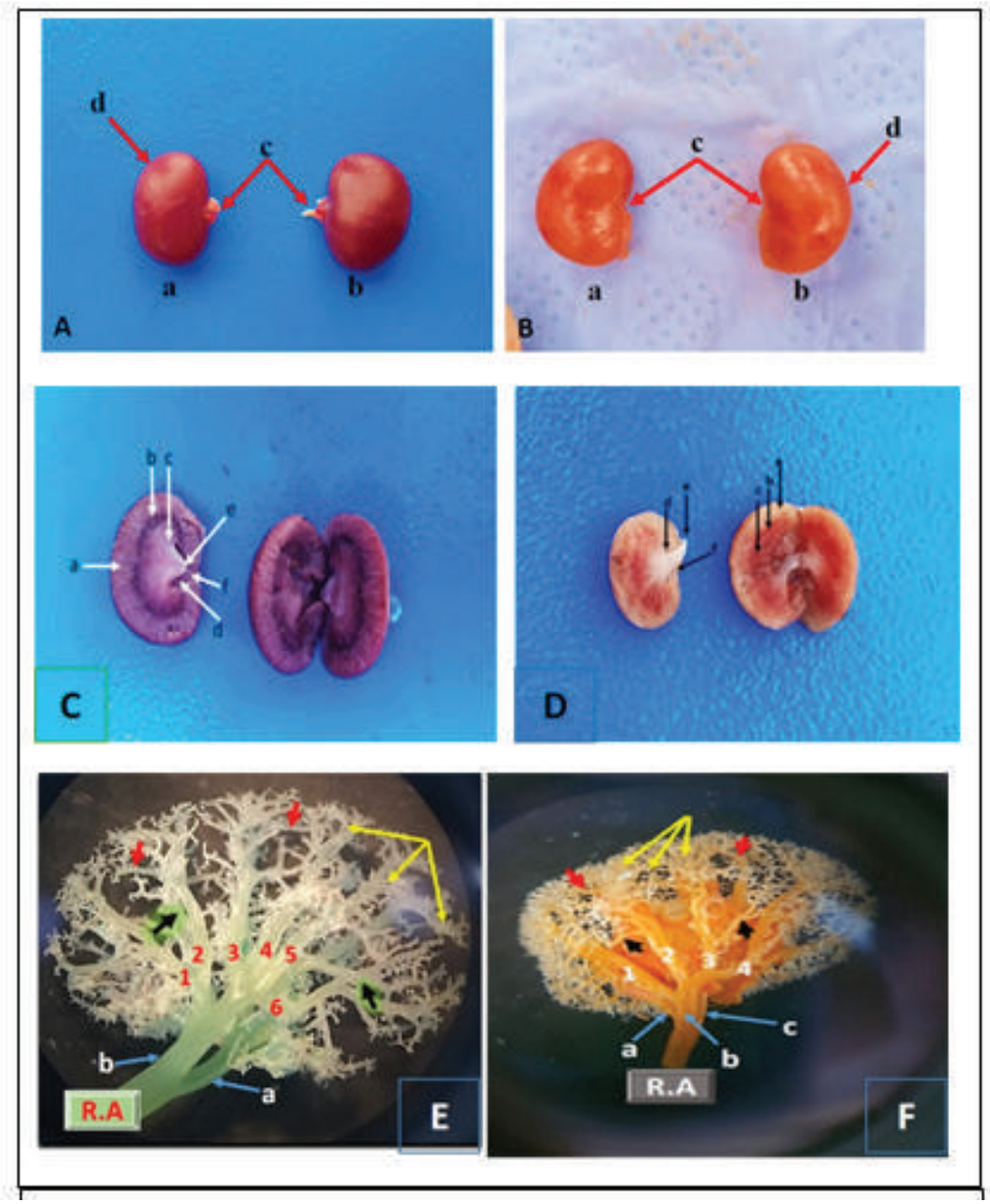


**Fig.2 A: Squirrel kidney a.right kidney b.left kidney c.hillus of kidney d.renal capsule without capsular vein. B: E: Mice kidney a.right kidney b.left kidney c.hillus of kidney d.renal capsule without capsular vein.**

**C: Longitudinal section of male squirrel kidney a.renal cortex b.renal medulla (external zone) c.renal medulla (internal zone) d.renal sinus e.renal papillae f.renal pelvis.**

**D: Longitudinal section of male mice kidney a.renal cortex b.renal medulla (external zone) c.renal medulla (internal zone) d.renal sinus e.renal papillae f.renal pelvis.**

**E: dorsal view blood supply (corrosion cast) of squirrel kidney showing:**

**R.A-Renal artery a-Anterior renal artery b-Posterior renal artery 1,2,3,4,5,6-interlobar renal artery (black arrow)-interlobular renal artery(red arrow)-arcuate renal artery (yellow arrow)-vasa recta and cortical radiated arteries**

**F: Dorsal view blood supply (corrosion cast) of mice kidney showing:**

**R.A-Renal artery a-Anterior renal artery b-Posterior renal artery 1, 2, 3, 4,-interlobar renal artery (black arrow)-interlobular renal artery(Red arrow)-arcuate renal artery (yellow arrow)-vasa recta and cortical radiated arteries.**

### Measurements

The biometrical measurements of the kidney revealed that the mean weight in squirrel (*Sciurus anomalus*) was significantly larger than that of mice (*mus musculus*) (1.25±0.03 gm., 0.49±0.02gm) respectively. Table (2) showed that there was no significant difference in all micromorphometric measurements between right and left kidneys in squirrel as well as in mice.

The measurements recorded in table (2) showed a significant differences between squirrel and mice where the means in squirrel appeared higher than those of mice.

**Table-2: Showing the anatomical measurements in squirrel and mice**

| Groups | Weight of kidney (gm) | Length of right kidney (mm) | Length of left kidney (mm) | Width of right kidney dorso-ventrally (mm) | Width of left kidney dorso-ventrally (mm) | Thickness from middle of right kidney (mm) | Thickness from middle of left kidney (mm) | Thickness of cranial pole of right kidney (mm) | Thickness of cranial pole of left kidney (mm) | Thickness of caudal pole of right kidney (mm) | Thickness of caudal pole of left kidney (mm) | Volume of kidney in water( ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Squirrel | 1.25±0.03 * | 17.16±0.2 * | 17.48±0.3 * | 10.24±0.7 * | 10.52±0.3 * | 8.18±0.4 * | 8.28±0.8 * | 7.58±0.9 * | 7.81±0.6 * | 6.63±0.2 * | 6.79±0.3 * | 4.37±0.5 * |
| Mice | 0.49±0.02 | 10.42±0.9 | 10.30±0.3 | 6.29±0.6 | 6.36±0.6 | 5.74±0.3 | 5.73±0.3 | 4.50±0.2 | 4.40±0.2 | 3.94±0.2 | 3.68±0.1 | 3.39±0.06 |

The index of cortex to medulla in squirrel was (1:2.8) while in mice was (1:1.5). Longitudinal section revealed outer most dark brown granulated area referred to cortex. The medulla consists of two distinctive region the outer dark and inner paler medulla with shallow striation (fig.2,C,D).

### Blood supply

The blood supply arises from abdominal aorta where each kidney was supplied usually by a single artery, called. Renal artery divided to the right side (right artery) and to the left side (left artery), the right artery appeared shorter than the left artery, because the left kidney being more distant from the median plane than the right one (Fig.1A,B). Renal artery enter the kidney at hilum and branched. The renal artery in mice divided into 3 branches called anterior renal artery, intermediate renal artery and posterior renal artery but in squirrel the corrosion cast revealed that the renal artery divided to 2 branches anterior and posterior renal artery. in both animals the 2nd order of artery branches intermediate renal artery divided in mice in 4 inerlober branches but in squirrel the interlober divided into 6 branches after that in both animals the interlober divided into interlobular, then to arcuate and cortical radiated artery and ends into

afferent, efferent and glomeruli.(Fig.2 E,F)

## Discussion

It has been recorded that any species of animals survive in different environments need physiological and anatomical features that arrange the body water. This could be done through the production and concentration of urine, rendering to the structure of the kidneys and urinary tract, which are developed as a response to several environmental aspects involving morphological differences [16].

The present study was revealed that there was a differences in location and size of kidneys between the squirrel and mice which belong to the size of body, age, family and climate of living ,these findings come in agreement with [17] in Guinea pig and ,[18] in jerboa (Jaculus jaculus) who mentions the kidney located retroperitoneal in caudal portion of the upper abdomen cavity on each side of spinal cord under the sub-lumbar region situated in the posterior part of the abdomen on each side of the vertebral column. In addition the present observations are contrast with that observation of, [19] in all studied mammals and [20] in desert rodents.

The left kidney was in squirrel longer and thicker than right kidney in mice was reversible and in both animal the left kidney more distant from the median plane than the right but the left kidney in squirrel was larger than right kidney, the value in this study were also similar to those results of (18) in jerboa and (21) in rabbit who registed the left kidney weight and dimensions more than right kidney but unlike result in albino rat[22], house mice[6], desert rodents [20,23] , guina pig[17] , and rabbit[24].

Our study in squirrels revealed there was a large amount of adipose tissue depositions completely surrounds of both kidney (fatty layer like cushion) act as insulator a protective layer protect against distorting pressure from adjacent organs giving additional support for kidneys capsule and after this layer adhesion connective tissue capsule But in mice little or light quantity of the adipose tissue and the adipose tissue which occupy the renal sinus and surrounded the renal vessels and origin of ureter (fig.3,a,b) respectively these results is agreement with [18] in jerboa (Jaculus jaculus), albino rat[22] , African gaint rat and wistar rat[4] and in house mice by [6] who noted also that the kidneys covering by highly attachment fibrous layer of connective tissue with abundant amount of perirenal fat tissue. The presence of large amount of fat tissue surrounding both kidneys are act as a good insulator and one of fixatives of kidney in its position.

Shape of kidneys in squirrel appeared semi bean, but in mice the shape was typical bean like structure, and in both animals kidneys smooth surface without any lobulation and fissured, these results was fit to result of [25] in pig kidney shape appeared as bean, the dorsal and ventral surfaces are flat, and the cranial and caudal poles [18] In jerboa, [26;27] dog ,sheep, cattle[26;27], and camel(28) , spotted deer[29], and musk deer[30] which showed that the kidney is firm, reddish - brown in color and disagree with [31] who mentioned that the kidney of buffalo was irregular oval in shape, solid in texture; red- brown in color like of [32] in camel .and disagree but only corresponding in color to the mice (*mus musculus*) but agreed with [4] and [6], [21], rabbit (oryctologus cuniculus) [30] , fat sand rat and jerboa[17] [20], and house mice [6] who mentioned normal texture and dark red bodies.

The kidney biometrical measurements are the mean weight of the kidneys in this research the anatomical results were revealed that the kidney of the mice (*mus musculus*) was small relatively, but in squirrel larger than one Anatomical diameter was in male squirrel) mean weight of the kidneys in this research was (1.25±0.03 gm.) a significant differences at $P \leq 0.0.5$ compared with mice. The

mean length, width and thickness of the right kidney were (17.16±0.2 mm, 10.24±0.7 mm, 8.18±0.4 mm) respectively and the mean length, width and thickness of the left kidney were (17.48±0.3 mm, 10.52±0.3 mm, 8.28±0.8 mm) respectively agreement with [33] in rat who reports the mean weight of kidneys was 0.95gm and the length of right kidney was 1.35 cm and the left was 1.49 cm. similar results of [18] in jerboa discovered the left kidney was weighted about (1.196±0.35gm) while the right one about (1.175±0.41) gram. This result differed with [5] in white rabbit New Zealand who observed that had a mean kidney weight of (0.510±0.012gm). The length of right kidney was ranged about (31.62 ± 0.174) mm, while the length of the left kidney ranged about (30.6 ± 0.159mm). The width of the two kidney were varied, the width of the right kidney are about (20.85 ± 0.155) mm, while the left kidney are about (20.04 ± 0.157) mm. the right thickness about (16.42±0.144) and left one about (17.02±0.13).

But in mice (*mus musculus*) both kidneys lesser than squirrel (*Sciurus anomalus*) with a significant differences at $P \leq 0.05$ was the mean weight kidneys in this research was (0.49±0.02 gm.). The mean length, width and thickness of the right kidney were (10.42±0.9 mm, 6.29±0.6 and 5.74±0.3 mm) respectively and the mean length, width and thickness of the left kidney were (10.30±0.3 mm, 6.36±0.6 mm, 5.73±0.3mm) respectively which corspoding of jebori(2016) who mention The length of right kidney was about (14.62 ± 0.174) mm, while the length of the left kidney ranged about (12.55 ± 0.183) mm, The width of the two kidney were varied, the width of the right kidney are about (5.57 ± 0.349) mm , while the left kidney are about (5.60 ± 0.045) mm. thickness the right thickness about (4.88±0.212) and left one about (5.19±0.201) and unlike the data reported by [6] mentioned that the weight of the right kidney was 0.076±0.013gm the left kidney was 0.072±0.011 gm. The mean length, width and thickness of the right kidneys were .647mm, 5.098±0.427mm, 3.624±0.198 mm respectively those of the left were 6.8±0.518mm, 4.768±0.481 mm, 3.468±0.123 mm respectively Table (2). The study showed the mean thickness of cranial and caudal pole of right squirrel kidney was (7.58±0.9, 6.63±0.2 mm) respectively but in left was (7.81±0.6, 6.79±0.3 mm) respectively and the mean thickness of cranial and caudal pole of right mice kidney was (4.50±0.2, 3.94±0.2mm) respectively but in left kidney of mice was (4.40±0.2, 3.68±0.1 mm) respectively (table 1).

The cortex to medulla index in squirrel (1:2.8) in mice was (1:1.5) Longitudinal section revealed outer most dark brown granulated area referred to cortex. The medulla consists of two distinctive region the outer dark and inner paler medulla with shallow striation.

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# Review of the Professional Ethics of Doctors as Chemical Castration Sanctions in Indonesia

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## Abstract

This study aims to determine the view of the medical ethics code regarding the doctor's profession as the chemical castor of child sexual offenders who has been decided by the court as mandated by Article 82 A paragraph (2) of Law Number 17 of 2016 concerning Child Protection in conjunction with Article 9 of Government Regulation Number 70 of 2020 concerning Procedures for Implementing Chemical Castration, Installing Electronic Detection Devices, Rehabilitation and Announcement of the Identity of Perpetrators of Sexual Violence Against Children. This research is a normative legal research. Based on the results, it is known that theoretically related to the authority of the medical profession as a party ordered by law to carry out the castration process for perpetrators of sexual abuse against children who have been decided by the court is contrary to the principles contained in the medical code of ethics and oaths. doctor, in this case the presence of such a thing makes the doctor feel that he is not doing his profession in accordance with the Code of Medical Ethics which has been agreed upon and highly upholds moral values, one of which is "informed consent" or consent from family or patient. all actions that result in a decrease in the patient's physical endurance, although not necessarily reduce his psychological endurance. Second, that chemical castration is not a type of medical service but a punishment, so it is not the domain of medical or health personnel to do it.

***Keywords:*** *Chemical Castration, Code of Medical Ethics*

## Introduction

Every year, crimes in the form of sexual violence in Indonesia have increased. The victims are not only adults, but now it has also spread to adolescents, children and even toddlers. The phenomenon of sexual violence against children is increasingly happening and has become global in almost all countries. [1]

Unlike other forms of abuse, "cases of sexual violence against children have a much more significant impact on children, both directly and in the long term, not only physically." Furthermore, this action will have a negative impact on emotional, social and emotional well-being. psychological victims of violence ". As it is known that in response to this, the Government issued a Government Regulation in Lieu of Law (Perpu) Number 1 of 2016 concerning the Second Amendment to Law Number 23 of 2002 concerning Child Protection to anticipate increased sexual abuse

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of children, known as Law Number 17 of the Year. 2016 (hereinafter referred to as Law 17/2016) regulates the weight of sanctions for perpetrators of sexual crimes against children, including the death penalty, life imprisonment and a maximum imprisonment of 20 years, as well as alternative crimes in the form of announcing the identity of the perpetrator.

According to the author's notes, so far the imposition of castration sanctions has never been applied in Indonesia after the enactment of Law 17/2016, there are only two cases where judges used the punishment of castration before this research was conducted. First, in case No. 69 / Pid.sus / 2019 / PN.Mjk, In this case, the defendant Muhammad Aris was legally and convincingly proven to have committed crimes of sexual violence against 9 (nine) children in the jurisdiction of the Mojokerto Regional Police, as determined by the Mojokerto District Court and later strengthened by the Surabaya District Court. High Court Decision number 695 / PID.SUS / 2019 / PT.Sby. According to the facts of the case, "the perpetrator raped 9 (nine) children with an average age of 6-7 years and was found guilty. Based on a court ruling, convicted of sexual abuse and violence against children is sentenced to 12 years in prison, a fine of Rp. 100 million, and 6 months in prison ".[2]

Second, the decision of the Surabaya District Court which stated that "the convict Rahmat Slamet Santoso was sentenced to chemical castration". where since 2015, Rahmat was found guilty of molesting 15 children while working as a Scout Guide in six public and private elementary and junior high schools. The judge sentenced the defendant to 12 years in prison with a fine of Rp. 100 million, three months in prison and three years of chemical castration. [3]

However, it should be noted that there is no technical protocol yet to enforce the criminal sentence of castration against the two convicts at the time of the verdict. The new government passed a regulation regulating the technical implementation of castration sanctions on December 7, 2020, through "Government Regulation Number 70 of 2020 concerning Procedures for the Implementation of Chemical Castration, Installation of Electronic Detection Devices, Rehabilitation and Announcement of the Identity of Perpetrators of Sexual Violence Against Children (hereinafter referred to as PP 70 / 2020).

On the other hand, at first glance, it seems that the medical profession has a completely different relationship with the enforcement of the castration sanction, because each has very different meanings. However, "there is a connection, that castration is a form of punishment in the health sector while doctors are health workers who act as executors who are given the obligation to punish". It is not "clearly stated in Law 17/2016 that castration is carried out by doctors," but "as a profession that is responsible for the health sector, it is clear that doctors are one of them." The following is contained in Article 82 A paragraph (2) of Law 17/2016: "The implementation of the actions referred to in paragraph (1) shall be under regular supervision by the ministry which is responsible for government affairs in the legal, social and health sectors..

Likewise, this lack of clarity is also contained in Article 9 of PP 70/2020 regarding the implementation of the castration process which states that the procedure for implementing the castration process is carried out as follows:

1. "the implementation of the chemical castration act is carried out after the conclusion as meant in Article 8 states that the perpetrator of intercourse is eligible to be subject to the act of chemical castration;

2. Within a period of not later than 7 (seven) working days from the receipt of the conclusion as referred to in letter a, the prosecutor will order the doctor to carry out the implementation of chemical

castration to the perpetrators of intercourse;

3. The implementation of the Chemical Castration as referred to in letter b shall be carried out immediately after the convict has completed serving the main sentence;

4. the implementation of the chemical castration act is carried out at a government-owned hospital or a designated regional hospital;

5. the implementation of the Chemical Castration Act was attended by prosecutors, representatives from ministries that administer government affairs in the field of law, ministries that carry out government affairs in the social sector, and ministries that carry out government affairs in the health sector;

6. the implementation of the chemical castration action is stated in the minutes; and

7. the prosecutor notifies the victim or the victim's family that the chemical castration act has been carried out.

Based on the mandate of Article 9 PP 70/2020, the medical profession has a great possibility to carry out the castration process, because it is recognized that doctors are responsible for the health sector, so it is clear that doctors are one of them. Furthermore, the argument that "the castration that the medical profession will perform is that castration has two distinct forms of procedure, namely surgical and chemical processes are very convincing." The results of castration surgery, also known as testicular surgery, are permanent, in chemical castration. however, drugs will be given regularly to reduce the amount of testosterone in the body, thereby reducing sex drive".[4]

This type of procedure requires experienced personnel, so doctors with experience are the most qualified to perform the procedure. This shows that the purpose of the article above is related to the doctor's duties as the party who will execute. Therefore, the question that deserves to be raised is "what is the view of medical ethics regarding the medical profession as the executor of chemical castration, as it is well understood that doctors are driven by the lofty ideals of their profession, and this code of ethics becomes a guideline for every doctor to behave. and act. relevant to his profession ".

### Research Methods

This research is a normative legal research, namely the process of finding a rule of law, legal principles, and legal doctrines in order to answer the legal issues at hand. (Marzuki, 2016) With regard to the normative legal research method, the technique of collecting legal materials used is document study or literature study. The approach used in this study is a statutory approach and a case approach. (Marzuki, 2016) by studying and examining the views of the medical code of ethics regarding responsibility as the party implementing (executor) of chemical castration sanctions on child sexual offenders.[5]

## Discussion

The view of the code of medical ethics regarding the authority of doctors as executors of chemical castration sanctions.

Today, various types of professional professions in Indonesia usually have a standard professional code of ethics. If it is related to the problem of chemical castration, as the theme that the author raises in this article, namely, there is a conflict between some of the ethics listed in the Code of Medical Ethics and the fact that doctors are a profession appointed by the court to be the executor of chemical castration. Doctors as a profession that has competence in the health sector with the implementation of chemical castration punishment in Indonesia then face a dilemma related to the existing medical ethics review of this phenomenon. The Indonesian Doctors Association (hereinafter abbreviated as IDI) has issued a fatwa

refusing doctors as executors of castration who are considered to injure the professional oath, given the questionable effectiveness of castration and the risk of other complications that convicts with castration must face.[6]

IDI Chairman Daeng M. Faqih said that IDI allowed Indonesia to impose a punishment in the form of chemical castration. However, IDI asks not to appoint medical personnel or health workers as executors. The reason IDI refuses to be the executor of chemical castration punishment is first, that chemical castration is not a type of medical service but a punishment, so it is not the domain of medical or health personnel to do it. Therefore, IDI has invited the government to appoint executors other than medical personnel. The second reason IDI refuses to be the executor is that the act of execution can lead to a conflict of norms, namely medical ethics, orders from the World Health Organization (WHO), and the Health Act which prohibits carrying out these actions, in this case, chemical castration.

Castration or castration is a surgical procedure and / or the use of chemicals with the aim of eliminating the function of the reproductive organs in the form of testes in males and ovaries in females. Based on its understanding, the castration procedure is then classified into physical castration and chemical castration. In physical castration, the operator performs total removal of the reproductive organs, namely the testes and ovaries. In chemical castration, such an action is not carried out, but is replaced by giving chemical compounds that can weaken or eliminate the function of sex hormones. Pathophysiologically, chemical castration is carried out by injecting the hormone anti-testosterone into the body of the convicted person.

Today, castration is carried out with the aim of serving as a criminal sanction against perpetrators of sexual crimes such as rapists and pedophiles in various countries. Each country applies different methods of castration. The Czech Republic and Germany are examples of several countries that apply physical castration punishment, namely the removal of the testicles of pedophile offenders as an effort to control the perpetrators' abnormal sexual urges. Russia and South Korea have imposed chemical castration penalties for perpetrators of sexual crimes who are at risk of repeating their crimes after consultation with a psychiatrist.[7]

Based on the principles of medical ethics contained in the Hippocratic Oath, causing death or disability to a person is against the principle of nonmaleficence or "do no harm". Then, the criminal is also not in a situation that allows him to give informed consent or consent, which is part of the principle of autonomy in the Hippocratic Oath. The fifth item on the hippocratic oath which reads "I will not use my doctor's knowledge for something that is against humanity even if threatened" adds to the basis of reinforcing reasons for doctors not to use their expertise whose impact is contrary to humanity. . Not only violating the hippocratic oath, the execution of chemical castration punishment which if carried out by a doctor will also violate the 2012 Code of Medical Ethics Article 5 which states that "Every action or advice that may weaken psychological or psychological endurance, must obtain the consent of the patient or his family and only be given. for the benefit and benefit of the patient.[7]

These two things, the fifth item of the hippocratic oath and Article 5 of the 2012 Medical Code of Ethics is a clear description of the principles found in the medical profession, namely, the principle of "do no harm" and the principle of "informed consent." The two pillars of medical principles will be misled if the doctor executes the chemical castration sentence. Chemical castration considering its side effects in the form of decreased levels of the hormone testosterone

which will affect the function of other organs, such as muscle atrophy, bone loss, reduced blood cells, and impaired cognitive function, of course it will be very dangerous for people affected so the principle of "do no harm "has been ruled out.[8]

In the case of carrying out chemical castration executions of child sexual offenders, doctors do not need to seek approval from the person who will be executed because chemical castration is a clear punishment if it has been decided by the court against that person. The presence of such things makes doctors feel that they are not doing their profession in accordance with the Code of Medical Ethics that has been agreed upon and highly upholds moral values, one of which is "informed consent" or consent from family and patients for all actions that result in decline. physical endurance of the patient, although not necessarily reduce his psychological endurance. So what if the execution of chemical castration still has to be carried out by doctors, considering that doctors are a profession that is considered very competent to carry out chemical castration executions. So based on the description above, in the opinion of the author regarding the executive authority of perpetrators of child sexual abuse by doctors as contained in Article 82 A paragraph (2) of Law 17/2016 Juncto Article 9 PP 70/2020 can be said to be contradictory or inconsistent with these principles. principles contained in the medical code of ethics and the doctor's oath.[9]

## Conclusion

Based on the results of this study, it is known that theoretically related to the authority of the medical profession as a party ordered by law to carry out the castration process for perpetrators of sexual abuse against children who have been decided by the court is contrary to the principles contained in the medical code of ethics and oaths. doctor, in this case the presence of such a thing makes the doctor feel that he is not doing his profession in accordance with the Code of Medical Ethics which has been agreed upon and highly upholds moral values, one of which is "informed consent" or consent from family or patient. all actions that result in a decrease in the patient's physical endurance, although not necessarily reduce his psychological endurance. Second, that chemical castration is not a type of medical service but a punishment, so it is not the domain of medical or health personnel to do it.

**Conflict of Interest**: Nil

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# Height-for-age in Children under 5 Years Old with Down Syndrome and Hypothyroidism

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## Abstract

**Background:** Children with Down syndrome tend to have accompanying comorbidities, such as hypothyroidism, and late detection of this ailment leads to reduced growth of the child. This study aimed to assess the growth patterns in children with Down Syndrome and hypothyroidism at diagnosis.

**Methods:** This cross-sectional study was conducted from September to December 2020 with 56 subjects at the Pediatric Endocrine Outpatient Clinic of Dr. Soetomo Hospital. Diagnosis of Down Syndrome was confirmed by karyotyping, while the weights and heights were assessed using a standardized curve for children with Down Syndrome and then calculated using Peditools. Fifty-six children with Down Syndrome and hypothyroidism were included, comprising 32 boys and 24 girls (mean age, 37.75 ± 34.26 months). Majority of the subjects had normal weight, height, and Body Mass Index (36/56 [mean z-score, −1.62 ± 2.36], 33/56 [mean height-z-score, −0.43 ± 2.74], and 30/56 [mean z-score, −2.00 ± 2.06], respectively). Furthermore, the mean onset of diagnosis was 17.07 ± 32.23 months, where 23 out of the 56 children had short stature and had gotten diagnosed at over 12 months of age.

**Conclusion:** From the results obtained, hypothyroidism can be said to be associated with reduced growth in children with DS.

***Key Words:*** *down syndrome, growth, short stature, hypothyroidism*

## Introduction

Short stature is one of the most recognized features in children with Down syndrome (DS), and in a healthy population of children with this condition, 35.7% were found to have short stature[1–3]. Consequently, several adapted growth charts are used to assess the growth of children with DS [4–7]. The prevalence of short stature is increased by 46.2% in children with DS and hypothyroidism compared to those without hypothyroidism [8]. Children with DS experience late growth until 12–15 years, when they are approximately 20 cm shorter than children at the same age but without the condition [9]. Short stature is one of many complications caused by hypothyroidism, which is, as a result, often suspected if it occurs in adolescence. It should be suspected in children with short stature, as a study conducted in India showed

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that almost 50% of these had hypothyroidism [10].

Hypothyroidism is a common comorbidity accompanying DS, and previous studies show that the incidence in people with this condition is 12 to 22 times that of individuals without[11]. Neonates suspected of having DS often demonstrate an increase in plasma thyroid-stimulating hormone (TSH), and hypothyroidism is found in 4%–8% of this population[12]. Thyroid hormone is associated with cognitive development in the central nervous system during childhood, and any abnormality in its function may interrupt brain development, and consequently, general growth and development[10,11]. Consequently, hypothyroidism is believed to impair the growth of children with DS and increase their chances of having short stature. Although the detection of congenital hypothyroidism should be performed for every baby born[13], it is different in babies with DS. A study stated that autoimmune hypothyroidism occurs at five (5) months, and many were diagnosed at eight (8) years[14]. Hence, detecting congenital hypothyroidism in babies with DS can happen after a few years[15].

Research on DS with hypothyroidism has not been widely studied in Indonesia, especially regarding the growth of children, although growth studies have been performed on subjects with Turner syndrome [16]. Therefore, this study aims to assess how hypothyroidism impacts the growth pattern of children with DS.

A greater understanding of the growth pattern of children with DS and hypothyroidism could lead to earlier diagnosis and intervention. Early intervention has proven to offer good results in preventing and treating growth disorders in patients with these conditions [17]. Indeed, current guidelines suggest evaluating thyroid function in DS just after birth and every six (6) months afterward to detect problems earlier [18]. Moreover, the high incidence of short stature in patients with hypothyroidism has been shown to improve with earlier diagnosis and appropriate therapy [17].

## Materials and Methods

This study used a cross-sectional design and was performed from September to December 2020. The data were obtained from the patients' medical records, and the subjects were interviewed to collect hypothyroid history and nutritional status. This process was performed during their visit to the Pediatric Endocrine Outpatient Clinic of Dr. Soetomo Hospital.

### Sample

This study involved children diagnosed with both DS and hypothyroidism and registered at the Pediatric Endocrine Outpatient Clinic of Dr. Soetomo Hospital. The inclusion criteria were patients aged 1–5 years, diagnosed with both conditions, while critically ill patients were excluded. Fifty-six (56) patients between 1 and 5 years of age (32 boys and 24 girls) were the subjects in this study. The weight-for-age, height-for-age, body mass index (BMI)-for-age, and BMI were presented.

### Down syndrome

The DS diagnosis was confirmed physically by referring to the Fried *et al.* [19] criteria, which evaluated the abundant neck skin, downturned mouth corners, general hypotonia, and flat face. Other indications are dysplastic ear, epicanthic eye-fold, the gap between the first and second toes, and protruding tongue. This diagnosis was confirmed by a karyotyping test.

### Instrument / Nutritional status

The nutritional status of children with DS was classified by Zemel *et al.* [2] after recalculation of the standard growth curve of children with the condition. Using the Peditools.org website, the z-score of each growth parameter was calculated. The instrument, peditools.org/downinfant/, was used for children aged 0–2 years, while peditools.org/downpedi/ was used for those older than 2 years. Then, the height-for-age was classified either as short stature or normal based on the DS curve and z-score classification by Peditools.

According to WHO child growth standards, 2006, a z-score of -3.00 is severely stunted, -3.00 to -2.01 is moderately stunted, while -2.00 to 1.01 is mild.

### Evaluation of data

A descriptive test was used to analyze the data using SPSS 17.0 software (IBM SPSS), while the mean and standard deviation of each element was used for the baseline and clinical characteristics. The Kolmogorov–Smirnov test was also used to evaluate the normality of each dataset.

### Ethical permission

This study received ethical approval from the Ethics Committee overseeing health research at Dr. Soetomo Hospital (Ref. No.: 1960/KEKP/IV/2020).

## Results and Discussion

This cross-sectional study included 56 children with DS and hypothyroidism, comprising 32 boys and 24 girls, at 57.1% and 42.9%, respectively, with an average age of 37.75 ± 34.26 months old. The baseline characteristics and history of these patients are presented in Tables 1 and 2. Most of the subjects had normal weights, heights, and BMI (36/56 [mean z-score, −1.62 ± 2.36], 33/56 [mean height-z-score, −0.43 ± 2.74], and 30/56 [mean z-score, −2.00 ± 2.06], respectively). Based on the nutritional status, 20 of the 56 subjects, at 35.7%, were revealed to be underweight, while 23, at 41.1%, had short statures. The mean duration of hypothyroid illness was 22.97 ± 23.24 months, while the mean onset age was 21.65 ± 39.76 months. Finally, the mean z-score of all criteria was within the normal limit of $-2 < z < 2$.

**Table I. Frequency of subject characteristics**

| Characteristic | N (%) |
|---|---|
| Sex | |
| - Boy | 32 (57.1) |
| - Girl | 24 (42.9) |
| Weight-for-age | |
| - Severely underweight | 12 (21.4) |
| - Underweight | 8 (14.3) |
| - Normal | 36 (64.3) |
| **Height-for-age** | |
| - Short stature | 23 (41.1) |
| - Normal | 33 (58.9) |
| **BMI-for-age** | |
| - Severely wasted | 11 (19.6) |
| - Wasted | 15 (26.8) |
| - Normal | 30 (53.6) |

**Table II. Baseline characteristics of subjects**

| Characteristic | Mean (SD) | Minimum | Maximum | N |
|---|---|---|---|---|
| Age (months) | 47.75 (34.23) | 2 | 129 | 56 |
| Illness duration (months) | 23.25 (23.63) | 0 | 90 | 56 |
| Onset age (months) | 17.07 (32.23) | 0 | 163 | 56 |
| Weight-for-age z-score | −1.62 (2.36) | −10.13 | 1.66 | 56 |
| Height-for-age z-score | −0.43 (2.74) | −5.66 | 4.88 | 56 |
| BMI-for-age z-score | −2.00 (2.06) | −9.68 | 0.80 | 56 |

Based on the results, 23, i.e., 41.1% out of the 56 children with DS and hypothyroidism, had short statures, while the remaining 33, at 58.9%, were classified as normal. This finding is consistent with the report by Nam *et al.*, which concluded that infants with DS are likely to have short statures, especially when accompanied by comorbidities, such as a history of infectious or non-infectious diseases [20]. Adequate and complete nutrient intake is also a strong predictor of nutritional status, including height-for-age in infants [21]. Furthermore, a study by the Leung brothers had similar findings to this investigation that hypothyroidism can stunt growth and cause short stature in the absence of immediate therapy to improve the growth gap [22]. Growth failure in the thyroid may be related to the need for cartilage maturation to T3 [23]. Histologically, individuals with chronic hypothyroidism will demonstrate a delay in bone maturation and resting on the epiphysial plate [24].

As seen in Table 2, the mean age at diagnosis of hypothyroidism was 17.07 months, while some subjects were diagnosed as late as 20–23 months old. This is a cause for concern, as delayed diagnosis causes physical and intellectual disorders [13,25], and substitution therapy can improve hypothyroidism symptoms [8]. A study conducted in Turkey reported the average age of diagnosis to be 49 months [26]. Early diagnosis is crucial to prevent sequelae, as although a delay in diagnosis can lead to poor growth, it can be reversed [8], unlike intellectual delays [27].

Zemel *et al.* conducted an epidemiological study on nutritional status and DS in the United States [2]. It showed that the mean z-scores of height-for-age (−1.7 ± 1.2), weight-for-age (−0.8 ± 1.2), Head Circumference (HC)-for-age (−1.6 ± 1.0), and BMI-for-age (0.9 ± 1.0) were accompanied by a BMI value of 21.1 ± 5.8. These values are similar to our study, although only individuals with DS and hypothyroidism were included. However, several studies reported no decrease in the z-score of the nutritional status as a result of hypothyroidism [28]. The hypothyroid condition is a hypometabolic state in which the basal metabolic rate (BMR) decreases [29,30], hence, some individuals with hypothyroidism experience excess nutrients rather than a lack [31]. Also, the BMR in healthy individuals and those with DS devoid of other comorbidities are similar, meaning that DS does not affect BMR[32].

Concerning the nutritional status of the patients in this study, 35.7% were underweight and severely underweight, compared with the research by Al-Fahham *et al.*, where the prevalence in the DS group was only 14.3%[3]. However, this previous research also found congenital heart disease (CHD) in DS patients, a common comorbidity in this condition, similar to

this study, where 25% of the sample also had CHD[15]. Conversely, Al-Fahham found that the incidence of underweight subjects in the CHD group at 34.2%, which was greater than that observed in this study. Hypothyroidism is a chronic disease that can affect an individual's nutritional status, especially during growth and development[8]. A study from Al-Aaraj *et al.* showed an improvement in the nutritional status of individuals with DS undergoing hypothyroidism therapy at height-for-age and BMI-for-age[8].

The main limitations of the current study were that it was single-centered and included only a small proportion of children with DS and hypothyroidism that had short stature, meaning more research of similar samples is required to confirm this this relationship. Also, it did not compare a population of children with DS without hypothyroidism. This should be considered in prospective studies, as it would assist in drawing conclusions about the association between both conditions. Also, it will be important in future studies to perform early screening of hypothyroidism when examining the incidence of short stature in a similar population of children. If possible, the Indonesian multi-center epidemiological research will be performed to obtain more representative results on these conditions.

## Conclusion and Acknowledgement

Hypothyroidism, as a comorbidity, has been shown to be associated with reduced growth of subjects with DS. Although late diagnosis is common, earlier detection could prevent these issues before they worsen, making the evaluation of the thyroid hormone status of patients born with suspected DS important. Finally, adolescents with short stature should undergo testing for hypothyroidism, and education should be provided to the community to create awareness of these associations and their consequences if left undiagnosed.

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# Determinants of Quality of Life Air Traffic Controller in AirNav Surabaya

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## Abstract

The quality of work life is affected by the quality of the work environment. A positive work atmosphere will create a conducive work life. This study aims to determine the factors that affect the quality of life of air traffic controller at AirNav Surabaya. This is a quantitative research with a cross sectional study method. Interviews were conducted on 38 workers who were drawn based on the purposive sampling method. Then, from these results a logistic regression test was carried out which showed that blood pressure, position, and workload had a significant relationship. To determine the effect of individual characteristics, work fatigue and quality of life were measured using a questionnaire, blood pressure using a tensimeter and workload using an oximeter. Data were analyzed using the SPSS 25 application with the chi-square test. Results of the chi-square test analysis showed that there was a relationship between age and quality of life (p = 0.009), blood pressure with quality of life (p = 0.032), years of service with quality of life (p = 0.031), position and quality of life (p = 0.029), fatigue with quality of life (p = 0.012) and workload with quality of life (p = 0.005), while there was no relationship between sex and quality of life (0.279) and marital status with quality of life (p = 0.560). From these results, a logistic regression test was carried out which showed that blood pressure, position, and workload had a significant relationship. This research suggests AirNav Surabaya to pay more attention to the quality of life of air traffic controller, maintain the nutritional intake, exercise regularly, and pay attention to rest times.

***Keywords:*** *Quality of Life, ATC, Work Load, Safety Aviation, Work Fatigue.*

## Introduction

All flight operations are always directed to optimize flight safety and cost efficiency. Safety is a matter that should be prioritized in the world of aviation, because it is an unsafe condition of transportation. In an effort to achieve work profitability and flight safety, the world of air transportation involves a potential element related to the safety policy, namely control arrangements for airspace and ground area of airplane movement. This potential element is Air Traffic Controller. Flight safety depends on the capabilities and quality of the controllers. [1]

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The purpose of air traffic services is to prevent collisions between aircraft in the maneuvering area and obstructions in the area, maintain and smoothen the flow of air traffic, provide information and advice that is useful for safety and efficient flight operations, provide information to the authorities related to the need for search and rescue assistance, as well as assisting the needs of that party. Therefore, air traffic controllers need to perform multiple functions at the same time such as thinking, listening, and talking. [2]

ATC is considered to be one of the most demanding and highly saturated professions. This job has a high level of stress due to the heavy burden of responsibility because they carry the lives of aircraft passengers and the entire crew at risk. [1, 3]

Based on the results of preliminary studies from interviews with several workers, it was found that some of these workers experienced various health problems both physically and psychologically, exhaustions like work stress and fatigue were also reported that many controllers are experience it due to the high workloads.

The concept of quality of life includes how to measure individual happiness from various aspects of their life. This evaluation includes a person's emotional reactions to life events, dispositions, a sense of fulfillment and life satisfaction, and satisfaction with work and personal relationships. [4]

Juanda Airport, located in East Java, is one of the busiest airports in Indonesia. The smooth running of flights that cannot be separated from the role of ATC in regulating, monitoring, and informing all matters related to flight for 24 hours makes this job a fairly high level of stress. This can have an impact on the performance of ATC officers which will affect the quality of work to reduce work productivity and affect their quality of life.

## Materials and Methods

This study is an observational research. The research design is a cross sectional study, which aims to analyze the variables that affect the quality of life of the Surabaya branch of the Air Traffic Controller (AirNav, Surabaya). This research was conducted at AirNav Surabaya Branch from September to December 2020.

Previously, an experiment to examine the effect of progressive muscle relaxation on reducing work stress in air traffic controllers was conducted. The study aims to determine the effect of relaxation on reducing stress levels in Air Traffic Controller (ATC). The study design used quasi experimental research with pre-test post-test control group design. The sample of the research was ATC employees in Makassar Air Traffic Service Center, which amounted to 60 people consisting of 30 people given relaxation therapy interventions and 30 people as a control group. Datacollection using questionnaires and also blood pressure measurements. Data collection was done using the stress scale questionnaire (Perceived Stress Scale) and using the job stress NIOSH questionnaire. [5]

The population in this research were all employees of Air Traffic Control at AirNav Surabaya as many as 83 people. The number of samples to be studied was taken using purposive sampling technique, which is a technique based on certain considerations made by the researcher himself based on previously known characteristics or characteristics of the population. The inclusion criteria in this study were selecting respondents who were active employees at AirNav Surabaya and served as controllers and supervisors. Meanwhile, the exclusion criteria are employees who are on leave. Based on the above inclusion criteria, a sample of 38 workers was obtained.

Data collection related to the demographic variables (age, gender, marital status, blood pressure) and job variables (tenure, position, work fatigue, workload) were obtained through filling out questionnaires to respondents at the research location and also conducting direct interviews. Data processing using SPSS 21 application with multivariate analysis, namely logistic regression LR backward method.

## Discussion

From the results of the research that has been done, several things can be discussed that can be discussed in this paper.

Relationship between Age and Quality of Life on Air Traffic Controller at AirNav Surabaya

Wagner, Abbot, and Lett [6] found that there are age-related differences in aspects of life that are important to individuals, and also, adult individuals express higher welfare in their middle adulthood.

This study shows the results of the analysis of the relationship between age and quality of life get a p-value of 0.020 < 0.05, which means that there is a relationship between age and quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya Branch. Based on the results of observations on ATC AirNav Surabaya employees, the division of tasks or jobs is not based on age categories. However, in general, workers who are old (senior) occupy structural positions or are at the managerial level. In addition, under the International Civil Aviation Regulations (ICAO), employees aged < 60 years are required to undergo a medical examination to obtain a medical examination facility in the form of 3rd medical examination, then obtain a health certificate which is valid for 1 year. While employees aged > 60 years have a valid health certificate for 6 months. If the employee does not pass the results of the inspection, they are not get permission to guide the aircraft.

However, there are still young workers who have a poor quality of life. This is because workers still have minimal experience so that the pressure when doing work has an impact on their psychology which affects their quality of life. This research is in line with research conducted by Hamzah [7] with the results of the Kendal Tau correlation test showing a significance value (p) of 0.001. The significance value (p) which is below 0.05 indicates a significant relationship between age and quality of life for people with heart failure at PKU Muhammadiyah Yogyakarta Hospital. Quality of life can be seen as follows and sometimes it can mean more than one at the same time: [8]

1. State of health,
2. Physical function
3. Perceived health status
4. Subjective health
5. Perceptions of health
6. Symptoms
7. Satisfaction of needs
8. Individual cognition
9. Functional disabilities
10. Mental disorders
11. Well-being

The Relationship between Gender and Quality of Life on Air Traffic Controller at AirNav Surabaya

In general, the welfare of men and women is not much different, but women are more related to positive aspects of relationships, while high welfare in men is more related to aspects of education and better work. [9]

The results of statistical tests obtained p value = 0.557, thus there is no relationship between sex to

the quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya Branch. Based on observations made by researchers, gender does not affect the quality of life in ATC because there is no equal distribution of tasks and workloads for employees, both men and women. In other words, the division of tasks for ATC employees at AirNav Surabaya Branch is not carried out based on gender, therefore both women and men have the same type of work and workload.

This is also supported by other research by Lim [10] that there is no difference in the quality of life between men and women due to the personality factors of each individual.

The Relationship between Marital Status and Quality of Life on Air Traffic Controller at AirNav Surabaya

Quality of life can be seen as a state of health, physical function, perceived health status, subjective health, perceptions of health, symptoms, satisfaction of needs, individual cognition, functional disabilities, mental disorders, well-being and sometimes it can mean more than one at the same time. [8]

The result of statistical test shows that the value of p = 1,000, so there is no relationship between marital status and quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya branch. In the research conducted, it was found that marital status did not affect employee performance on their job. The workers continue to do their jobs in a professional manner, without bringing family conflicts into the work environment. In addition, the data in the research are homogeneous, with the status of employees who are married as many as 37 people and 1 person who is not married and there is the possibility of the influence of other variables that require further research.

However, this study is not in line with the results of research conducted by Astuti [11] which states that the relationship between marital status and quality of life of the elderly is found that as many as 12 elderly (85.7%) who are married have a high quality of life.

The Relationship between Blood Pressure and Quality of Life on Air Traffic Controller at AirNav Surabaya

Hypertension and quality of life have a reciprocal relationship, hypertension can affect quality of life and vice versa, quality of life can affect hypertension. In hypertension, the domains related to quality of life include physical and mental, social, satisfaction with therapy and general feeling of comfort. [12]

Based on statistical tests, it was found that there was a relationship between blood pressure and quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya with p value = 0.024. Based on observations of blood pressure on the controller, it was found that more results were in the normal category due to the obligation for the controllers to check blood pressure before working. In addition, ATC workers are also required to stay focused while on duty. Performance level is the dominant factor that causes high blood pressure in ATC operators.

According to research conducted by Grivit [13] shows that there is a relationship between hypertension and the quality of life of residents in Kolongan Village, Central Tomohon District, Tomohon City. More respondents who have a good quality of life do not suffer from hypertension than respondents who suffer from hypertension and respondents who have a poor quality of life suffer from hypertension more than respondents who do not suffer from hypertension.

The Relationship between Years of Service and Quality of Life on Air Traffic Controller at AirNav Surabaya

The years of service is the length of time a person works in the scope of work which is calculated in months or years. If the longer the period is, it can

produce better work productivity, that is, it can be done by mastering and developing a thought in doing work. So and vice versa, can have a negative influence on the quality of life of workers, where the work period is carried out continuously which can cause health problems. [14]

Working period is a factor associated with work fatigue. The longer working period will cause boredom and boredom will cause work fatigue. The longer the working period gives a negative effect that is disrupting the body's resistance and causing work fatigue. The longer a person works, the the feeling of beiing familiar with the work will affect the level of endurance to the fatigue they experience. [15]

The statistical test results obtained p value = 0.027, which means that there is a relationship between the years of service and the quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya.

Most of the ATCs at AirNav Surabaya have long years of service. Based on the observations of researchers, AirNav has provided a work environment that is quite comfortable and safe, but the work carried out is the same as before and has no variation so that sometimes they feel bored and tire easily. However, workers claim that the work they do is worth the incentives they receive. As work life should include occupational safety and health, justice, individual choice, participation in decision-making, opportunities for growth, meaningful work, the ability to control time and place of work, protection from injustice, treatment and the opportunity to meet social needs. [16]

There are ATCs who have a poor quality of life, but are still in a new years of service. Employees with new tenure do not have good experience in doing their jobs so they still feel pressured because they are more afraid of making mistakes in every job. This of course can affect the quality of life of ATC employees. Meanwhile, employees with long tenure have had work experience that affects their work knowledge and skills. Work experience that employees have indirectly trains employee work attitudes to be more skilled, fast, calm and able to analyze difficulties and be ready to overcome them. [17]

The Relationship between Work Fatigue and Quality of Life on Air Traffic Controller at AirNav Surabaya

International Civil Aviation Organization (ICAO) defines fatigue as a physiological condition of reduced mental or physical abilities due to loss of sleep, length of time awake, circular phase, and excessive workload that can interfere with the ability to carry out security and safety during the operational process carried out [18].

According to Aroem [19], poor quality of life is always associated with the inability to carry out daily activities as usual, characterized by fatigue, disturbed sleep, and feeling hopeless. Fatigue is a system in the body that signals that something is causing interference with the body and will recover after rest. Fatigue due to work is called work fatigue and is one of the problems in the workplace, both formally and in the formal sector. [20]

Based on the results of the statistical test conducted, the value of p = 0.024 was obtained, so there is a relationship between fatigue and quality of life in the Air Traffic Controller (ATC) at AirNav Surabaya Branch.

ATC fatigue is met by a variety of factors caused by the interplay of professional job requirements and demands on job performance, plus ATCs have to make decisions under time pressure. Stress will increase when you have to make an immediate decision. On the other hand, ATC's working time is very constant in terms of busy and busy work schedules, plus situations and environments that can be said to be

isolated. The work environment can be a physical and mental stress factor for ATC. All of the factors mentioned above contribute to ATC fatigue and job stress which can affect ATC's ability, responsiveness, and alertness.

The Relationship between Work Load and Quality of Life on Air Traffic Controller at AirNav Surabaya

Workload is ATCO's main problem. In their analysis, they found that the best predictor of ATCO workload is traffic load, namely the number of aircraft managed by the controller. Other factors that contribute to the workload include the number of flight altitude transitions, average airspeed of the aircraft, variations in direction, aircraft proximity, and weather conditions. [21]

The results of statistical tests obtained p value = 0.010, so there is a relationship between workload and quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya Branch. Based on observations in the field, the workload experienced by ATC operators is predominantly mental workload because in doing their work they are required to concentrate heavily in monitoring navigation, radiation and supervising and carrying out aircraft monitoring so as to ensure safety and regularity of aircraft traffic. According to Budiman [1], the workload experienced by an ATC controller is located from peak hours.

This research is in line with the research conducted by Lopez [22], which shows the Workload factor shows a significant relationship with the four dimensions of Quality of Work Life on Air Traffic Controller in Equador Airport.

The Relationship between Position and Quality of Life on Air Traffic Controller at AirNav Surabaya

Kirsten [23] states that the type of position or quality of work affects the personal needs of a worker, personal life outside of work, relationships with fellow workers which of course will affect the quality of life of the worker. The way people respond to their work can impact their personal happiness and quality of life, the effectiveness of their performance, and the success of their organizations. [24]

The results of this study indicate a relationship between position and quality of life with a value of $p = 0.020 < 0.05$, which indicates that there is a relationship between position and quality of life on the Air Traffic Controller (ATC) at AirNav Surabaya based on research conducted, employees as a supervisor have a lower job risk because they are required to focus and be responsible only when extraordinary events occur, where this is incidental. So that the pressure that occurs due to work does not take place continuously, then in terms of salary, the supervisor position is higher than the position of controller. Whereas employees with controller status are required to always focus on every time they carry out their work, where this happens continuously and causes pressure.

Analysis of Determinants of Quality of Life for Air Traffic Controllers (ATC) at AirNav Surabaya

Based on the results of the study, it was found that most of the quality of life of ATC employees at AirNav Surabaya was quite good. In accordance with the results of observations, the quality of life of the controllers is indicated by various programs to support the quality of life of the controllers, such as periodic health checks, shift systems, pay attention on incentives and others. However, there are still some controllers that show poor quality of life results. This indicates that the program held is not optimal or still requires improvement from both management and individual factors.

In this research, statistically through multivariate analysis showed no effect or relationship of age, years of service, work fatigue on quality of life. Meanwhile,

those that affect the quality of life are blood pressure, position and workload. The improvement of the quality of life of the ATC positions is because the higher the position of an ATC, the higher the incentives they get. And based on the results of ATC research, whose blood pressure is classified as abnormal, it is dominant to have high blood pressure, which is generally experienced by employees who are at managerial level positions. Workers admit that this affects their quality of life. Not only that, the quality of life of ATCs is also influenced by their workload where workers with high positions have light workloads and vice versa, so it can be concluded that the higher the ATC positions, where they are included in the managerial division or supervisors who have high blood pressure, so that the workload faced is also reduced which will affect the quality of life of the workers.

## Conclusion

The results of this study indicate that there is a significant relationship between age, blood pressure, length of service, work fatigue, workload and position on the quality of life of the ATC of Surabaya AirNav. However, after the Logistic Regression test is carried out to find out the strongest variables that have a relationship to the quality of life, namely blood pressure, position and workload. Meanwhile, gender and marital status did not have a relationship with the quality of life in this study. Based on this, the researcher suggests that the management of the AirNav Surabaya needs to pay attention to the quality of life experienced by the Air Traffic Controller so that it can still have a positive impact on physical health, psychological health, social relationships and spiritual conditions and for ATCs themselves have to pay attention to their health conditions, physical and spiritual. Maintain nutritional intake, exercise regularly, pay attention to rest times, take time for recreation to gather with family or socialize, and get closer to God.

**Ethical Approval:** Hasanuddin University

**Conflict of Intereset:** None

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# Characteristics and Outcome of Hospitalized Children with COVID-19: A Single Center Experience

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## Abstract

**Objectives:** Are to describe the clinical, and laboratory features of patients hospitalized with COVID-19 and to compare some of these parameters between patients with and without MIS-C.

**Methods:** A cross sectional study, undertaken over a 10 months period from 1st of march to 31 of December 2020 at child's central teaching hospital in Baghdad city , Iraq.

**Results:** In total, 80 children hospitalized with a diagnosis of COVID-19. The mean age of the patients was 6.05±5.1years.60% were male and 46.35% had no comorbidities . Different types of malignancies were the most common comorbidity accounting for 22.5% of the patients. A history of contact with infected person was reported in 27.5% of the patients. Chest X-ray revealed that 52.5% of the patients had normal findings. 11.25% developed multi systemic inflammatory syndrome. Only two hematological abnormalities were significant in pediatric COVID-19 cases for development of MIS-C which is elevated NLR and CRP. In this study, there were no deaths linked to COVID-19 admission..

**Conclusions :** The majority of children admitted to the hospital with confirmed COVID-19 have a mild course and a good outcome. malignancy is the commonest comorbidity associated with COVID-19 admissions. Elevated Neutrophil: lymphocyte ratio and CRP are independently associated with development of MIS-C in pediatric COVID-19.

***Keywords****: Children, Coronaviruses , COVID-19 .*

## Introduction

SARS-CoV-2, the novel coronavirus that causes the disease COVID-19, first presented in December 2019 in Wuhan City, China, and on January 30, 2020 was declared a public health emergency of international concern, as it spread quickly around the globe through human-to-human transmission [(1)]. In Iraq the first case was reported on February 24th, 2020 [(2)] .since that time more than 1,139,373 cases reported [(3)].

Despite the global spread, clinical patterns of COVID-19 remain largely unclear, particularly among children[(4)]. Many studies show that pediatric COVID-19 has a milder course than adult COVID-19, that children have a better prognosis, and that deaths are exceedingly rare [(5)]. On other hand, Since April 2020, the number of children and young people who have presented with a hyperinflammatory illness involving many organs and mimicking Kawasaki disease shock syndrome has increased. The United Kingdom, the United States, and the World Health Organization have used several labels for this presentation, including multisystem inflammatory syndrome in children (MIS-C) and pediatric inflammatory multisystem syndrome temporally linked with SARS-CoV-2 infection. [(6)].

Until far, neither the WHO nor the US Centers for Disease Control and Prevention (CDC) have recommended any specific treatment for children. The goal of treatment for children with COVID-19 is to prevent organ failure, acute respiratory distress syndrome(ARDS), and hospital-acquired infections. To achieve this, supportive care is commonly used, which includes enough hydration, calorie intake, and ventilator support. (7).

The goal of this study is to describe the clinical and laboratory characteristics of pediatric COVID-19 patients who are hospitalized, as well as to compare those parameters between those who are hospitalized with and without MIS-C.

## Materials and Methods

A cross sectional study, conducted over a period of 10 months from 1st of march 2020 to 31 of December 2020 at child's central teaching hospital in Baghdad city in Iraq.

Data was collected after obtaining consent and ethical approval from the patient's parents and family members, who were explicitly told about the study's goal. The information was gathered through a questionnaire created by the researcher. The questionnaire was verified for inconsistencies in data quality and secret coding.

80 children <16 years old with laboratory-confirmed COVID-19 and admitted to the child's central teaching hospital were included in the study.

All children included in the study had a thorough medical history and physical examination. The results of laboratory and radiographic tests, as well as patient management and outcomes, were gathered.

In our study, A COVID-19 case was diagnosed based on positive test for SARS-CoV-2 infection by a positive real-time reverse transcription polymerase chain reaction test of a specimen using deep nasopharyngeal swab.

A temperature equal or more than 38.0°C was considered as fever. Normal levels of hematological indices including absolute neutrophil count, absolute lymphocyte counts and platelets count and subsequently neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio (PLR) determined based on normal values for age. Multisystem Inflammatory Syndrome in Children (MIS-C) was defined according to CDC criteria[8]

## Statistical Analysis

Statistical analyses were performed by using SPSS software version 25.0 (SPSS, Chicago). Continuous data were subjected to normality test (Shapiro Wilk test), Data with normally distribution were presented as mean and standard deviation, and analyzed with Student t-test. Data with non-normal distribution were presented as median and range and analyzed with Mann Whitney U test. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Receiver operating characteristic curve (ROC) was used to evaluate the prognostic value of NLR and CRP in predicting MIS-C. A p- value less than 0.05 was considered to indicate a statistically significant difference.

## Results

### Demographic Characteristics of the Patients:

This study included 80 children with COVID-19. The mean age of the patients was 6.05±5.1years (range <1-16years). The age group 1-3 years was the most frequent accounting for 37.5% followed by age group 8-11 years (23.75%) and 12-16 years (15%). Infants constituted (11.25%) of the cases . Males had a preponderance over females (60% versus 40%). The residency of about one-fourth of the patients were urban. About half of the patients had no comorbidity at presentation. Different types of malignancies were the most common comorbidity accounting for 22.5% of the patients, while asthma was reported in 15% of the patients. A history of contact with infected person was reported in 27.5% of the patients. The mean hospital

stay was 3.15±1.94 days. Chest X-ray revealed that 52.5% of the patients had normal findings. The most common lesion was diffuse haziness of the lung accounting for 26.25% of the patients followed by lung infiltration (11.25%) and finally patchy lesion (10%). Ten patients (12.5%) required $O_2$ therapy, while 9 patients (11.25%) developed multi systemic inflammatory syndrome (Table 1).

**Table 1: Demographic and clinical characteristics of the patients (n=80)**

| Variables | Values |
|---|---|
| Age, years | |
| <1 | 9(11.25%) |
| 1-3 | 30(37.5%) |
| 4-7 | 10(12.5%) |
| 8-11 | 19(23.75%) |
| 12-16 | 12(15%) |
| Mean±SD | 6.05±5.1 |
| Range | 16 days-16 years |
| Gender | |
| Male | 48(60%) |
| Female | 32(40%) |
| Residence | |
| Urban | 61 (76.25%) |
| Rural | 19(23.75%) |
| Comorbidity | |
| No comorbidity | 37(46.35%) |
| Malignancy | 18(22.5%) |
| Asthma | 12(15%) |
| Cerebral palsy | 4(5%) |
| Others | 4(5%) |
| Contact with infected person | |
| Yes | 22(27.5%) |
| No | 58(72.5%) |
| Hospital stays, days | |
| Mean±SD | 3.15±1.94 |
| Range | 1-9 |

***Cont...*** **Table 1: Demographic and clinical characteristics of the patients (n=80)**

| | |
|---|---|
| Radiological Findings(chest x ray)<br>Normal<br>Haziness<br>Bilateral Infiltration<br>Patchy lesion | <br>42(52.5%)<br>21(26.25%)<br>9(11.25%)<br>8(10%) |
| Need for O2 therapy<br>Yes<br>No | <br>10 (12.5%)<br>70(87.5%) |
| Multisystemic inflammatory syndrome<br>Yes<br>No | <br>9(11.25%)<br>71(88.75%) |

**Presenting Symptoms**

Nineteen patients (23.75%) were asymptomatic at presentation. So far, fever was the most common presenting symptom reported in 47.5% of the patients followed by cough (21.25%), SOB (20%) and vomiting (16.25%). Less common presentation included diarrhea (8.75%), rash (7.5%) loos of appetite (5%) headache (3.75%) and conjunctivitis (3.75%) ,loss of taste and smell was not seen in any case (Table 2).

**Table 2: Presenting symptoms**

| Presenting symptoms | Frequency (%) |
|---|---|
| Asymptomatic<br>Fever<br>Cough<br>SOB<br>Vomiting<br>Edema of hands and feet<br>Diarrhea<br>Rash<br>Loss of appetite<br>Headache<br>Conjunctivitis | 19(23.75%)<br>38(47.5%)<br>17(21.25%)<br>16(20%)<br>13(16.25%)<br>9(11.25%)<br>7(8.75%)<br>6(7.5%)<br>4(5%)<br>3(3.75%)<br>3(3.75%) |

A patient can have more than one presenting symptoms

**Hematological Indices**

The mean WBC, neutrophil, lymphocyte and platelet count was 8.77±5.96×$10^3$/ml, 6.26±4.52×$10^3$/ml, 2.1±1.55×$10^3$/ml and 284.19±107.85×$10^3$/ml, respectively. On the other hand, mean NLR was 4.83±7.1, while PLR was 241.05±320.2. The mean CRP concentration was 40.71±60.4 mg/L (Table 3).

**Table 3: Hematological indices (n=80)**

| Variables | Values |
|---|---|
| **Total WBC ×$10^3$/ml**<br>Mean±SD<br>Range | <br>8.77±5.96<br>1.07-23.8 |
| **Neutrophil×$10^3$/ml**<br>Mean±SD<br>Range | <br>6.26±4.52<br>0.75-18.9 |
| Lymphocyte×**$10^3$/ml**<br>Mean±SD<br>Range | <br>2.1±1.55<br>0.2-7.0 |
| Platelets×**$10^3$/ml**<br>Mean±SD<br>Range | <br>284.19±107.85<br>41.0-515 |
| **NLR**<br>Mean±SD<br>Range | <br>4.83±7.1<br>0.24-44.0 |
| **PLR**<br>Mean±SD<br>Range | <br>241.05±320.2<br>7.32-2050 |
| **CRP, mg/L**<br>Mean±SD<br>Range | <br>40.71±60.4<br>0-168 |

Admission Diagnosis

So far, pneumonia was the most common diagnostic entity encountered in 51.25% of the patients. Fever without a source came next with 21 patients (26.25%) followed by gastroenteritis (13.75%), seizure (12.5%) and Kawasaki (7.5%) as shown in table 4.

**Table 4: Diagnosis at admission (n=80)**

| Diagnosis | Frequency (%) |
|---|---|
| Pneumonia | 41(51.25%) |
| Fever without focus | 21(26.25%) |
| Gastroenteritis | 11(13.75%) |
| Febrile Seizure | 10(12.5%) |
| Kawasaki | 6(7.5%) |
| Histiocytosis | 1(1.25%) |

Some patients had more than one diagnosis

**Association of demographic and Clinical characteristics with MIS-C**

Four factors were found to be significantly associated with development of MIS-C. Most patients (60%) with no MIS-C had **no comorbidity** compared to 10% of those who develop MIS-C, with a significant difference. The mean **hospital stay** among patients with MIS-C was 5.44±2.92 days which was significantly longer than those without MIS-C (2.86±1.58). The **median NLR** in patients with MIS-C was 6.21(range=0.24-44.0) which was significantly higher than that without MIS-C (median = 2.25, range=0.5-39). Finally, in patients who developed MIS-C, the **median of CRP** was 112.0 mg/L (range= 0-168 mg/L) compared with 32.0 mg/L (0-128 mg/L) in those without MIS-C, with a significant difference (Table 5).

**Table 5: Association of demographic characteristics with patients' outcome**

| Variables | No MIS-C (n=70) | With MIS-C (n=10) | p-value |
|---|---|---|---|
| **Age, years** mean±SD | 6.1±4.28 | 5.67±4.32 | 0.777† |
| **Gender** Male Female | 44(62.86%) 26(37.14%) | 4(40%) 6(60%) | 0.168‡ |
| **Residence** Urban Rural | 54(77.14%) 16(22.86%) | 4(40%) 6(60%) | 620‡ |

***Cont...*** **Table 5: Association of demographic characteristics with patients' outcome**

| | | | |
|---|---|---|---|
| **Comorbidity** | | | |
| No comorbidity | 42(60%) | 1(10%) | **0.003**‡ |
| Malignancy | 14(20%) | 4(40%) | 0.157 |
| Asthma | 9(12.86%) | 3(3%) | 0.156 |
| Cerebral palsy | 3(4.39%) | 1(10%) | 0.438 |
| Others | 3(4.29%) | 1(10%) | 0.438 |
| **History of contact** | | | |
| Yes | 51(72.9%) | 7(70%) | 0.850‡ |
| No | 19(27.1%) | 3(30%) | |
| **Hospital stays, days** | | | |
| Mean±SD | 2.86±1.58 | 5.44±2.92 | **<0.001**† |
| **Need for $O_2$ therapy** | | | |
| Yes | 63(90%) | 7(70%) | 0.074 |
| No | 7(10%) | 3(30%) | |
| Total WBC $\times 10^3$/ml | | | |
| Median (range) | 7.7(1.07-23.8) | 9.05(4.3-21.9) | 0.235‡ |
| Neutrophil$\times 10^3$/ml | | | |
| Median (range) | 6.4(0.75-18.9) | 6.65(1.4-18.3) | 0.085‡ |
| Lymphocyte$\times 10^3$/ml | | | |
| Median (range) | 1.6(0.2-7.0) | 1.35(0.2-5.9) | 0.393‡ |
| Platelets$\times 10^3$/ml | | | |
| Median (range) | 288(41-515) | 247(166-515) | 0.344‡ |
| NLR | | | |
| Median (range) | 2.25(0.5-39.0) | 6.21(0.24-44.0) | **0.013**‡ |
| PLR | | | |
| Median (range) | 175.34(7.32-2050) | 162.2(4.68-218.3) | 0.454‡ |
| CRP, mg/L | | | |
| Median (range) | 32.0 (0-128) | 112.0(0-168) | **0.038**‡ |

† Student t-test, ‡ Chi square test, ‡ Mann Whitney U test

**Prognostic Value of NLR and CRP**

Receiver operating characteristic (ROC) curve was used to find out the cut off value of NLR and CRP in predicting MIS-C. **For NLR**, the area under the curve (AUC) was 0.827, 95%CI=0.670-0.984, $p$=0.006. The sensitivity and specificity of the test at cut off value of NLR= 3.34 were 71% and 82%, respectively. **For CRP**, the AUC was 0.748, 95%CI=0.512-0.984, $p$=0.039. The sensitivity and specificity of the test at cut off value of CRP= 101.7 mg/L were 71% and 89%, respectively (Figure 1).

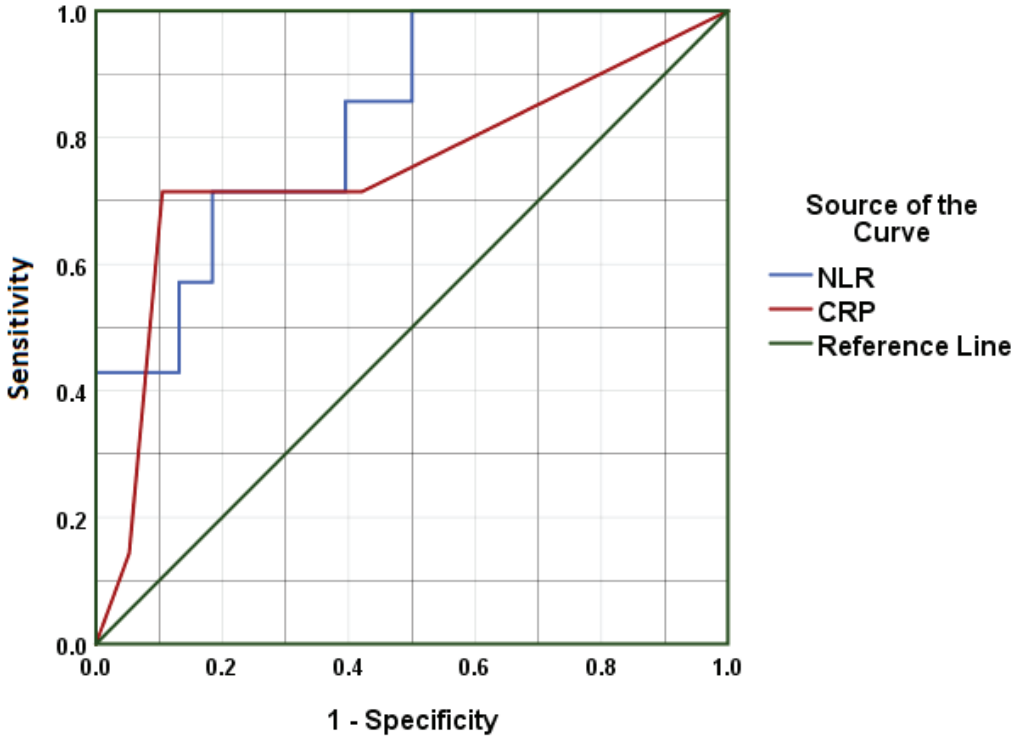


**Figure 1: Receiver operating characteristic curve for NLR and CRP in predicting development of MIS-C in children with COVID-19**

Antimicrobials were used in all cases (100%) and oxygen need was seen in 50% of study cohort. COVID-19 investigational treatments were not administered to any case in study cohort. intravenous immunoglobulin was given to the cases with MIS-C for suspected Kawasaki disease . During the study period no mortality was documented in the study cohort.

## Discusion

Despite the rising incidence of COVID-19 cases in children, little is known about their clinical and analytical features. In contrast to adults with COVID-19, studies demonstrate that changes in leukocyte indices are mainly inconsistent in children. As a result, leukocyte indices do not appear to be accurate indicators of illness severity in children (9). We are conducting this research to better understand the clinical and laboratory features hospitalized COVID 19 cases in pediatric age group, and more critically, to find a valid disease severity predictive biomarker..

In the current study, the mean age of the patients was 6.05±5.1 years (range <1-16years), lower than what's is found by Lu X et al [10] and Dong Y et al [11]. male were predominant this agree with Dong et al who reported that 56.6% of the patients in their study were boys[4].

In this study, only 27.5 percent of cases had a positive history of contact with a confirmed case of COVID-19, which is significantly lower than the 70 percent of patients in other studies conducted in China, Saudi Arabia, and Oman.[12,13,14]. This may be attributed to the lack of accurate and frequent testing and most importantly documentation, all these could have led to the underestimation of reported cases.

Pediatric patients with COVID-19 in this study were more likely to have underlying comorbidities (53.75%), this percentage is more than what has been described in other studies for example 39% by DeBiasi et al [15] . however, Shekerdemian et al found

that underlying comorbid conditions were present in 83 percent of the patients with COVID19 who had admitted to intensive care unit [16].

Different types of malignancy were the most common comorbidity in this study accounting for 50%. this is much higher than what found by DeBiasi et al (1%) and While other studies the most common comorbidity was sickle cell anemia in Al Yazidi et al [14], asthma in DeBiasi et al [15]. sickle cell not seen in any case in our study this is probably because other hospital in Baghdad city is concerned with sickled children.

Fever was the most common presenting symptom in children hospitalized with COVID-19 in the current study; this was similar to results reported by Al Yazidi et al [14], Qiu et al [17], Derespina et al [18].

The most common Initial syndromic diagnoses in our study was pneumonia, this agree with result by Lu x et al study at Wuhan Children's Hospital located in Wuhan, the epidemic center of this novel infection who found that pneumonia was diagnosed in 64.9% of children with COVID 19 infection [19].

The complete blood count revealed no significant changes in children hospitalized with COVID-19 in this study, which is consistent with findings by Henry et al [9].In terms of inflammatory markers, Children with COVID-19 rarely exhibit high inflammatory markers, according to Al Yazidi et al [14] and Liguoro et al [20], which is consistent with the findings of this study. However, Kainth et al[21] , Zachariah et al[22] have linked elevated inflammatory markers to severe illness in children.[22 ] .

Half of patients in our study had normal findings on chest radiograph, which is similar to what Liguoro et al found , that only 50% of children with confirmed COVID-19 had chest X-ray abnormalities , this could be attributed to milder involvement by the disease so chest X-ray may fail to identify typical lesions, and it is primarily used in the neonatal period and infancy [20] .

All the patients in the current study received antibiotic empirically, which could be explained by physician's worries about COVID-19 's potential severity and risk of co-infection with other viruses or bacteria. antibiotic use in children with COVID-19 has been shown to range from 19.4% to 100.0% by Wang et al [23],while al Yazidi et al found that Antimicrobials were used in 68% of children admitted with COVID- 19 [14].

In our analysis, nine patients (11.25 percent) had a diagnosis of MIS-C, which was defined as a severe COVID 19 presentation with unknown pathogenesis [24]. Patients with MIS-C have greater comorbidity, according to our findings. Harman et al similarly found a link between underlying comorbidities and severe COVID-19 [25] .

longer stay at hospital was found in patients with MIS-C about 5.44±2.92 days, likewise, Sinaei R et al [26] in case series of children with MIS-C in Iran reported that the average length of PICU stay for MIS-C cases was 6 days.

Regarding laboratory result two factors was found to be significant in children who develop MIS-C which was the NLR and CRP. This goes with results found in a case series by Feldstein LR et al showed that patients with MIS-C had higher neutrophil to lymphocyte ratio and C-reactive protein level [27]. Also agree with Zachariah et al in a case series done at New York City who found that patient with severe disease had significantly higher C-reactive protein.

This could be of benefit since those are simple, rapid tests, of low cost and widely available at ER and can be used as an early signal for development of MIS-C.

Finally, the outcome for all children in this study was favorable, with no reported mortality, which was similar to result by Al Yazidi et al.

## Conclusions

Mild course and favorable outcome signify the

majority of pediatric cases of COVID 19 Malignancy is the commonest comorbidity associated with COVID-19 admissions. Elevated NLR and CRP are independently associated with development of MIS-C in COVID-19 among children.

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**Conflict of Interest** :none to declare.

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# Mung Bean Sprouts (*Vigna radiata*) Ethanol Extract on Alanine Aminotransferase (ALT) Activity and Malondialdehyde (MDA) Levels in Toluene-Induced Rats

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## Abstract

**Objective** –This study aimed to evaluate the effect ofethanol extract from mung bean sprouts on alanine aminotransferase (ALT) activity and malondialdehyde levels in toluene-induced white rats.

**Methods**–This research is an experimental laboratory with a research design that is post-test only control group design using 40 rats (Rattus norvegicus) which were divided randomly into 5 groups, G1 (control without toluene), G2 (900 mg/kg toluene), G3 (mung bean sprouts extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean sprouts extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean sprouts extract 1000 mg/kg + 900 mg/kg toluene). ALT measurement using spectrophotometer method. Measurement of MDA using the ELISA method. The data collected will be statistically tested with the help of SPSS with a significant ($p < 0.05$).

**Results**–The results of the measurement of ALT activity ($p=0.972$) and MDA levels ($p=0.190$) in each group had no significant effect ($p>0.05$) in each treatment.

**Conclusion**–It can be concluded that there was no significant effect of ethanol extract of sprouts on alanine aminotransferase (ALT) activity and malondialdehyde levels in white rats *(Rattus norvegicus*) induced by oral toluene.

***Keywords:*** *Alanine aminotransferase, Mung bean sprouts, malondialdehyde, toluene*.

## Introduction

The painting industry requires toluene solvent as a paint thinner[1]. However, its use has side effects on health problems because toluene is volatile and inhaled[2]. Toluene can pass the blood-brain barrier and cause neurotoxicity, nephrotoxicity[3], headaches, fatigue, nausea, and loss of consciousness[4]. Toluene will be converted by cytochrome p450 into benzyl alcohol[5. T]his reaction also produces anionsuperoxide radicals[6].Increased production of anions superoxide can cause an imbalance between free radicals and antioxidants, known as oxidative stress.It can damage cell[7], increase the malondialdehyde (MDA), and increasethe excretion of Alanine aminotransferase (ALT) enzyme into the blood circulation[8]. Oxidative

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stress conditions can trigger degenerative diseases such as diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder disease, cancer, and schizophrenia [9].

The body has endogenous antioxidants (superoxide dismutase, glutathione peroxidase, and catalase) that reduce free radicals[10]. The imbalance of free radicals with antioxidants causes oxidative stress conditions[11]. Mung bean sprouts come from green bean seed plants that have germinated. During germination, there may be an increase in compounds that can act as antioxidants and affect antioxidants such as flavonoids, vitamin C, quercetin[12]. Therefore, the germination time of mung bean sprouts can increase the nutritional content of bean sprouts and improve the nutrition that can be used as an additional antioxidant to see the free radicals formed[13].

On this basis, this study aimed to evaluate the ethanol extract of mung bean sprouts against the activity of alanine aminotransferase (ALT) and malondialdehyde (MDA) in white rats (*Rattus norvegicus*) induced by oral toluene. This research hypothesizes that bean sprouts extract can reduce MDA and ALT activity.

## Materials and Methods

### Experiment design

This study is a laboratory experiment with a research design that is post test only control group design using 40 male rats (Wistar novergicus strains) weighing 150-230 grams which are divided into 5 groups, G1 (n=8, control without toluene), G2 (n=8,900mg/kg toluene) G3 (n=8,250 mg/kg bean sprout ethanol extract + 900mg/kg toluene), G4 (n=8,500 mg/kg bean sprout ethanol extract + 900 mg/kg toluene), and G4 (n= 8, 1000mg/kg bean sprout extract + 900 mg/kg toluene). All procedures have been approved by the Ethics Research Committee of the Faculty of Veterinary Medicine, Airlangga University (2.KE.008.01.2021)

### Mung bean sprout ethanol extract

Mung bean sprouts (*Vigna Radiata*) were bean sprouts with an age of 4 days; the extract was made with ethanol as a solvent. First,Mung bean sprouts were air-dried, mashed and the extraction process was carried out by dissolving it with ethanol as a solvent. Then, the ethanol extract of Mung bean sprouts mixed with ethanol was evaporated until the remaining ethanol mung bean sprouts extract. Finally, bean sprout extract was given to groups of rats G3 (250 mg/kg), G4 (500 mg/kg), and G5 (1000 mg/kg) in the morning.

### Toluene

Toluene is given orally with a hefty dose of 900 mg/kg/BW; toluene is administered using a glass syringe. Toluene (EMSURE, GERMANY, 1,08325,2500) was used at a concentration of 98%.

### Blood samples and blood analysis

Blood samples were taken from the heart of rats. After being taken, the blood samples were put into a vacutainer tube and centrifuge for 15 minutes at a speed of 3000 rpm.Measurement of alanine aminotransferase (ALT) activity using a spectrophotometer with a wavelength of 340 nm. Measurement of malondialdehyde (MDA) using the ELISA method (using an ELISA reader) with an absorbance spectrophotometer of 450 Nm. The MDA sensitivity level is 0.023 nmol/mL.

## Statistical Analysis

Data analysis techniques used statistical software packet for social science (SPSS) version 25 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test. The homogeneity test used the Levene test. Data that were normally distributed and had homogeneous variants were tested using One-way

ANOVA and continued with the post hoc Least Significant Difference (LSD) test. All datapresented as mean±SD. All statistical analyzes used a significant level ($p$<0.05).

## Results

The results of measurements of alanine aminotransferase (ALT) and malondialdehyde (MDA) can be seen in Table 1.

**Table 1. Results of ALT and MDA measurements.**

| Variable | Group | | | | | ANOVA p-value |
|---|---|---|---|---|---|---|
| | G1 (n=8) | G2 (n=8) | G3 (n=8) | G4 (n=8) | G5(n=8) | |
| ALT (U/L) | 68,40±4,77 | 69,60±2,60 | 69,40±18,20 | 69,40±13,70 | 73,00±10,33 | 0,972 |
| MDA (nmol/mL) | 3,60±1,18 | 4,07±0,55 | 3,56±1,58 | 4,67±2,16 | 5,44±0,58 | 0,190 |

G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900 mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.

Based on Table 1, the results of the One Way-ANOVA test, the average ALT and MDA are not significant (p>0.05). The results of ALT measurements can be seen in Fig.1.

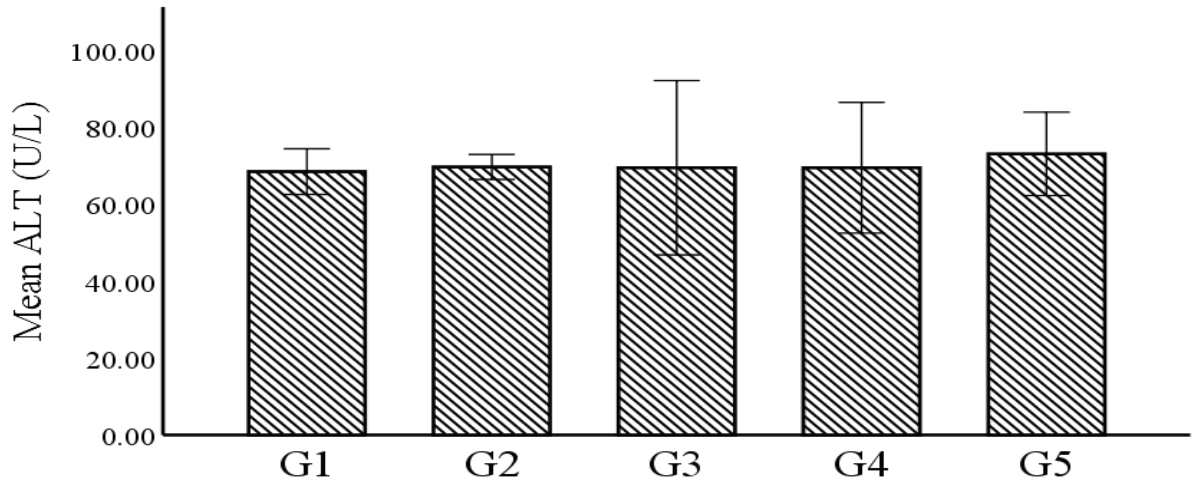


**Fig. 1 The Average ofALT in Each Group.G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900 mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.**

Based on Fig.1, the results of the one-way ANOVA test on ALT activity did not indicatea significant difference (p>0.05) where there is no difference between G1 and G2, G3, G4, and G5.

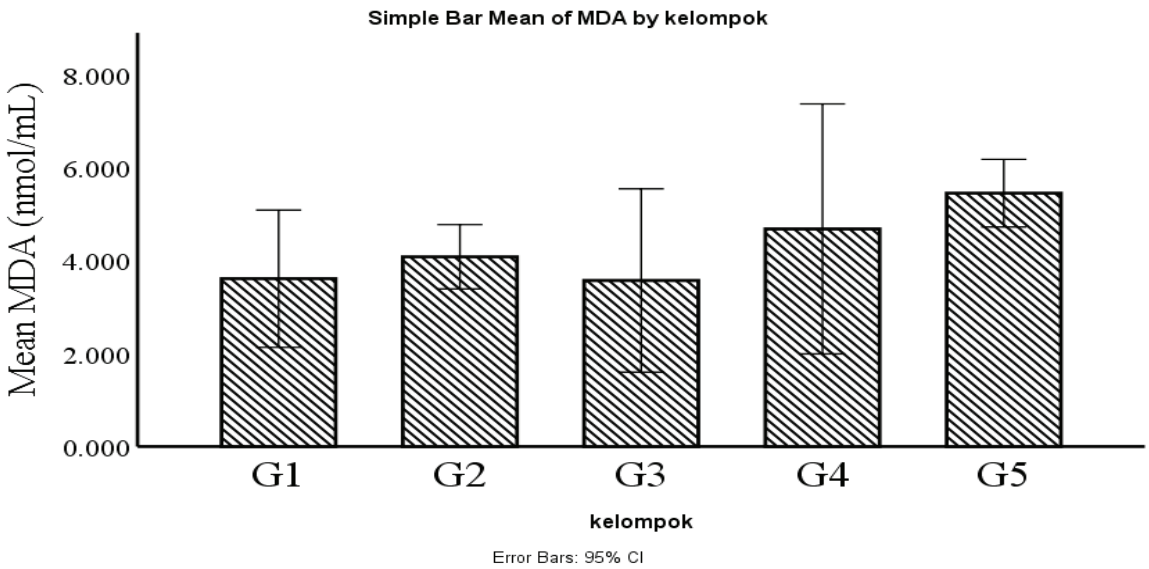


**Fig. 2 The Average of MDA Levels in Each Group. G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.**

Based on Fig.2, the results of the ANOVA test on the examination of MDA activity showed no significant difference ($p>0.05$). There was an increase between G1 (3.60±1.18) and G2 (4.07±0.55), but the increase was not significant ($p>0.05$).

## Discussion

The results of this study indicate that the measurement of alanine aminotransferase (ALT) between G2 (68.40±4.77) is higher than G1 (69.60±2.60). The results of One-Way ANOVA showed no significant difference between each group. These results follow the study of Tas, Ogeturk14, which stated an increase in ALT in white rats (*Rattus norvegicus*) with inhalation of toluene exposure[15]. This difference may occur because previous researchers used a larger dose of inhalation exposure[16]. There are antioxidant mechanisms in the body that can still prevent free radicals formed due to toluene[17]. Several studies have also stated that ALT activity can be used as a parameter to see liver damage[18]. ALT can be caused by the mechanism of the inability of antioxidants to reduce free radicals so that ROS are formed, which can cause damage to cell components and cause an increase in the excretion of ALT enzymes into blood vessels[18,19].

Another important finding is that the body20 can metabolize toluene, toluene binds to cytochrome p450[21]. It causes an increase in the formation of superoxide anion radicals in monooxygenase metabolism[16, 22], an increase in superoxide anion radicals will be converted by the enzyme superoxide dismutase (SOD) into hydrogen peroxide[23]. When reacted with metal ions, hydrogen peroxide can cause Fenton and Haber Weiss reactions and cause more dangerous hydroxyl radicals[24]. In addition, the increase in free radicals causes oxidative stress conditions that will cause necrosisf[25]. Necrosis conditions can cause ALT excretion out into the blood vessels and increase ALT activity in blood vessels[26].

The current study found that the administration of bean sprouts ethanol extract at G3 (69.40±18.20), G4 (69.40±13.70), and G5 (73.00±10.33) had no

significant effect (p> 0.05) on the ANOVA test. These results explain the administration of bean sprout extract has not been able to affect ALT activity. Mung bean sprouts contain flavonoid compounds that can be used as free radicals formed and prevent cell damage caused by free radicals[27].

Results Measuring the average level of malondialdehyde (MDA) found that G2 (4.07±0.55) was higher than G1 (3.60±1.18). The results of the One-Way ANOVA test showed no significant differences in each group (p>0.05). These results follow the research of Iqbal, Mansyur[28,] which states that toluene can increase MDA levels but will be different from the study of Ayu, Tualeka[29,]which says that exposure to toluene requires large doses to cause an effect on MDA. Toluene can increase freeradicals. Free radicals can react with lipids to cause lipid peroxidation and produce MDA[30]. Exposure to toluene can cause an increase in free radicals and cause oxidative stress conditions. Research Coskun, Oter[31,] who researched the induction of 3000 ppm toluene, stated that oxidative stress levels significantly affect the increase in MDA levels. Examination of MDA levels is often with oxidative stress levels[30,32].

The current study found that the administration of mung bean sprout extract in G3 was lower than in G2. Therefore, giving bean sprouts extract to G3 can reduce MDA levels. The results of the One-Way ANOVA test stated that there was no significant difference (p>0.05) between G3 and G2. Bean sprouts contain flavonoid compounds that can reduce free radicals[33]. Research by El-Newry, Shaffie[34] proves that flavonoids can make free radical scavengers to prevent the increase of free radicals in alcohol-induced rats.

## Conclusion

Overall, this study showed that ethanol extract from mung bean sprouts could not reduce ALT and MDA in toluene-induced white rats.Endogenous antioxidant status in rats plays a vital role in increasing ALT and MDA in toluene treatment. This endogenous antioxidant can reduce free radicals caused by toluene induction.

**Conflict of Interest** : The authors declared no conflict of interest. Furthermore, all the authors agreed that the manuscript is submitted to the Indian Journal of Forensic Medicine and Toxicology.

**Ethical Clearance:** : The Ethics Research Committee approved this study of the Faculty of Veterinary Medicine, Airlangga University (2.KE.008.01.2021)

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# Workers Knowledge about First Aids of Emergency Accidents at Industrial Sector of Al-Najaf City in Iraq

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## Abstract

Unintentional accidents and sudden illnesses are two of the most common causes of disability and death among workers, especially those under the age of forty . Using a simple random sample, twenty workers in the industrial workshops were randomly selected . A questionnaire consisting of two main groups was used. The result revealed that out of a total of 201 workshop workers included in the study, 59 (29.4%) of them had overall fair knowledge and 125 (62.2%) of the participating workers had poor knowledge, in addition to none having good knowledge. The results showed that only 17 (8.5%) of the workers had a positive attitude towards first aid. With regard to the relationship between the social and demographic data of the participants and their levels of knowledge, no an important correlation was not identified except for the monthly income, number of children and age, while with regard to workers' attitudes towards first aid, residency in urban areas, years of experience, marital status, education level and receiving information about first aid showed significant correlation with their social relationships - demographic data.

***Keywords :*** *aids , emergency accidents , industrial sector*

## Introduction

First aid is the first most essential care given to an injured or an ill individual in a life-threatening circumstance, first aid means the assessments and interventions that can be performed by a bystander to the victim immediately with minimal or no medical equipment First aid is generally of a series of Simple steps, sometimes life-saving medical techniques, that a caregiver takes, either with or without a formal medical. The bulk of first-aid medical emergencies occur in workshops. With respect to the prevalence of these medical emergencies, little is understood [(1)]. When given proper first aid, the severity of the injury and resulting damage to the brain is reduced. First aid procedures must be simple to understand, execute, and provide a level of analgesia, with all necessary supplies readily accessible. Furthermore, the procedure must not have a detrimental impact on subsequent specialized assessment and care. The goal of first aid is to stop the progression of a burn by adequately cooling the affected area and providing symptomatic relief [(2,3)]. Until competent medical services can be given, first aid is used to treat any accident or sudden illness. The goal is to keep the condition from getting worse, to ensure a quick recovery, and to keep the patient alive. Human life is priceless. The majority of injuries are minor and can be treated without seeking medical help, such as

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bruises, mild fractures, sprains, and strains, to name a few. When properly applied, first-aid knowledge can make the difference between a temporary or permanent injury, a quick recovery, or a long-term disability [4]. Accidents and illnesses inevitably occur in a variety of settings and at different times in one's life. In several nations, programs are often missed. This is expressed in a lack of knowledge and understanding of common illnesses and first-aid procedures. Accident-related involuntary injuries, in particular, are a global public health concern, and they are one of the leading causes of death and disability [3,5] . First aid for acute illness or injury was given as assistive behavior and primary care. The first aid provider's priorities include sustaining life, alleviating pain, preventing further disease or injury, and facilitating recovery. In any case, first aid can be started by anyone and requires self-care. The assessments and procedures for first aid must be medically sound and based on empirical evidence or in the absence of such evidence, on the consensus of experts. Require first aid skills, at the level of a more first aid for acute injury or illness can be described as therapeutic primary care given and assistance. The primary aid provider's priorities include sustaining life, alleviating pain, preventing further injury or illness, and facilitating rehabilitation [6,7]. Managers and supervisors in the workplace are expected to be highly competent when it comes to managing first aid for minor accidents and severe emergencies. With this knowledge, supervisors will be in a better position to protect employees in any dangerous situation [8]. Truly mishaps these days are a lot of because of the enormous number of groups and constant illnesses widespread in our Arab society, for example, coronary illness, asthma, auto crashes, introduction to compound toxins and other perilous mishaps [9,10].

## Materials and Methods

Its part will clarify the current study's methodology as well as all of the various phases that have occurred, from the beginning of its acceptance to the conclusion of the data analysis. A convenience sample is a non-probability sampling technique that selects a cumulative sample of 201 participants from Al-Najaf City's industrial sector. These individuals showed a willingness to participate in the study on a voluntary basis during the duration of the study. Using a simple random sample, twenty workers in industrial workshops were randomly selected, then 230 workers were randomly selected, through an equation used to determine the sample size. Finally, the total number of questionnaires valid for analysis was 201. The study instrument's reliability was determined using Cronbach's Alpha coefficient test, which was also performed separately for information questions. That test resulted in reasonable reliability based on the Cronbach's Alpha value of (0.761) for the information scale. Furthermore, data were collected from (20) workers in the industrial sector using a specially designed questionnaire. The data was collected using a developed and updated questionnaire and a self-administered technique. In addition, the researcher interacted with the workers and inquired about socio-demographic data that existed at the time of the study in order to select participants at random. The researcher then received verbal consent from the chosen workers to participate in the analysis. The subject of study and the correct way to fill out the questionnaire sectors were then clarified for each chosen section. It took about (30-40 minutes). Finally, since many of the selected workers could not read or write, they completed questionnaires under the supervision of the researcher. In total, 201 questionnaires were obtained and used for statistical analysis, with 11 questionnaires being invalid due to filling errors and 15 not being returned at all, and three questionnaires lacking socio-demographic data. The data was obtained from December 28th, 2020, to February 24th, 2021.

## Results

The result showed that out of a total of 201 workshop workers included in the study, 59 (29.4%) of them had overall fair knowledge and 125 (62.2%) of the participating workers had poor knowledge, in addition to none having good knowledge. The results showed that only 17 (8.5%) of the workers had a positive attitude towards first aid. With regard to the relationship between the social and demographic data of the participants and their levels of knowledge, an important correlation was not identified except for the monthly income, number of children and age, while with regard to workers' attitudes towards first aid, residency in urban areas, years of experience, marital status, education level and receiving information about first aid Showed significant correlation with their social relationships - demographic data.

**Table (1): Overall participant 's Knowledge about first aids**

| Levels of knowledge | Frequency | Percent | mean of score | Overall evaluation |
|---|---|---|---|---|
| poor | 125 | 62.2 | 1.60 | poor |
| Fair | 59 | 29.4 | | |
| Good | 17 | 8.5 | | |
| Total | 201 | 100.0 | | |

**Table (2): participant 's responses for first aid domains**

| First aid domains | Levels | frequency | Percent | Mean score | Evaluation |
|---|---|---|---|---|---|
| General information about first aid | Fair | 113 | 56.2 | 1.684 | Fair |
| | Good | 25 | 12.4 | | |
| | poor | 63 | 31.3 | | |
| Wounds and bleeding | Fair | 44 | 21.9 | 1.554 | poor |
| | Good | 17 | 8.5 | | |
| | poor | 140 | 69.7 | | |
| Bones and joints injuries | Fair | 58 | 28.9 | 1.599 | poor |
| | Good | 20 | 10.0 | | |
| | poor | 123 | 61.2 | | |

***Cont...*** **Table (2): participant 's responses for first aid domains**

| | | | | | |
|---|---|---|---|---|---|
| Other medical conditions | Fair | 47 | 23.4 | 1.60 | poor |
| | Good | 16 | 8.0 | | |
| | poor | 138 | 68.7 | | |
| Burns | Fair | 76 | 37.8 | | |
| | Good | 11 | 5.5 | 1.580 | poor |
| | poor | 114 | 56.7 | | |
| Bites, Stings, and Foreign Bodies | Fair | 65 | 32.3 | 1.598 | poor |
| | Good | 22 | 10.9 | | |
| | poor | 114 | 56.7 | | |
| | Total | 201 | 100.0 | | |

**Table (3): Mean differences by (Independent T- test) of participant's Knowledge about first aids according to their residency& Receive information.**

| Demographic data | Rating | N | Mean | SD. | T value | Df. | p-value |
|---|---|---|---|---|---|---|---|
| Residency | urban | 148 | 1.6556 | 0.38390 | 3.59 | 199 | 0.001 HS. |
| | rural | 53 | 1.4434 | 0.32273 | | | |
| Receive information | no | 140 | 1.4801 | 0.31530 | 7.68 | 199 | 0.001 HS. |
| | yes | 61 | 1.8740 | 0.37473 | | | |

## Discussion

Internationally, injuries and accidents in industrial cities are among the most dangerous life-threatening things, in addition to that the workers' lack of knowledge of how to use first aid to provide it to the injured. This table shows the characteristics of our sample mainly the age that ranging from (33-51) that reflect the reproductive age in our society with such qualifications that met such work. This outcome is consistent with studies [11,12]. In relation to residency area, the majority of workers (73.6 %) were from urban. This result can be interpreted those

lived in nearest areas from the industrial sector. This study is consistent with a research from 2011 [13,14]. According to level of education, (26.9%) of the respondents were unable to read and write with years of experience (41.8%) this result reflects that such work needs experiences rather than level of education. In Compatible with studies [15,16]. More than two third of workers have low level of knowledge regarding first aid (62.2%) and (29.4%) of them have moderate knowledge followed by (8.5%) were good. Such result can be interpreted by those workers doesn't pay attention to such vital issue to save their life also there is no formal or governmental agency advocate training courses or monitoring to detect emergency cases. A study conducted in Singapore in 2020 by Pisharody and others shows that workers have knowledge of first aid through courses and television, so the media is essential for the transfer of general knowledge [17]. Other studies also demonstrated the need workers to take special first aid courses and not to rely exclusively on television or the Internet [18,19].

This part shows that evaluation of knowledge about General information about first aid is fair, while evaluation of knowledge was poor for (wounds and bleeding, bones and joints injuries, Other medical conditions, Burns and Bites, Stings, and Foreign Bodies) about first aid. The percentage of workers' information about general information was, those whose level was fair (56.2%), and the people whose information percentage was poor (31.3%), and a small group of respondents was good (12.4%). In general, when the group was tested more closely regarding their knowledge of wounds and bleeding, their level was bad and the rate of right was low (21.9%) and the percentage of poverty was the highest (69.7%) and the percentage whose level was decent (8.5%) was the lowest. In the questionnaire collected through the study, bone and joint injuries played a role in the ranking, if we note in Table (3), and respectively from fair to success (28.9 %, 61.2 %, 10.0 %). We remember that the largest proportion of individuals do not have adequate knowledge of first aid (61.2 %). Bearing in mind the necessity for workers to obtain first aid for other chronic health problems such as asthma, epilepsy, diabetes, food poisoning, etc. They found the highest percentage of those with insufficient knowledge (68.7 %) and the proportion of respondent's fair (23.4 %), and the few who had good information regarding other medical conditions (8.0 %). This is because they did not realize the benefits of their knowledge of first aid. Also, there is no governmental or supervisory body specialized in the health aspect to take care of them. And previous studies found support for this study [20,21].

## Conclusions

According to the study, the Ministry of Health, the Ministry of the Environment, and the Ministry of Municipalities should work together to include this industrial sector in occupational health and safety initiatives.

**Ethical Clearance** : Taken from University of Kufa ethical committee

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# Self-Efficacy with the Quality of Life of Pulmonary Tb Patients

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## Abstract

**Aim**: It is known that the relationship between self-efficacy and quality of life in patients with pulmonary tuberculosisat Pertamina Bintang Amin Hospital in Bandar Lampung 2020.**Methods:** This type of research was quantitative with a cross sectional approach. The population was all pulmonary TB patients at the Pertamina Bintang Amin Hospital Bandar Lampung, with a sample size of 62 people. Data analysis in this study used the chi-square test.**Results:** The results of the univariate analysis showed that most of the respondents' self-efficacy was in poor condition, namely as many as 33 people (53.2%). Most of the respondents' quality of life was in the medium category, namely 27 people (43.5%). The results of the bivariate analysis showed that the p-value = 0.001). 1) demographic factors such as the type of education level are an indicator of the potential vulnerability of pulmonary TB infection, 2) Most of the respondents' self-efficacy was in poor condition, namely 33 people (53.2%). Most of the respondents' quality of life was in the medium category, namely 27 people (43.5%), and 3) there was a relationship between self-efficacy and the quality of life of patients with pulmonary tuberculosis.

***Keywords:****Self-Efficacy, Quality Of Life, Pulmonary Tuberculosis*

## Introduction

In this globalized world era, the health level of a country will affect the health level of other countries. In other words, the health level of a country will influence each other. This is due to one of the fast growing tourism industries. The tourism industry encourages people to move from one place to another.

Move people by themselves along with the overall health level of these people, like TB disease, a disease caused by bacteria. A disease caused by infectious bacteria that has serious potential, especially affecting the lungs.

The bacteria that bring about TB are outspread when contaminated person hoops or sneezes. Nearly all people who are contaminated with the bacteria that induce tuberculosis possess no indications. When indications do occur, they are ordinarily hoop (sometimes spotting), mass loss, evening perspiration, and feverishness. Medication isn't constantly required for persons without indications. Sufferers with active indications shall require a long route of medication

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incriminate some antibiotics.

Given that TB treatment requires a long and tiring duration, preventive measures are the wisest actions. WHO as a world institution must monitor and encourage member countries to always take preventive measures against TB disease because the health level of a country contributes to the level of health globally. In order to provide additional information that is comprehensive and complex treatment of TB disease, this research is important to carry out in order to provide data on the ins and outs of TB disease sufferers. This research was conducted in Lampung Province, Indonesia. This research is also intended for WHO to put pressure on health stakeholders in Indonesia to continue to care for and be responsive to this pulmonary TB disease.

Tuberculosis is one of the very significant contagious sicknesses to paying attention to, with so major morbidity and death globally[1]. The World Health Organization (WHO) approximates that there were 10.4 million new TB occurrences and 1.4 million TB deaths globally in 2015[2]. Mycobacterium tuberculosis is the causative factor of TB, and while it could cause infection in biological structures there out the lungs, such as the lymph nodes, bones, and meninges, M. tuberculosis mainly contaminates the lungs and leads to pulmonary TB[3]. With a large number of individuals with pulmonary TB sufferers, they will be in pain even more if they are exposed to Covid-19. TB sufferers will be in pain multiple times and could induce death.

Pulmonary TB disease

Pulmonary TB disease

The person's body is occupied by a large number of commensal bacteria (macrobiotic) which is in contact closely with the person's invulnerable structure and exerts an important effect on a person's physical fitness. Numerous contagious and non-contagious illnesses are connected to the human macrobiotic, particularly the intestine macrobiotic[4]. Designated the tight relation between the intestine macrobiotic and illnesses of the neural structure, liver, and lungs, the intestine macrobiotic is put forward to be implicated in the intestine-brain (Foster & Neufeld, 2013), intestine-liver[5], intestine-liver[6]gut-lung [6], and intestine-lung [5,7], the ending is known one [7-9]. Much research has centered on revealing the role of this axis in the expansion of lung illness, such as asthma[10], chronic obstructive pulmonary disease (COPD) [11], and pneumococcus and Staphylococcus aurous pneumonia [12,13].

So far, some attempts have been taken to disclose changes in the intestine microbiota after M. tuberculosis contamination utilizing murine specimen or clinical models[14,18]. However, if there is a microbiota signature in the microbial structure of the gut or the metabolic potential that may differentiate healthy from M. tuberculosis contamination has not been assessed. Here, we characterize shifts in the intestine microbiota in stool specimen of clinical pulmonary TB sufferers. We notice substantial shifts in the construction and metabolic pathways of the intestine microbiota in TB sufferers, and illustrated that bacterial execution in the intestine could likely be used to differ healthy from TB patients.

The 2017 Global Tuberculosis Report shows that new tuberculosis cases aggregated to 6.3 million, or the equivalent of 61% of tuberculosis occurrences (10.4 million). Tuberculosis is still one of the big ten inducesof death in the globe with an predicted death rate of 1.3 million sufferers [19]. Further, most of the predictedoccurrences of tuberculosis happened in the Southeast Asia Region (45%), and 25% in Africa. The five countries with the highest incidence of cases with incidence estimates based on samples taken (best estimate value) are India (2,790), Indonesia (1,020), China (0.895), the Philippines (0.573), and

Pakistan (0.405) (Indonesian Ministry of Health, 2018) . Indonesia is one of the 5 countries that have the biggest burden of tuberculosis [19]. Data for 2017 shows that the sum of tuberculosis cases was 425,089 cases, this number increased when compared to the incidence in 2016 which was 360,565 cases [20].

Meanwhile, the incidence in Lampung Province, referred to the results of the Tuberculosis Commonness Survey for 2013-2014, was 1,600,000 cases, while the incidence was 1,000,000 cases and the mortality was 100,000 cases [21]. Whereas for Bandar Lampung City in 2018, the prevalence of pulmonary TB based on a doctor's diagnosis history, has a higher prevalence than the average of districts/cities in Lampung Province, namely 0.48% of the weighted value of 3,878 people [20].

**Self-Efficacy and Quality of Life**

To be able to cope with the growth rate of TB sufferers and carry out treatment efforts, it is important for all health stakeholders to know the factors that cause it. As the results of[22]research on the causes of TB at the LubukAlung TB Special Hospital, West Sumatra, it is closely related to the patient's quality of life. The grade of life of pulmonary TB patients at the LubukAlung Special Hospital, West Sumatra, shows that more than half (62.5%) of pulmonary TB sufferers have a poor grade of life.

Quality of life is an indicator factor because quality of life is the main criterion in evaluating the success of health service interventions [23]. Factors that affect quality of life include coping skills with life pressures, identification of new roles, opportunities and availability of social support, demographic, socio-economic factors, cultural and value influences, health factors, self-efficacy, role tension and family burdens, and programs. health training [24].

Factors that can improve the quality of life include self-efficacy. Self-efficacy is part of personality attitudes, which are related to personal beliefs about self-competence and abilities [25]. [26]describe the results of two experimental tests of behavior change self-efficacy theory. Study one investigated the hypothesis that systematic desensitization effects change avoidance behavior by creating and reinforcing personal efficacy expectations. The reduction in total arousal anxiety to threat visualized by desensitization treatment resulted in a marked increase in self-efficacy. As expected, microanalysis of the suitability between self-efficacy and performance revealed self-efficacy to be a very accurate predictor of behavior change assessment after complete desensitization. These findings also support the view that self-efficacy mediates arousal anxiety. The second study investigated the efficacy process and behavior change during treatment with a participant model. Self-efficacy was shown to be a superior predictor of the number of behavioral improvement phobias obtained from mastery of multiple threats at different phases of treatment.

Research conducted by [27-29], regarding the relations between self-efficacy and quality of life of pulmonary tuberculosis patients at RSUP Haji Adam Malik Medan, found that there was a connection between self-efficacy and grade of life. People who own right self-efficacy have a 5,850 times opportunity of showing a better grade of life than people who own poor self-efficacy.

Researchers identified knowledge gaps about the causes of TB, unfitting healthcare-seeking behavior, and the disgrace against TB. Around 83% of TB suspects had heard of TB which is similar to research done in Northern Ethiopia where 86% of research contributors were aware of TB[30]but under than the 99.1% notified from India[31].

A small proportion of people with traditional faith such as animist and religious beliefs, such as Satan and sorcery are the most common causes of TB. This is

not only the case in the Indonesian context. This is the case in Tanzania, a large amount of people as well as mention that magic can be the cause of TB[32]. Freezing air, alcohol, smoking and shortage of sanitation were usual causes felt in different researches[30,32]. These traditional beliefs may have contributed to the dissemination of TB because nearly all people with such beliefs can not have visited health easinesses. A research from Ethiopia indicated that 46% of suffers looking for care in a health easiness did so after informal treatment had failed. In addition, bad suffers though of TB causes such as the "bad eye" were related to long puts off in looking for medical care[33],although it was not statistically significant in this study. In our study, only 33.7% of respondents were aware that TB was caused by microorganisms higher than those found in Vietnam (22%)[34]. People who are literate are more likely to undrstand the causes of TB which are consistent with prior reports[30,32]. Men are more likely to know the causes of TB than women. Bad knowledge among women and uneducated personals about the causes of TB shall lead to unsuitable health care looking for behavior[35].

A large amount of research contributors felt the disgrace of TB on possibility for marriage, community relations and reproduction relations. larger than half of the contributors felt that others will take into account them inferior and a third thought that others will keep away from them because of their diseases. Such perceptions may have a profound effect on the community, psychic and mental well-being of victims and their families. This could have terrible effect in communities where informal social organization plays an important role in the everyday life of personals. TB sufferers may by design hide their position to stay away isolation. They may try to live with it as long as possible, becoming a source of contagion to others. The social isolation of TB patients was also described in Ghana[36] and Nepal [37]. In Ghana, people feel that TB sufferers shall not sell their products in the market. In Nepal, there is a common presumption that healthy persons should not meet someone who has TB and not go to see a home where there is a family member who has TB. In the research, disgrace was not affected by gender and other socio-demographic variables. Many studies however indicated that women have more damage self-esteem, social isolation and disgrace compared to men[38].

The healthcare-seeking conduct of the research contributors was bad. A large number of them do not look for aid for their disease because of wrong understanding and shortage of financial resources, mainly for transportation. Most of them did nothing because they thought the disease was not serious. Alike arguments are addressed in Northwestern Ethiopia[39], Vietnam[34] and China[40]. In this study, health care seeking behavior was not influenced by gender, reading and writing ability, marital status, and knowledge of the causes of TB, information about TB medication, perceptions of disgrace, age, job, or acquaintance with TB patients. But those who have been on anti-TB medications are more likely to take appropriate action for their disease. Alike the findings were reported from another study in Northwestern Ethiopia[39]. In a study from Tanzania, apprehended disgrace was also not included with a specific type of healthcare-seeking conduct[41]but in another research, healthcare-seeking conduct was influenced by understanding[34,42], gender [34, 45] and education [34,46].

In research, the researcher attempted to evaluate several attitude factors connected to knowledge and disgrace against TB in rural communities that could potentially become barriers to the national TB control program. Nevertheless, this study was not without drawbacks. First, the researchers did not conduct focus group discussions to triangulate findings. Second, the disgrace questionnaire was not validated[47].

Accordingly, still there is little knowledge of TB in the society area of TB in many countries. We looked

at inappropriate healthcare-seeking behavior and stigma against TB. Alike to the TB control program in Ethiopia should educate rural communities, especially women and uneducated individuals, about the causes and importance of early diagnosis and treatment of TB [47].

Based on the presurvey at the Lung Poly Hospital, Pertamina Bintang Amin hospital Bandar Lampung, the number of pulmonary TB patients increased from 1,648 patients in 2018 to 1,733 in 2019. Based on the results of interviews with 10 pulmonary TB patients, it was found that 7 people (70%) had a poor quality of life. In addition, as many as 6 people (60%) have personal beliefs about self-efficacy or lack of self-efficacy. The purpose of this study was to describe 1) what were the characteristics of the respondents with pulmonary TB 2) what was the respondent's health condition based on self-efficacy, and 3) how was the relationship between respondents with pulmonary tuberculosis and self-efficacy, Lung at the hospital Pertamina Bintang Amin Bandar Lampung in 2020?

Everyone tries to contribute to life. However, to be able to contribute requires good self-condition. In fact, his health is supported by many factors. One of them is self-efficacy. Self-efficacy is very effective in overcoming various problems, including health. Curing a disease is not enough only from medical factors but also non-medical factors: self-efficacy. For this reason, the treatment of the patient's disease must also be accompanied by an increase in the patient's self-efficacy. Many patients are afraid of pre-treatment programs, such as what happened to women with cervical cancer. However, several factors need to be controlled in a self-efficacy improvement program including considering their region of origin, background, and focus on giving women confidence to overcome barriers to cervical cancer screening [48, 49].

Additionally, two studies reported their findings from two experimental tests of behavior change self-efficacy theory. The first study concluded that the reduction in anxiety was due to overall sensitization of the threat of increased self-efficacy. Microanalysis becomes a very accurate predictor of the rate of behavior change after complete desensitization of self-efficacy and performance. These findings also support the view that self-efficacy mediates anxiety. The second trial investigated the process of efficacy and behavior change during treatment. The conclusion is that self-efficacy has been shown to be a superior predictor of increased phobic behavior obtained from mastery of multiple threats at various phases of treatment [26]. Thus we conclude that self-efficacy plays a major role in survival and management of all the factors that make poor health conditions better.

In order for this research to be focused, the researcher asks research questions as a guide, namely, 1) what are the characteristics of the respondent? 2) What is the respondent's health condition based on self-efficacy? 3) How is the relationship between respondents with pulmonary tuberculosis and self-efficacy?

## Research Methods

This study used a cross sectional study design. The population in this study were all patients with pulmonary TB and visited the hospital. Pertamina Bintang Amin Bandar Lampung in 2020 with a sample of 62. Data collection used a questionnaire. The statistical test used is the Chi Square test using the SPSS version 25.0 application and data processing uses the SPSS version 20 application.

## Research Result

### 1. Characteristics of Respondents

**Table 1.Frequency Distribution of Respondents by Gender, Age, Education, Occupation**

| Respondent Characteristics | Amount | Percentage |
|---|---|---|
| Gender | | |
| Man | 34 | 54,8 |
| Women | 28 | 45,2 |
| Age | | |
| <20 | 8 | 12,9 |
| 20-45 | 36 | 58,1 |
| 45-60 | 18 | 29,0 |
| Education | | |
| Primary school | 5 | 8,1 |
| Junior High | 31 | 50,0 |
| High school | 24 | 38,7 |
| College | 2 | 3,2 |
| Profession | | |
| Labor | 18 | 29,0 |
| Private employees | 4 | 6,5 |
| Farmer | 10 | 16,1 |
| Civil servants | 1 | 1,6 |
| Jobless | 16 | 25,8 |
| Entrepreneur | 13 | 21,0 |
| Total | 62 | 100,0 |

Based on table 1, it is known that most of the respondents were male, as many as 34 people (54.8%). Most of the respondents were 20-45 years old, as many as 36 people (58.1%), most of the respondents had junior high school education, as many as 31 people (50%), and most of the respondents worked as

entrepreneurs, namely 18 people ( 29%).

## 2. Univariate Analysis

**Table 2. Frequency Distribution of Respondents based on self-efficacy and quality of life**

| Self-Efficacy | Total | Percentage |
|---|---|---|
| Low | 24 | 38,7 |
| Moderate | 27 | 43,5 |
| High | 11 | 17,7 |
| Quality of Life | | |
| Not good | 33 | 53,2 |
| Good | 29 | 46,8 |
| Total | 62 | 100,0 |

Based on table 2, it is known that most of the respondents' self-efficacy was in poor condition, namely as many as 33 people (53.2%) and the quality of life of the respondents was mostly in the moderate category, namely as many as 27 people (43.5%).

## 3. Bivariate Analysis

Authors present the relationship between Self Efficacy and Quality of Life for Patients with Pulmonary TB as shown in table 3.

**Table 3. Relationship between Self Efficacy and Quality of Life for Patients with Pulmonary TB**

| Self-Efficacy | Quality of Life | | | | | | Total | | Pvalue |
|---|---|---|---|---|---|---|---|---|---|
| | Low | | Medium | | High | | | | |
| | n | % | n | % | N | % | N | % | |
| Not good | 19 | 57,6 | 12 | 36,4 | 2 | 6,0 | 33 | 100 | 0,002 |
| Good | 5 | 17,2 | 15 | 51,7 | 9 | 31,1 | 29 | 100 | |
| Total | 24 | 38,8 | 27 | 43,5 | 11 | 17,6 | 62 | 100 | |

The results showed that respondents with self-efficacy were in the poor category, as many as 19 people (57.6%) had a low quality of life, 12 people (36.4%) had a moderate quality of life, and 2 people (6.0%) had a moderate quality of life. high quality of life. In addition, respondents with self-efficacy were in the good category, as many as 5 people (17.2%) had a low quality of life, 15 people (51.7%) had a moderate quality of life, and 9 people (31.1%) had a high quality of life. high life. Chi square test results, obtained p value = 0.001, so that the p value <α (0.001 <0.05), then Ha is accepted. So it can be concluded that there is a relationship between self-efficacy and quality of life for patients with pulmonary tuberculosis in the hospital Pertamina Bintang Amin Bandar Lampung in 2020.

## Discussion

With regard to research question 1, what are the characteristics of the respondents?

Based on table 1, it is known that most of the respondents were male, as many as 34 people (54.8%). Most of the respondents were 20-45 years old, as many as 36 people (58.1%), most of the respondents had junior high school education, as many as 31 people (50%), and most of the respondents worked as heads of households with irregular income. , namely as many as 18 people (29%).

As many as 54.84% of male sufferers described that in a patrilineal setting where men are tasked with earning a living, it means that men work outside the home. This research contradicts the results of research which states that most of the cadres are: women; the highest age group is 21-45 years[50]. With regard to men who work as breadwinners, this situation is very dangerous because it relates to the condition of family welfare. Family in Indonesia currently has two children, so there are approximately 102 people who live with economic disabilities and are under threat of pulmonary TB virus. Even worse, the sufferer is at a very productive period, at the age of 20-45 years. This situation can predict Indonesia's gloomy future [51].

It seems that education is an important factor in improving public health conditions. This can be seen from the data which shows that 58.1% have a junior high school education. This result is in line with research conducted even though it was carried out at one level above it, namely a high school graduate[50]. From the results of these studies, schools need to be encouraged to campaign for a healthy lifestyle to avoid pulmonary TB infection. Educational factors are in fact directly proportional to the economic ability of the sufferer.

With regard to research question 2) what is the respondent's health condition based on self-efficacy?

The results of this study are in accordance with the theory put forward by [25], self-efficacy is a person's belief in their ability to organize and carry out the actions needed to achieve goals. In other words, people who have strong efficacy believe that they are more confident in their capacity to carry out a behavior. Beliefs about self-efficacy have a significant impact on goals and achievement by influencing personal choices, motivations, and emotional patterns and reactions. Sources of self-efficacy include prior experience (prior experience). Previous experiences are previous successful experiences that will happen over and over again. It is seen as a very effective way of developing a strong sense of self-efficacy.

According to research, most of the respondents' self-efficacy was inadequate due to the lack of self-confidence of respondents about their ability to organize and carry out actions in order to achieve recovery from the pulmonary tuberculosis. Self-efficacy can be influenced by age where the higher the age, the more experience. Then self-efficacy is also influenced by the environment, where the support

system in the family environment will increase one's self-confidence.

With regard to research question 3) how is the relationship between the responses of pulmonary TB patients to self-efficacy?

The outcomes of this research are in accordance with the thesis proposed by[52], pulmonary tuberculosis is an contagious disease induced by Mycobacterium tuberculosis which strikes the lungs and almost all other biological structurs of the body. These bacteria can enter through the respiratory area and digestive area (GI) and open wounds on the skin. But mostly through respiration of droplets that come from people who are contaminated with these bacteria. Pulmonary tuberculosis, which is one of the chronic diseases, will certainly have an influence on the quality of life of the sufferer. Ekasari, Riasmini [24], that the quality of life is an individual's thought of their life in society in the context of existing culture and value oders related to goals, expectations, standards, and also attention. Quality of life in this case is a very broad concept which is influenced by the physical condition of the individual, psychologically, and the level of independence. as well as individual relationships with the surroundings.

The outcomes of this research are in line with [22] research on factors related to the quality of life of pulmonary TB patients at the Lubuk Alung Special Hospital, West Sumatra, showing that 62.5% of pulmonary TB patients have a poor quality of life.

According to the researchers, most of the respondents' quality of life was in the moderate category because not all respondents who had pulmonary tuberculosis had decreased their quality of life. The physical, psychological, and level of independence of each individual will differ from one another, where most are still able to live their lives in a moderate quality due to the chronic course of pulmonary tuberculosis. Based on the answers to the questions posed through the questionnaire, it is stated that the quality of life of the respondents is that there are still many respondents who are not satisfied in enjoying their daily lives due to pulmonary tuberculosis.

The outcomes of this research are in accordance with the opinion expressed by self-efficacy, which refers to an individual's belief that he is capable of carrying out a task. The higher the self-efficacy, the more confident a person will be in his ability to succeed. Thus, people with low efficacy were more likely to reduce efforts or give up. Meanwhile, people with high self-efficacy will try harder to master challenges. Self-efficacy can create positive energy to engage in their tasks which will increase efforts and motivation to achieve goals.

In addition, according to [53]; In [23], an increase in the quality of life also occurs in clients who are given mentoring treatment such as psycho education or social support as a basis for increasing the self-efficacy of clients who are undergoing treatment and need routine control which takes up a lot of time. Self-efficacy can be maximized if the client is willing to accept the illness and carry out therapy regularly so that it can improve the client's standard of life.

The outcomes of this research are in line with the research conducted by[29], regarding the relationship between self-efficacy and quality of life for pulmonary tuberculosis patients at Haji Adam Malik General Hospital Medan. It was proved that there was a relations between self-efficacy and quality of life (p-value = 0.016 (p <0). , 05), OR = 5.850 (95% CI = 1.554-22.023).

Accordingly, there is a relationship between self-efficacy and quality of life for pulmonary tuberculosis patients because self-efficacy is one of the factors affecting the quality of life of respondents

who experience pulmonary tuberculosis where the higher the level of self-efficacy, a person will be more confident in his ability to carry out the recommendations in achieve healing. Conversely, respondents with low efficacy are more likely to reduce efforts or give up. This is consistent with the results of the study where respondents with self-efficacy in the poor category tended to have a low quality of life (57.6%), while respondents with self-efficacy in the good category had a moderate quality of life (51.7%). Self-efficacy will create positive energy for respondents in undergoing medical therapy so that it will motivate them to make treatment efforts. For this reason, health education and support systems are needed, especially from families in order to increase self-efficacy in patients with pulmonary tuberculosis in undergoing routine treatment and control so that with confidence in themselves that patients are able to carry out regular therapy will be able to improve the standard of life of these patients.

## Conclusion

Based on the exposure, this study presents the conclusion that 1) demographic factors such as the type of education level are an indicator of the potential vulnerability of pulmonary TB infection, 2) Most of the respondents' self-efficacy was in poor condition, namely 33 people (53.2%). Most of the respondents' quality of life was in the medium category, namely 27 people (43.5%), and 3) there was a relationship between self-efficacy and the quality of life of patients with pulmonary tuberculosis.

### Suggestion

1. It is hoped that health workers will intervene in improving the quality of life of patients with pulmonary tuberculosis by carrying out health education and also providing family education as a support system in order to increase self-efficacy in patients with pulmonary tuberculosis health education and support systems, especially from families in in order to improve self-efficacy in patients with pulmonary tuberculosis. As a matter of consideration for the health office with regard to efforts to improve the quality of life of pulmonary TB patients by providing counseling on self-efficacy in pulmonary TB patients.

2. It is hoped that the respondent will add information, insight and knowledge to patients with pulmonary tuberculosis about the importance of self-efficacy in healing the disease so that it can improve the quality of life of pulmonary TB sufferers who are under treatment.

3. For this reason, it is suggested that this study be used as a reference for further research, and it is hoped that further researchers can expand the scope of research or examine other factors not examined in this study.

**Ethical Clearance :** The researcher conducted an ethical test has been carried out with the number No. 1288 / EC / KEP-UNIMAL / XII / 2020

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# Ultrasound Assessment for Thyroid Examination in Patients with Hypothyroidism

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## Abstract

Patients with overt hypothyroidism show decreased echogenicity of the thyroid at ultrasonography **.** The purpose of the present study was to evaluate the effectiveness of ultrasonography for diagnosing the most common hypothyroidism and to guide patients to appropriate treatment show decreased echogenicity of the thyroid by ultrasonography. This study have been conducted on 80Patients, the age group between 20-70 years, in the period of January 2020 to October 2021. Thyroid ultrasonography was carried out by the type of ultrasound (US) machine used in the study is a sameness -X300 ultrasound device with 7.5MHz probe. The volume of the thyroid lobe noticed calculations according to the formula of the: width x length x thickness. The assessment of the 80 patients with hypothyroidism who were included in our study, (57) patients with decreased echogenicity, 23 patients had been with normal echogenicity, the lowest was found in patients homogenous enhancement 42.5%, non- homogenous 57.5%, thyroid the gland has a medium gray scale homogeneous echo pattern and the level of echogenicity is higher than Size of thyroid, The higher percentage of nodules (51.3%) of the size of the thyroid was normal. But the higher percentage of nodules type (40.0%) for the study group was solid compared to the lower percentage (2.5%) was solid and cystic. Ultrasound had high specificity and more accuracy to assess patients with hypothyroidism. An association between hypo echogenicity at thyroid US and high level of thyroid stimulation hormone (TSH suggesting decreased echogenicity at the ultrasonography diagnostic**.**

***Keywords:*** *Hypothyroidism, Ultrasound thyroid, TSH, Central hypothyroidism*

## Introduction

Hypothyroidism is the most widely recognized thyroid problem, influences ladies often as more as possible, and the frequency increments with age [(1)] brings about low degrees of thyroid chemical [(2)] Hypothyroidism is described by an inadequacy in the T4 and T3 chemicals [(3-4)]. There are essentially two kinds of hypothyroidism, essential hypothyroidism, which is brought about by thyroid organ infection itself [(5)] Primary hypothyroidism is characterized as high serum TSH levels with typical or decreased free thyroxin (FT4) levels [(6)] . The most predominant etiology of essential hypothyroidism is iodine insufficiency [(7)] Hypothyroidism is named essential when there is low thyroid organ movement; it

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represents over 90% of the cases. Focal hypothyroidism addresses under 1% of the cases [8] and is brought about by either low degrees of TSH emission by the front pituitary (optional hypothyroidism) or low degrees of thyrotropin-delivering chemical (TRH) discharge by the hypothalamus [9]. become ultrasound the main imaging strategy utilized for thyroid infections [10] . The thyroid organ is unmistakably appropriate for high-recurrence sonography (utilizing 7-15 MHz transducer) which works with the recognition of clinically non-obvious knobs of 2-3 mm size and permits a more precise portrayal of the. It is likewise used to decide the size and numbers of thyroid knobs evaluate the volume of tissue in thyromegaly cases, and separate thyroid masses from adjoining [11]. the diminished echogenicity of the thyroid organ is related to plain hypothyroidism. Echogenicity has been shown hypothyroidism. Diminished echogenicity or inconsistency in the reverberation design during a thyroid in patients with raised thyroid-invigorating chemical (TSH) could be taken as early indications of thyroid disappointment [12]. US Characteristics of the Thyroid Gland [13] . Examination of the accuracy of ultrasonography to evaluate ultrasound discoveries of patients with hypothyroidism.

## Patients and Methods

The present study had been executed between October 2020 and February 2021, eighty patients, with age group (20–70) years; diagnosed with hypothyroidism by ultrasound the radiology department in Baghdad medical city/ institute of radiology, patients, present with a variety of symptoms include (Swelling of the thyroid gland, swelling, difficulty swallowing Hoarseness presence .patient has a thyroid-stimulating hormone (TSH) test which was high more than (0.4-4.2 ulU/ml), which indicated the patient has hypothyroidism. A thyroid ultrasound is performed to determine the size and shape detect abnormalities of the thyroid such as enlargement, hypothyroidism. Ultrasound examination in supine position: The patient lies down with his or her neck on a pillow or other soft support. His or her neck is stretched slightly. The ultrasound technician applies a water-based gel on your neck to help transmit the sound waves. Next, the technician moves a wand, called a transducer, back and forth on the skin of his or her neck. The system was used sameness -X300 ultrasound device with 7.5MHz that is found in the Institute of X-Ray in medical city/ Baghdad. The device had a thyroid probe of about 5–7 MHz Axial scans of the whole gal of each lobe, to compare echogenicity and ND at the upper AP diameters is measured. The size of both lobes as normal amassment is (50 mm Length, 30mm width, 20mm depth). Texture, either homogenous or non-homogenous contacting nodules or not if nodules Echogenicity either normal slightly echogenic or hypo echoic which mean decrease in echogenicity. Identify focal lesions, measure the main lesions and identify the dominant one (according to size). –sizes and texture (solid, cystic, or complex) found at right or left lobes and their number either few <4 nodules, multiple>4 nodules. After y ultrasound, you can resume your normal activities.

## Results

The results of the present study that were explained in the table (1) showed the distribution study group according to, gender, which showed that the patient with hypothyroidism was male 10(12.5) which less than70 females (87.5%)

**Table (1) : The distribution of study group according to gender**

| | | No.(n=80) | %( 100%) |
|---|---|---|---|
| Gender | Male | 10 | 12.5 |
| | Female | 70 | 87.5 |

Table (2) represents the distribution of study group according to Size of thyroid, Nodules (Type, lobe and Number) .The higher percentage (51.3%) of size of thyroid was normal. But the higher percentage of nodules type (40.0%) for study group was solid compared to lower percentage (2.5%) was solid and cystic. The higher percentage (38.8%) non nodules lope that followed by 26.3% was right lobe compared to lower percentage was (10.0%) for Left lobe. The higher percentage of cement mantle was (90.0%) was compared to lower percentage was (10.0%) was 2-3m. The higher percentage of number nodules was (45.0%) have a few nodules compared to lower percentage was (15.0%) with multiple nodules.

In table (3) show the distribution of nodules for study group by echogenicity. The higher percentage (28.8%) was solid for decrease echogenicity cases compared with 11.3% of echogenicity cases, while lower percentage of nodules was solid and cystic among decrease echogenicity cases and echogenicity cases. The association between size of thyroid and echogenicity was significantly (P=0.040).

Table (4) represented the distribution of number nodules for study group by echogenicity. The higher percentage (31.3%) of cases was few nodules for decrease echogenicity cases compared with 13.8% of normal cases. The lower percentage (11.3%) with multiple nodules was decrease echogenicity cases compared with 3.8% of normal cases. The association between number nodules and echogenicity was significantly (P=0.047).

**Table (2): Distribution of study group according to Size of thyroid, Nodules (Type, lope and Number)**

| | | No.(n=80) | %( 100%) |
|---|---|---|---|
| Size of thyroid | Normal | 41 | 51.3 |
| | Large | 28 | 35.0 |
| | Small | 11 | 13.8 |
| Nodules Type | Non | 31 | 38.8 |
| | Solid | 32 | 40.0 |
| | Cystic | 9 | 11.3 |
| | Solid &Cystic | 2 | 2.5 |
| | Cystic &Complex | 6 | 7.5 |
| Nodules lobe | Non | 31 | 38.8 |
| | Right lope | 21 | 26.3 |
| | Left lope | 8 | 10.0 |
| | Right lope& Left lope | 20 | 25.0 |
| Number of nodules | Non | 32 | 40.0 |
| | Few nodules | 36 | 45.0 |
| | Multiple nodules | 12 | 15.0 |

**Table (3) : The distribution of nodules for study group by echogenicity.**

| Nodules | | Texture and homogenous | | Total |
|---|---|---|---|---|
| | | Echogenicity | Decrease Echogenicity | |
| Non | No. | 9 | 22 | 31 |
| | % | 11.3% | 27.5% | 38.8% |
| Solid | No. | 9 | 23 | 32 |
| | % | 11.3% | 28.8% | 40.0% |
| Cystic | No. | 2 | 7 | 9 |
| | % | 2.5% | 8.8% | 11.3% |
| Solid &Cystic | No. | 0 | 2 | 2 |
| | % | 0.0% | 2.5% | 2.5% |
| Cystic &Complex | No. | 3 | 3 | 6 |
| | % | 3.8% | 3.8% | 7.5% |
| Total | No. | 23 | 57 | 80 |
| | % | 28.8% | 71.3% | 100.0% |

(Kappa P=0.040 (P≤0.05) S)

**Table (4): Distribution of Number nodules for study group by Echogenicity**

| Number nodules | | Echogenicity | | Total |
|---|---|---|---|---|
| | | Normal | Decrease Echogenicity | |
| Non | No. | 9 | 23 | 32 |
| | % | 11.3% | 28.8% | 40.0% |
| Few nodules | No. | 11 | 25 | 36 |
| | % | 13.8% | 31.3% | 45.0% |
| Multiple nodules | No. | 3 | 9 | 12 |
| | % | 3.8% | 11.3% | 15.0% |
| Total | No. | 23 | 57 | 80 |
| | % | 28.8% | 71.3% | 100.0% |

(Kappa P=0.047 (P<0.05) S)

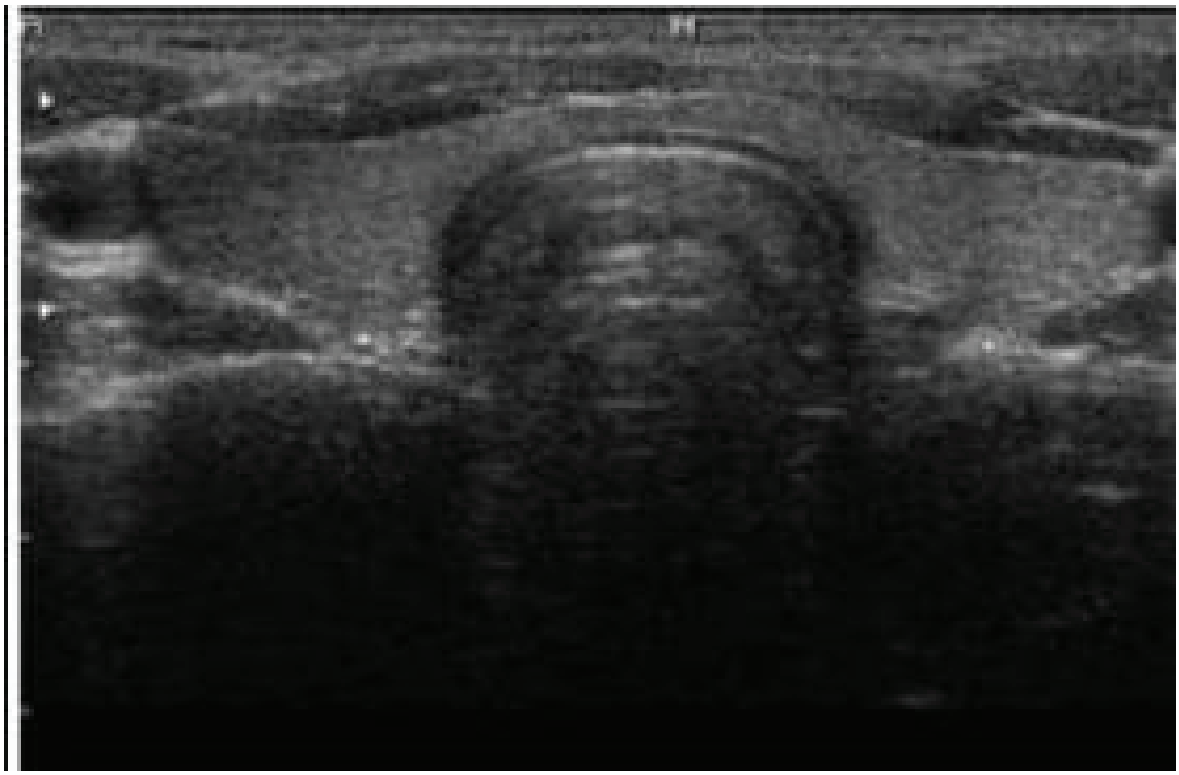

**Figure (2) Ultrasound examination of the Hypothyroidism a 29-year-old woman showing homogeneous in texture.**

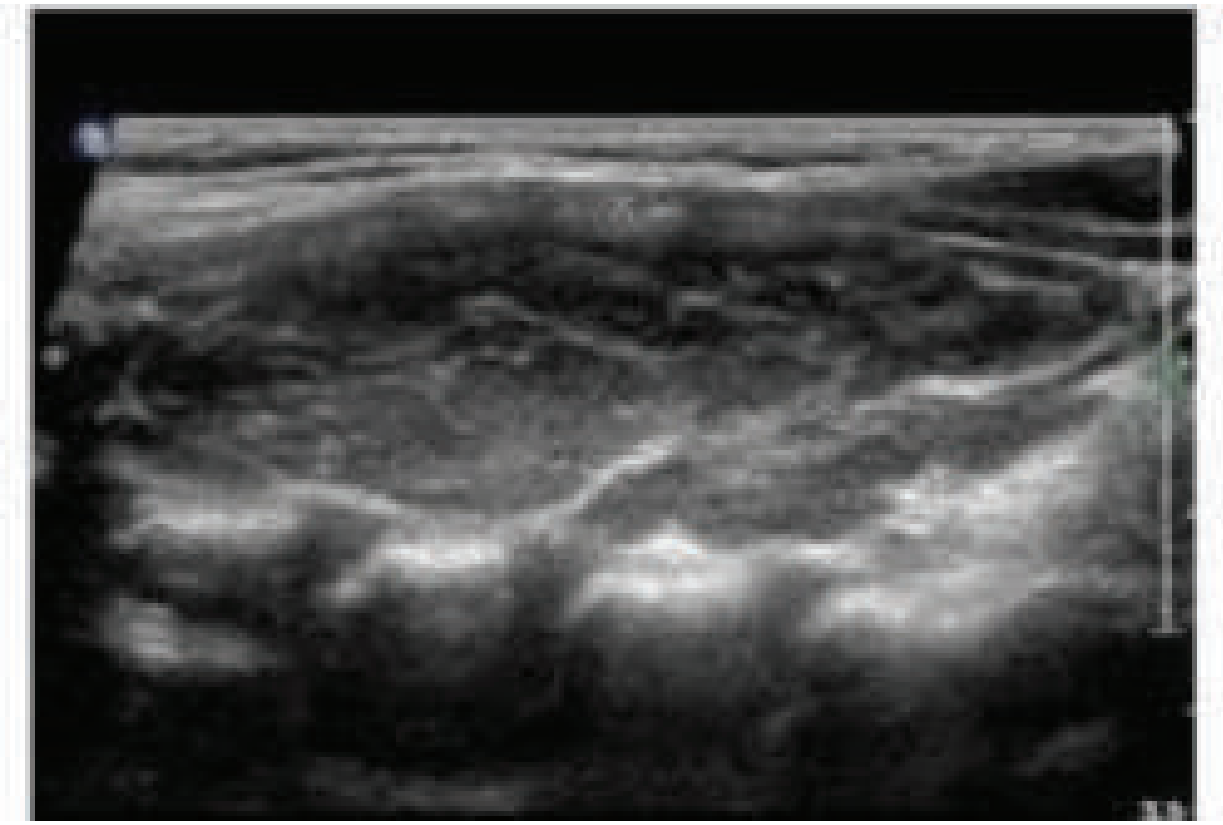

**Figure (3) Ultrasound examination of the of Hypothyroidism a 45-year-old woman showing non homogeneous in texture**

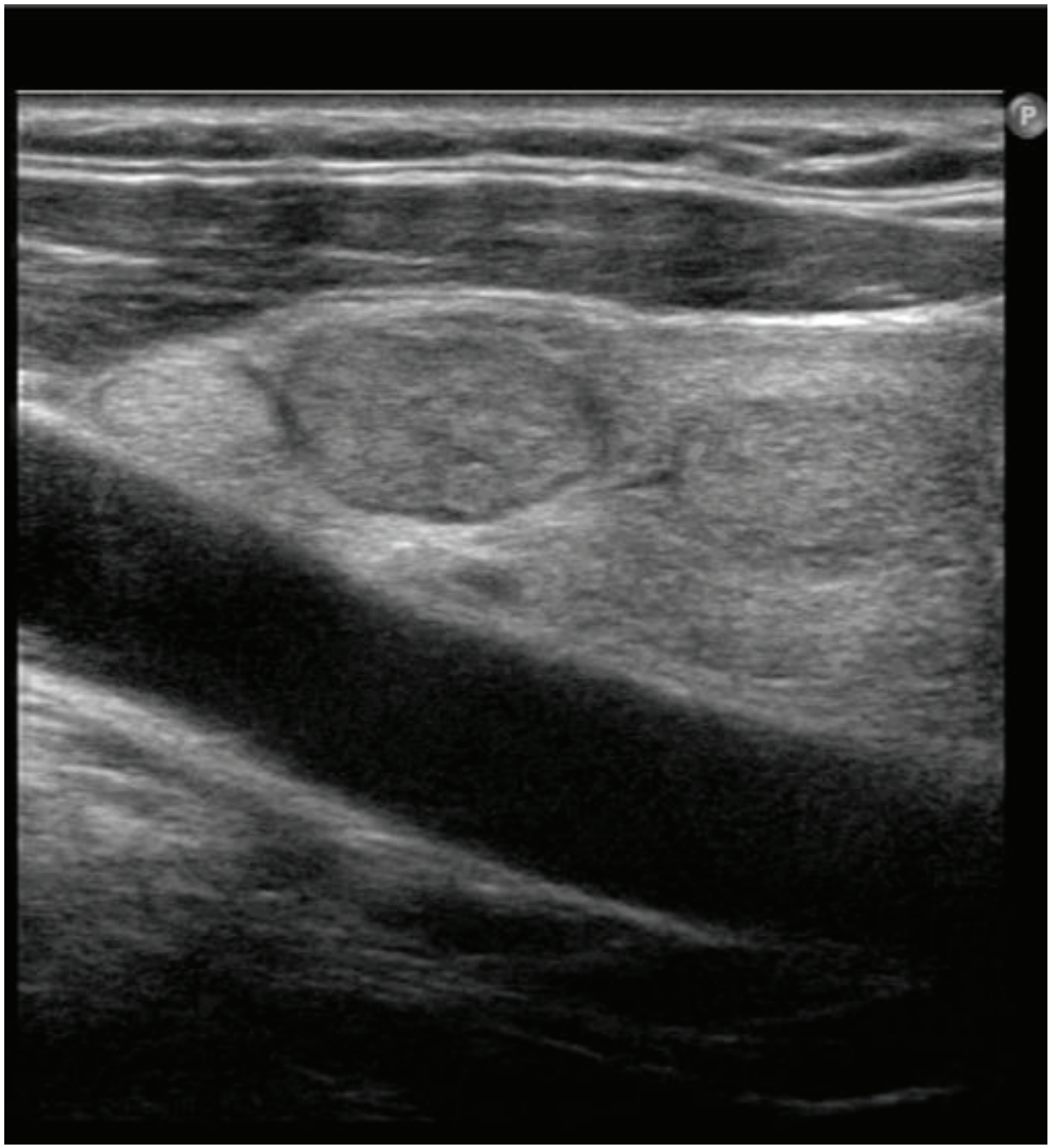

**Figure (4) benign hypo echoic, well-circumscribed solid thyroid nodule with peripheral "halo" in the right lobe show typical benign patterns in ultrasound-mode**

## Discussion

The study was achieved on 80 patients who had clinically hypothyroidism who were primarily investigated with U/S. Age patients ranged from 20 to 70 years; In the investigation, we surveyed the capacity of thyroid ultrasound to distinguish hypothyroidism the best larger part of patients; were female which exhibited that a huge level of patients were ladies 70 (87.5%) in examination with male 10(12.5%). the predominance of hypothyroidism was more normal among ladies than men. These outcomes were likewise in concurrence with an investigation acted in the United Kingdom [(14)], which showed that the female: male proportion was 6:1. Another examination [(15)], shown in table 1 our study found

an association between the decreased echogenicity of the thyroid and overt hypothyroidism. Were included in (57) patients with decreased echogenicity, 23 been with normal echogenicity and all of the patients had a high TSH, and results were correlated with homogeneity. Normal echogenicity the lowest was found in patients Thyroid Texture homogenous 42.5%, Non- homogenous 57.5%, our study found an association between the decreased echogenicity of the thyroid and overt hypothyroidism. Were included in (57) patients with decreased echogenicity, 23 been with normal echogenicity and all of the patients had a high TSH, and results were correlated with homogeneity. been with normal echogenicity the lowest was found in patients Homogenous enhancement 42.5%, Non- homogenous 57.5%, the thyroid gland has a medium grayscale homogeneous echo pattern and the level of the echogenicity thyroid gland has a medium grayscale homogeneous echo pattern and the level of echogenicity is higher than Size of thyroid, showed an association study between low echo the thyroid gland and hypothyroidism In the current study y group according to Echogenicity. The higher percentage was (71.25 %) of Decrease Echogenicity compared to a lower percentage (28.75%) was echogenicity Ultrasound assessed thyroid nodules, types, and sizes, and the results were that the highest percentage of nodules (51.3%) were normal and (40.0%)were solid compared with the lowest ratio of (2.5%)whose composition was cystic solid and the percentage of nodules increased with age . The study included the type of thyroid nodules with echo the higher percentage (28.8%) was solid for decrease echogenicity cases compared with 11.3% of echogenicity cases, while a lower percentage of nodules were solid and cystic among decrease echogenicity cases and echogenicity cases. The association between the size of the thyroid and echogenicity was significant (P=0.040). of number nodules for the study group by echogenicity. The higher percentage (31.3%) of cases was few nodules for decrease echogenicity cases compared with 13.8% of normal cases. The lower percentage (11.3%) with multiple nodules was decreased echogenicity cases compared with 3.8% of normal cases. The association between number nodules and echogenicity was significant (P=0.047).

## Conclusion

In the US, (71.25 %) of decrease echogenicity (28.75%) was echogenicity there 35.0% were of large thyroid and of Small thyroid. US reports showed that 40.0 % cases solid nodules, 11.3% of cases cystic, but 7.5% cystic and complex. The thyroid gland hypo-echogenicity and heterogeneity constitute findings of great importance in the diagnosis when correlated with hypothyroidism. And thyroid ultrasonography is useful to detect hypothyroidism there was an association between decreased echogenicity and thyroid dysfunction and high TSH levels, even in subjects with normal thyroid function, or subclinical or overt hypothyroidism.

**Ethical Clearance**: Taken from Middle Technical University ethical committee

**Source of Funding** : Self

**Conflict of Interest** : Nil

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# Alpha-Mangostin and Gamma-Mangostin Isolated from Mangosteen (*Garcinia mangostana* L.) as Promising Candidates against SARS-CoV-2: A Bioinformatics Approach

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## Abstract

The world is endangered by the COVID-19 pandemic caused by SARS-CoV-2, people are dying in thousands every day, and without an actual treatment, it seems that bringing this global health problem to a quit is impossible. Natural products have been in constant use since ancient times and are proven by time to be effective. Medicinal plants from Indonesia may lead to the discovery of the novel drugs. Mangosteen or *Garcinia mangostana* L. is a native tropical fruit from Southeast Asia and is known to contain bioactive compounds. Interestingly, the main xanthone derivatives are alpha-mangostin and gamma-mangostin, these compounds have a variety of pharmacological activities such as antiviral activity. In summary, this study showed potential pharmacological benefits of alpha-mangostin and gamma-mangostin isolated from mangosteen against SARS-CoV-2. Thus, mangosteen exhibits as a valuable plant and a candidate for future drug development to fight SARS-CoV-2. However, further trials, such as *in vitro* and *in vivo* evaluation, are needed to prove the validity of these findings.

***Keywords:*** *Alpha-mangostin, COVID-19, Gamma-mangostin, Garcinia mangostana L., SARS-CoV-2.*

## Introduction

Indonesia is covered by many vegetations, including tropical rain forests. In addition, Indonesia is one of the top five countries in the world that has high plant diversity, including approximately 6,000 medicinal plants[1,2,3,4,5]. Consequently, Indonesia is rich in medicinal plants used by its population in curing many diseases. On the other hand, there has been around 92 million people globally who have been infected by SARS-CoV-2 (the causative agent of COVID-19) and more than 2 million deaths as the fast result of this pandemic. In Indonesia, there are more than one million cases and more than 25,000 deaths. Data was retrieved from Johns Hopkins University online website that tracks COVID-19 cases in real-time[6].

Mangosteen or *Garcinia mangostana* L. is appertain to the family of Clusiaceae and genus *Garcinia*. *Garcinia* is a large genus which consists of around 400 species originated from East India and Southeast Asia, including Indonesia[7]. Pratiwi *et al*. stated that the mangosteen production centers in Java are Blitar, Purwakarta, Bogor, Banyuwangi, Subang, Ciamis, Sukabumi, Cilacap, Purworejo, and Banjarnegara[8]. Moreover, based on the morphological and cytological studies, it can be suggested that mangosteen originates from Southeast Asia. As a matter of fact, mangosteen is a plant that has been

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used as traditional medicine for hundreds of years worldwide[9].

Mangosteen contains bioactive compounds such as xanthones, tannins, and some vitamins. In fact, mangosteen's pericarp has many important benefits for health. The main compounds in the content of mangosteen's pericarp are xanthones; such as alpha-mangostin, gamma-mangostin, beta-mangostin, and so on. The main xanthone derivative, such as alpha-mangostin and gamma-mangostin, have a variety of pharmacological activities such as antiviral activity[10,11,12].

## Materials and Methods

### Data retrieval

We extracted phytocomponents of mangosteen from PubChem, an open chemistry database at the National Institutes of Health (NIH), USA. We revealed the Canonical SMILES of alpha-mangostin and gamma-mangostin and submitted them to the SwissADME web server for further analysis.

**Table 1. Alpha-mangostin and gamma-mangostin revealed from the PubChem database.**

| Compounds | Formula | Molecular Weight | IUPAC Name | Canonical SMILES |
|---|---|---|---|---|
| Alpha-mangostin | $C_{24}H_{26}O_6$ | 410.46 g/mol | 1,3,6-trihydroxy-7-methoxy-2,8-bis(3-methylbut-2-enyl) xanthen-9-one | CC(=CCC1=C(C2=C(C=C1O)OC3=C(C2=O)C(=C(C(=C3)O)OC)CC=C(C)C)O)C |
| Gamma-mangostin | $C_{23}H_{24}O_6$ | 396.43 g/mol | 1,3,6,7-tetrahydroxy-2,8-bis(3-methylbut-2-enyl)xanthen-9-one | CC(=CCC1=C(C2=C(C=C1O)OC3=C(C2=O)C(=C(C(=C3)O)O)CC=C(C)C)O)C |

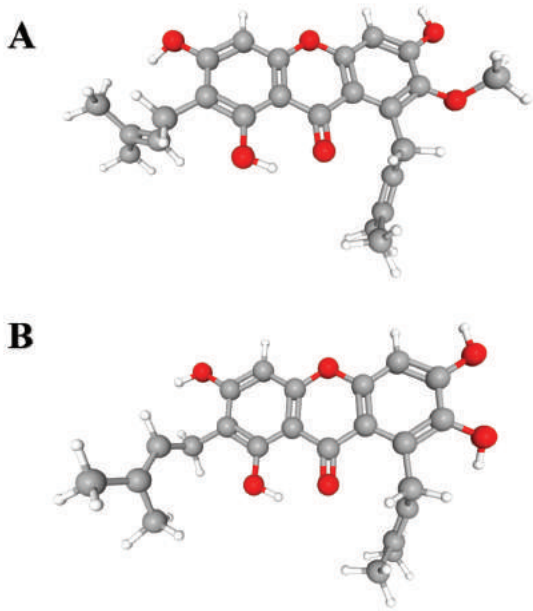


**Figure 1. Chemical structures of alpha-mangostin (A) and gamma-mangostin (B) isolated from mangosteen.**

Pharmacokinetics and drug-likeness predictions

In the present study, we predicted the pharmacokinetic properties and druglike nature of the phytocomponents using the SwissADME web server and identified gastrointestinal absorption prediction for oral drug probability[13] and Lipinski parameter for the drug-likeness prediction based on Lipinski *et al*.[14]

**Biological activity prediction**

We performed PASS (Prediction of Activity Spectra for Substances) web resource as a strong potential tool to predict the biological activity. This web resource estimates the predicted activity spectrum of a compound as probable activity (Pa)[13].

## Results and Discussion

We successfully revealed pharmacokinetics, drug-likeness, biological activity predictions of alpha-mangostin and gamma-mangostin from mangosteen as presented in Table 2 and Figure 2. In addition, phytochemical screening, based on ethnomedicinal data, is considered as an effective approach for the discovery of new therapeutic agents. The major bioactive secondary metabolites of mangosteen are xanthone derivatives. The major constituents from the xanthone fraction in mangosteen were found to be alpha-mangostin and gamma-mangostin. More than 60 other xanthones were isolated from its different plant parts, including 3-isomangostin, β-mangostin, gartanin, mangostanin, 1-isomangostin, garcinone B, 9-hydroxycalabaxanthone, mangostanol, mangostinone demethylcalabaxanthone, 8-deoxygartanin, and garcinone D[1,10]. The majority of investigations are focused on the extraction and structure elucidation of xanthones from the pericarp of mangosteen. Recently, the presence of these compounds in the stem, seed, and heartwood was reported by many researchers[2,15].

**Table 2. Pharmacokinetics, drug-likeness, biological activity predictions of alpha-mangostin and gamma-mangostin.**

| Compound | Pharmacokinetics Prediction (Gastrointestinal Absorption) | Drug-likeness Prediction (Lipinski) | Antiviral Activity Prediction |
|---|---|---|---|
| Alpha-mangostin | High | Yes | Herpesvirus (0.423) and rhinovirus (0.390) |
| Gamma-mangostin | High | Yes | Herpesvirus (0.453), rhinovirus (0.393), picornavirus (0.311), influenza (0.267), cytomegalovirus or CMV (0.244), hepatitis B (0.235), poxvirus (0.231), and HIV (0.191) |

Mangosteen is an important medicinal plant in traditional medication system. Studies of mangosteen's pharmacological properties has started since the 1990's. Mangosteen is an important medicinal plant in the family of Clusiaceae. In the recent history, this plant is reported for its various medicinal properties. In Asia, the pericarp of mangosteen is used as antimicrobial, antiparasitic

agents, and for wound healing. The pericarp decoction of mangosteen is administered to relieve gonorrhea and diarrhea. Mangosteen stem bark and leaves are recognized to have anti-inflammatory properties for many skin disorders. In the Philippines, leaves and bark is adopted as a medication for diarrhea and various urinary problems. In Thai traditional medicine, the pericarp is used as the medication of skin infections and wounds. In addition, mangosteen root stew is used by women to treat menstrual disorders. Moreover, mangosteen has also been used for medical purposes in Caribbean and Latin America, for example as a digestive aid in Brazil[16]. Traditional medicinal properties of mangosteen are employed for hemorrhoids, tuberculosis, mycosis, fever, abdominal pain, leucorrhoea, and convulsants[10].

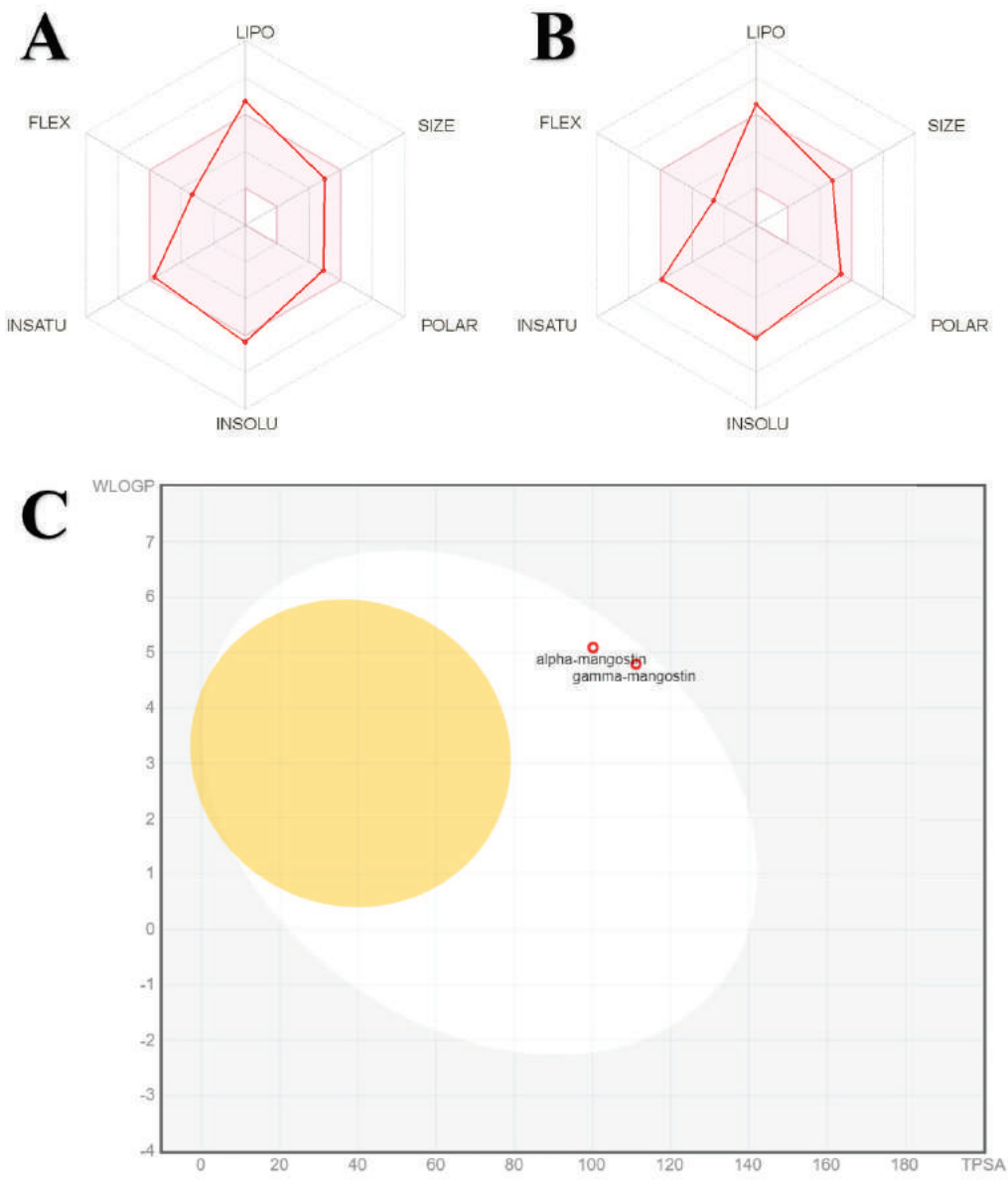


**Figure 2. Radar-like representation of the drug-likeness of alpha-mangostin (A) and gamma-mangostin (B) predicted by SwissADME web server. BOILED-Egg plot to globally estimate their gastrointestinal absorption and brain penetration, two major ADME behaviors impacting pharmacokinetics (C).**

In addition, alpha-mangostin and gamma-mangostin from mangosteen inhibited HIV-1 with $IC_{50}$ values of 5.1 and 4.8 µM, respectively[17]. Vlietinck *et al*. discovered the role of α-mangostin as a non-competitive inhibitor of HIV-1 protease by inhibiting the HIV virus replication cycle[18]. Patil *et al*. performed *in vitro* and *in vivo* studies, and revealed that α-mangostin, a xanthonoid from *Garcinia mangostana*, is a promising natural antiviral compound against chikungunya virus[19]. Moreover, a study by Tarasuk *et al*. stated that alpha-mangostin inhibits both dengue virus production and cytokine/chemokine expression[20]. In line with this, Sugiyanto *et al*. and Yongpitakwattana *et al*. demonstrated the inhibitory effect of alpha-mangostin to dengue virus replication and cytokines expression in human peripheral blood mononuclear cells and dendritic cells. In addition, gamma-mangostin reported to inhibit hepatitis C virus and SARS-CoV-2[21,22].

Bioinformatics provide more efficient target discovery and validation approaches, thus helps ensure that more drug candidates are successful during the approval process, making it more cost-effective[23]. Notably, the work of Lipinski *et al*. analyzed orally active constituents to describe physicochemical ranges for high probability opportunities as an oral drug. This called Rule-of-five delineated the relationship between pharmacokinetics and physicochemical parameters. Lipinski's rule of 5 helps in distinguishing drug-like and non-drug like molecules. It predicts a high probability of success or failure due to drug-likeness for molecules complying with 2 or more of the following rules, such as molecular mass less than 500 Dalton, high lipophilicity, less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, molar refractivity should be between 40-130[14].

In the present study, an attempt was made to investigate a more extensive pharmacological appearance of phytoconstituents by the application of PASS web resources. The proposed *in silico* method extends further to generate novel bioactivities of selected phytochemical leads, related side-effects, and their mechanisms. In addition, the recent version of PASS predicts approximately 3750 pharmacological activities, specific toxicities, biochemical mechanisms of action, and metabolic terms on the basis of the structural formula of drug-like substances with average fidelity ~95%[24]. This might be further validated through *in vitro* as well as *in vivo* trials. In line with this, the present study revealed the use of PASS in exploring hidden pharmacological potential of alpha-mangostin and gamma-mangostin as an antiviral (Figure 3).

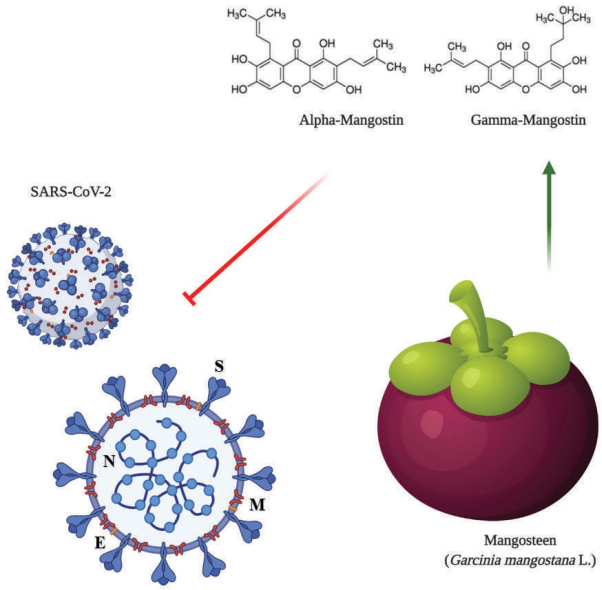


**Figure 3. Alpha-mangostin and gamma-mangostin isolated from mangosteen as a promising candidate against SARS-CoV-2. E: Envelope protein, M: Membrane protein, N: Nucleocapsid phosphoprotein, and S: Spike protein. This figure created in BioRender.**

## Conclusion

In summary, this study showed the potential pharmacological benefits of alpha-mangostin and gamma-mangostin isolated from mangosteen against SARS-CoV-2. Thus, mangosteen exhibits as a valuable plant and establishes as a candidate for future drug development to fight SARS-CoV-2. However,

further trials, such as *in vitro* and *in vivo* evaluation, are needed to prove the validity of these findings.

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

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**Ethical Approval**: No ethical approval needed.

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# Comparative Evaluation of Dexmeditomidine & Fentanyl in Terms of Cardiovascular Stress Response During Anesthetic Airway Management in Major Surgical Procedures

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## Abstract

**Background-** airway management both in operation theatre and during emergencies remains challenge because of cardiovascular stress response & inflammatory mediators during procedure exhilarating the stress response itself leading to catastrophic events. This study is aimed to evaluate comparative efficacy of dexmedetomidine over fentanyl because of it have not only as sympatholytic also as anti-inflammatory properties. Study was orchestrated at tertiary care centre in western India after approval of institutional scientific & ethical committee. Methodology Study group include 50 patients of either sex aged between 18 to 55 years who were admitted for major surgical procedure and given consent for inclusion. Study group divided in two group for record & statistical analysis of parameters like heartrate, mean arterial pressure at various interval during surgery to ascertain the superiority of dexmedetomidine vs fentanyl in stabilizing cardiovascular stress response.

***Keywords:*** *Airway management, Anaesthesia, cardiovascular stress, Dexmedetomidine, inflammatory mediators, fentanyl*

## Introduction

An airway management is first and very essential part of anesthetic management and critical care of the patient that why it's core in competency based medical education. The medical science has been fighting against the challenges of cardiovascular stress response to tracheal intubation for long time. Airway maneuvers like Laryngoscopy, tracheal intubation & extubation and inflammatory mediators released during these all are to blame for incitement to cardiovascular instability & stress. This is sympathetic stress response which results in raised blood pressure and heart rate due to rise in plasma concentration of catecholamine and other mediators. Extensive research work is done indicating that increase in sympathoadrenal activity with inflammatory substance release due to airway manipulation may result in hypertension, tachycardia and arrhythmias which can be minimized by use of dexmedetomidine [1,2,3] Anesthetists for many years trying to maintain stability of cardiovascular system in vivo. [4] Laryngoscopy and tracheal intubation are

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associated with a sympathetically driven increase in blood pressure by 40-50% and heart rate by 20% which is threatful for patients having cardiovascular and cerebrovascular disease [4,5,6] already so much research work done in defining role of drug regimens like opioids, barbiturates, benzodiazepines, beta blockers, calcium channel blockers, vasodilators have been used from time to time for attenuating the stress response to laryngoscopy and intubation. [4,5]

## Aim of the Study

comparative evaluation of dexmedetomidine & fentanyl in terms of cardiovascular stress response during anesthetic airway management in major surgical procedures to ascertain superiority of any drug.

## Material and Methods

This study was carried out at C.U. Shah Medical College and Hospital, Surendra Nagar, after obtaining scientific & ethical committee approval. Informed written consent was taken from all patients. 50 patients in the age group 18-55 year of either sex, belonging to (American society of anesthesiologists) ASA grade I and II scheduled for elective surgical procedures under General anesthesia were included. Group F: Inj Fentanyl citrate 50 µg /ml - 2ml ampoule & for Group D: Dexmedetomidine (100µg/ml- 2ml ampoule) Normal saline (25 ml)

**Inclusion criteria were 1.** Patients aged between 18-55 years 2. Patients of either sex male or female 3. Patients with ASA grade I & II 4. Patients scheduled for elective surgical procedure under general anesthesia.

**Exclusion criteria: were 1.** Patients with anticipated difficult airway 2. Patients with cardiac, coronary, renal, hepatic, cerebral diseases and peripheral vascular diseases. 3.Obese patients (BMI>30) or malnourished 4. Patients on drugs like antihypertensive, sedative, antidepressant

**Technique of anesthesia/Procedure:** 50 patients aged between 18 to 55 yrs belonging to ASA grade I & II were randomly divided into 2 groups, each group consists of 25 patients Group F [Fentanyl group] Group D [Dexmedetomidine group]. After confirmation of NBM status an intravenous line was secured with 18G cannula & preloading with 10 ml/kg of Ringer lactate done over 30 min for all patients. In premedication Inj Glycopyrrolate 0.2 mg, Inj Ondansetron 4 mg & Inj Ranitidine 150 mg IV was given. Basal systolic blood pressure (SBP)(T0), diastolic blood pressure (DBP) (T0), Mean arterial pressure (MAP)(T0), heart rate and SpO2 (T0) were recorded after 5 min of settling in the OR. Group F [Fentanyl group] patients received IV.Inj Fentanyl 2 µg/kg in 10ml normal saline. Group D [Dexmedetomidine group] patients received IV. Inj Dexmedetomidine 1µg/ kg in 25 ml normal saline infused over 10 mins with the help of syringe pump.

Systolic blood pressure (SBP)(T1), Diastolic blood pressure (DBP)(T1), Mean arterial pressure (MAP)(T1), heart rate and SpO2 (T1) were recorded. All patients were pre- oxygenated for 3 mins with 100% oxygen & patients were induced with Inj Propofol 2mg/kg (1%) IV. After successful check ventilation with 100% oxygen, Intravenous Inj succinylcholine 2mg/kg given to facilitate laryngoscopy & intubation. Oxygenation continued by positive pressure mask ventilation using Bains circuit.

After induction, endotracheal intubation was done. SBP, DBP, MAP, Heart rate, SpO2 were recorded. Anesthesia maintained with 50% N2O, 50% O2, Isoflurane, controlled ventilation with intravenous atracurium 0.5 mg/kg as loading dose and 0.1 mg/kg as maintenance dose. SBP, DBP, MAP, Heart rate, SpO2 were recorded at 1 (T2), 3 (T3), 5 (T4), & 10min (T5) after laryngoscopy & intubation.

**Table 1 parameters coding explained**

| Sequence of SBP, DBP, MAP, HeartRate, spo2 Recording | CODING: T- time, 0to5 numerical codes minutes as below mentioned |
|---|---|
| Basal reading when the patient is shifted to OT, | T0 |
| At Induction (with propofol + succinyl choline) | T1 |
| At 1 min after intubation | T2 |
| At 3min after intubation | T3 |
| At 5min after intubation | T4 |
| At 10min after intubation | T5 |

**Results**: Statistical analysis was performed by descriptive and inferential statistics using the student's unpaired *t*-test, Graph Pad Prism (version 5.0; Graph Pad Software Inc., California, USA). All data are presented as mean ± SD (standard deviation). Demographic data analysis and justification of study population was inferred by student's t test. ($p<0.01$) – Statistically highly significant, ($p<0.05$) – Statistically significant, ($p> 0.05$) – Statistically Not Significant (NS),

**Table 2: Age and sex wise distribution**

| AGE GROUPS IN YEARS | FENTANYL | | | DEXMEDETOMIDINE | | |
|---|---|---|---|---|---|---|
| | Male M | Female F | Total M & F | Male M | Female F | Total M & F |
| 18-36 | 6 | 6 | 12 | 6 | 6 | 12 |
| 37-55 | 7 | 6 | 13 | 6 | 7 | 13 |
| total subjects 50 | 13 | 12 | **25** | 12 | 13 | **25** |

**Table 3: Comparison of demographic parameters in Group F(Fentanyl) and Group D(Dexmedetomidine) and results of t-test:**

| Parameters | Group F Mean ± SD | Group D Mean ± SD | T | P |
|---|---|---|---|---|
| Weight (kilograms) | 55.160±5.836 | 53.880±5.805 | 0.7775 | 0.4407 NS |
| AGE (years) | 38.000±13.143 | 37.08±12.747 | 0.2512 | 0.8027 NS |

**Table 4: Showing the intergroup comparison of mean heart rate (bpm) changes in response to laryngoscopy and intubation between Fentanyl Group F and Dexmedetomidine Group D**

| TIME | Group F | Group D | p- value | t-value | Remarks |
|---|---|---|---|---|---|
| T0 (Basal) | 89.560 ± 11.612 | 88.320± 12.058 | 0.7127 | 0.3704 | NS |
| T1Induction | 85.92±10.234 | 71.320±11.721 | <0.0001 | 4.691 | S |
| T2 (1 min) | 103.08±11.637 | 81.020±12.021 | <0.0001 | 6.599 | S |
| T3 (3min) | 101.24±11.773 | 78.600±11.284 | <0.0001 | 6.942 | S |
| T4(5 min) | 97.600±11.77 | 76.400±10.468 | <0.0001 | 6.922 | S |
| T5 (10 min) | 94.160±10.16 | 76.080±11.083 | <0.0001 | 6.013 | S |

**Table 5: table 5 showing the intergroup comparison of Systolic (SBP), diastolic (DBP) & Mean arterial Blood Pressure (MABP) changes (mmhg) in response to laryngoscopy and intubation between fentanyl Group F and Dexmedetomidine Group D**

| parameter | TIME | Group F | Group D | p- value | t-value | Remark |
|---|---|---|---|---|---|---|
| SBP | T0 (Basal) | 122.40 ± 9.412 | 122.56± 9.996 | 0.9538 | 0.05827 | NS |
| | T1 (Induction) | 118.56±7.906 | 112.28±10.386 | 0.0200 | 2.406 | S |
| | T2 (1 min) | 126.72±7.602 | 113.28±8.615 | <0.0001 | 5.849 | S |
| | T3 (3min) | 123.32±7.353 | 112.04±8.890 | <0.0001 | 4.889 | S |
| | T4(5 min) | 120.0±6.758 | 111.52±9.184 | 0.0005 | 3.719 | S |
| | T5 (10 min) | 118.84±7.341 | 111.36±8.190 | 0.0014 | 3.401 | S |
| DBP | T0 (Basal) | 78.120± 8.151 | 78.000± 9.443 | 0.9618 | 0.04810 | NS |
| | T1 (Induction) | 74.440±7.638 | 67.440±9.845 | 0.0072 | 2.809 | S |
| | T2 (1 min) | 82.560±5.817 | 70.560±10.666 | <0.0001 | 4.939 | S |
| | T3 (3min) | 78.880±6.900 | 66.800±8.602 | <0.0001 | 5.477 | S |
| | T4(5 min) | 76.120±6.888 | 65.160±8.004 | <0.0001 | 5.190 | S |
| | T5 (10 min) | 75.920±7.164 | 63.920±8.597 | <0.0001 | 5.361 | S |
| MABP | T0 (Basal) | 92.88± 7.884 | 92.360± 9.092 | 0.8295 | 0.2165 | NS |
| | T1 (Induction) | 89.160 ±7.128 | 81.880 ± 9.597 | 0.0038 | 3.045 | S |
| | T2 (1 min) | 96.720± 5.997 | 84.240 ±9.558 | <0.0001 | 5.530 | S |
| | T3 (3min) | 93.040± 6.419 | 81.320± 8.148 | <0.0001 | 5.649 | S |
| | T4(5 min) | 90.000 ± 6.158 | 80.120 ±7.732 | <0.0001 | 4.998 | S |
| | T5 (10 min) | 89.64 ± 6.903 | 79.160 ±7.930 | <0.0001 | 4.984 | S |

## Discussion

Airway management which includes llaryngoscopy and tracheal intubation are considered as the most critical events during administration of general anesthesia in major surgeries as they provoke transient but marked sympathoadrenal response manifesting as hypertension and tachycardia. [7,8]

These responses are transitory, variable and may not be significant in otherwise normal individuals but in patients with cardiovascular compromise like hypertension, Ischemic heart disease, Cerebrovascular disease even these transient changes or stress can provoke ventricular failure, pulmonary edema, myocardial ischemia, ventricular dysrhythmias and cerebral haemorrhage[3]. Not only airway maneuvers but also the inflammatory mediators released during it threat to such patient which indicates the need of sympatholytic and anti-inflammatory drug in such procedures. [3,7,8].

Methods like use of inhalational anesthetic agents, lidocaine, opioids, direct acting vasodilators, calcium channel blockers and β-blockers have been tried for blunting cardiovascular stress because of laryngoscopy and intubation. [9,10,11]

Each has its pros & cones for example: opioids respiratory depression and chest wall rigidity were potential problems, use of halothane was associated with dysrhythmia, calcium channel blockers lead to reflex tachycardia, direct acting vasodilators needed invasive hemodynamic monitoring and lidocaine showed in consistent results in blunting the hemodynamic responses to laryngoscopy and intubation [12,13,14,15]

Beta blockers are administered for blunting sympathoadrenal stress response to airway manipulation, but their action is more targeted on heart rate rather than blood pressure stability. So, we need a drug which can is effective not only in stabilizing cardiovascular reflexes but also can control inflammation, post-operative nausea and vomiting (PONV) [16,17,18,19,20]

The search for the ideal technique or agents which can control all the side effect of airway manipulation stated above resulted in this study where we are checking supremacy of dexmedetomidine vs fentanyl as anesthetic agent in cardiovascular domain affected by airway manipulation.

### Fentanyl

Fentanyl is advocated attenuation of sympathetic response to laryngoscopy and intubation [21]. But blunting of sympathetic response is highly dose dependent which results in to undesirable respiratory depression [22,23]. Fentanyl at 6 μg/kg completely abolishes sympathetic response, whereas at 2 μg/kg significantly attenuates the hemodynamic response, during laryngoscopy and intubation [24]. In this study Intravenous fentanyl was given at the dose of 2 μg/kg diluted in 10 ml normal saline 10 min before induction [24,25].

### Dexmedetomidine

In our study we have given dexmedetomidine as I.V. infusion 1 μg/kg in 25 ml normal saline over 10 min. If Bolus dose of Dexmedetomidine is given rapidly, it leads to an initial transient increase in blood pressure and reflex decrease in HR because of peripheral α-2 adrenoceptor stimulation of vascular smooth muscle. So, in the present study we have given dexmedetomidine administration slowly over 10 min[25]. It has been proven that dexmedetomidine and fentanyl as premedication attenuates the sympathoadrenal response to laryngoscopy and endotracheal intubation.

The statistical analysis of outcomes shows that the population in groups are standardized in age, sex & weight distribution (table 2,3)

Heart rate variation analysis in the two groups (table 4) demonstrated that there was no statistically significant difference until induction of anesthesia ($P > 0.05$). The increase in mean HR after administration

of Propofol was statistically lower in Group D as compared with Group F. Analysis of the postinduction, postintubation values of the mean HR variation from the baseline values of the two groups showed a statistically significant difference until 10 min ($P < 0.05$).

Kharwar *et al*. [26] observed that there was a decrease in pulse rate from baseline by 17.80% in the dexmedetomidine Group Fs compared with the fentanyl group, in which the decrease was 6.99% from baseline after induction.

Statistical analysis showed that there was no significant change in Systolic & diastolic blood pressure between the two groups at Basal level-significant at T1 (Induction) level, but highly significant At T2 (1 min), At T3 (3min), At T4(5 min), At T5 (10 min) level. Mean arterial BP also follow the same discourse.

Gandhi *et al*. [25]observed that Dexmedetomidine produces more significant attenuation of increase in SBP & DBP during laryngoscopy and intubation as compared with fentanyl, which is in concordance with our study. With respect to MAP, our findings are confirming the statements of previous researchers [25,26,]

The above observations can be justified by ability of dexmedetomidine to reduce sympathetic nerve activity, inhibit the release of sympathetic impulses, and relieve nerve tension primarily according to the highly selective nature of the drug [3]

On using equipotent doses of fentanyl and dexmedetomidine, we found that Dexmedetomidine significantly depressed sympathetic response to laryngoscopy and intubation in terms of HR, SBP, DBP, and MAP compared with fentanyl. There are studies done previously which can justify the superiority of dexmedetomidine over fentanyl in cardiovascular domain. Hyperinflammatory responses damage organs like lung, heart and kidney paving for unfavourable pathological changes, including tissue and cell degeneration, necrosis, changes in hemodynamic like inflammatory hyperaemia, increased vascular permeability (inflammatory exudation), fluid exudation and cellular exudation (inflammatory infiltration) resulting in systemic inflammatory response syndrome: which facilitates secondary multiple organ injury and dysfunction, affecting postoperative outcomes like prolonged hospitalization and increased medical costs. [27,28,29,30,31,32,33,34] Previous researchers proves efficacy of dexmedetomidine as anti-inflammatory agent & potent sedative also because it suppresses the production of lipopolysaccharide-induced proinflammatory mediators, including Tumour necrosis facto-α, Interleukin-6, and CRP, both in vivo and in vitro [31,32,33,34]. Interleukin-10 is a cytokine that inhibits the production of IL-6 and TNF-α decreasing intra & postoperative inflammation [35]. Yang and Hong also suggested that dexmedetomidine could inhibit nuclear factor-κB activity and activate cholinergic anti-inflammatory pathways [36].

The conclusion is the dexmedetomidine should be preferred for administration during airway management over traditional optional like fentanyl because it has versatile properties which benefit the patients not only intraoperatively to minimize cardiovascular risks but also postoperatively as potent anti-inflammatory properties.

**Ethical Clearance**- was Taken from Institutional Ethics Committee – Human Research: C. U. Shah Medical College. **Source of funding**- Self. **Conflict of Interest** - Nil

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# A Tear Inflammatory Biomarker in Dry Eye Disease

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## Abstract

**Background:** Dry eye disease is a chronic ocular condition and significantly impacts visual function with multifactorial origin. It is characterized by tear instability and inflammation on the ocular surface. The inflammatory response initiates by synthesis and release of cytokines, Recently, there has been increasing scientific interest using tear film biomarkers that play a role in pathophysiology of dry eye disease. The objective of this study to explain relationship of tear film biomarker in dry eye.

**Methods:** The research design of this study used literature review. The data was collected from electronic database in PubMed, Google Scholar, and website of science and predominantly published in the last 10 years.

**Result:** The defect on the tear film component due to oxidative stress lead to ocular surface epithelial exposure, and intracellular signaling pathways are triggered, involving an inflammatory mediator which plays a role in the pathophysiology of dry eye. Using biomarker lead to better diagnosis, drug development, and effective management for dry eye disease.

**Conclusion:** In conclusion, TNF-a, IL-6, IL-8, IL-1 by number studies to consistently reflect disease severity and strong correlation with schirmer tear test and another test for the ocular surface in dry eye disease.

***Keywords:*** *Dry eye disease, Tear Biomarker, Inflammation*

## Introduction

In recent years, the epidemiology of DED is changing. The new paradigm impacting younger patients, mostly related to the rise of smartphone use. In America, an estimated 16 million had been diagnosed with DED and many countries worldwide with a range of 9.5-90 %. The prevalence is higher among women than man and increases with age[1]. In Indonesia, the majority of DED is 37,6 % by 40-49year age group and 1,4 times higher for men than women[2,6].

Defect on tear film components due to oxidative stress lead ocular surface epithelial exposed and triggered intracellular signaling pathways. The inflammatory response initiates the synthesis and release of cytokines, matrix metalloproteinases (MMPs), and chemokines. Pro-inflammatory cytokine

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could activate T-cell to raise inflammation, and MMPs promote extracellular degradation, causing exposure to epithelial barrier[3,6].

Recently, there has been increasing scientific interest in the use of tear biomarkers in DED. Biomarker or biological marker is an objective indicator medical sign or biological stated of normal and pathogenic processes[4]. Using biomarker will lead to better diagnosis, drug development, and effective management for dry eye disease. However, it is challenging to access ocular specimen—alternatively, many studies identifying protein biomarker in tear fluid. A biomarker originated anatomically close to the disease and noninvasive for dry eye disease is crucial to increase specificity. This article aims to describe an overview of the use of tear fluid biomarker in diagnosing and managing DED.

**Pathophysiology Dry Eye Disease**

Dry eye disease can divide into several significant subtypes, such as aqueous deficient and evaporative. The etiology of aqueous deficiency is disruption of the sensory drive to the lacrimal gland, decreased reflex tearing due to nerve damage (refractive surgery), and systemic drug (such as beta-blocker, diuretic, antihistamine), and aging. Aqueous deficiency most common is related to systemic inflammation, such as Sjogren syndrome. Sjogren syndrome is a chronic autoimmune disorder characterized by lymphocytic infiltration in the exocrine gland, resulting in dry mouth and dry eye[3,5].

Evaporative dry eye resulting in low covering of lipid to the surface of the eye. The most common cause by Meibomian gland dysfunction (MGD). The obstruction of the Meibomian gland leads to tear film instability, hyperosmolarity, and increased tear evaporation. Disruption Meibomian gland function impacts the quantity and quality of Meibomian secretion, affecting changes in tear film composition. The risk factor of MGD including hormonal aspect (imbalance androgen and estrogen), systemic medication (administration of 13-cis retinoid acid), and topical medication (e.g. beta-blocker, prostaglandin analogs, topical epinephrine) [4].

**Diagnosis Dry Eye Disease**

To assess DED required a combination of symptom and sign. Dryness symptom assessed on validated questionnaires such as ocular surface disease index (OSDI), MacMonnies dry eye questionnaire, and dry eye questionnaire (DEQ). Clinically, DED routinely assessed using a Schirmer tear test to evaluate production aqueous, tear break up time (TBUT) to measure tear film stability, defect on ocular surface-specific corneal and conjunctival staining. The tear film quality could be assessed using an osmometer that measures tear film osmolarity [5].

***Tear Film Composition***

The tear film is composed of three main layers. The outermost layer is formed predominantly of lipid, which function is to prevent water evaporation and reduce the tension of the ocular surface. The middle is an aqueous layer and plays a role in protecting against pathogens and particle and hydration ocular surface. Its contain insoluble and soluble component such as proteins, electrolytes, peptides, and small molecules. The innermost layer is a mucin layer that resides directly at the surface of the cornea. Mucin originated from the goblet cell. The primary function is to maintain the hydration of the ocular surface[7].

Dysfunction of the lacrimal functional unit cause tear film instability and change any component of the tear fluid. The lacrimal functional unit consists of the lacrimal gland, ocular surface, ocular nerves, goblet cell, and accessory gland (e.g. Meibomian). Hyperosmolarity would activate innate immune response by generating a pro-inflammatory microenvironment on the ocular surface[9]. Initially, natural immune response mediated by pattern recognition receptor such as a toll-like receptor. Receptor recognition and triggers activation of inflammasomes to secretion pro-inflammatory cytokines such as interleukin -1b[10,11]. The murine

model demonstrated that hyperosmolar condition activated protein kinase and stimulates cytokines (IL-8, IL-6, TNF-a), matrix metalloproteinases (MMP9, MMP1)[10,11]. Another study showed that hyperosmolarity induces apoptosis of corneal epithelial cell in vitro condition.

Hyperosmolar stress also activates adaptive immune response through activation antigen-presenting cells (APCs), mainly corneal dendritic cells, and recruitment of inflammatory mediators. Hyperosmolar state on the ocular surface also increased the level of chemokines such as CCL20 and CXCL9 in tear film[9,10]. Mature APCs migrate the regional lymph nodes and prime CD4+ T cell, including T helper 1 (Th1) and Th17. These inflammatory mediators interferon-gamma (IFN-g) through angiogenesis and lymphangiogenesis. IFN-g is secreted by T helper 1 and NK cell related loss of conjunctival goblet cell. Goblet cell produces mucins. In vitro study demonstrated goblet cell is susceptible to IFN-g with significantly reduced proliferation[12,13,14].

**Tear Inflammatory Biomarkers**

**Interleukin-6**

Interleukin-6 (IL-6) is an important pro-inflammatory cytokine that is produced by T-lymphocytes and activated macrophages. IL-6 promptly and transiently acute responses to infection, inflammation, immune response, and hematopoiesis. However, dysregulated continue synthesis of IL-6 play a significant effect on chronic inflammation. Interleukin 6 is a representative cytokine with increased expression in tear fluid and conjunctival epithelium and has been known as one of the key molecules in DED.

In many studies, the expression of IL-6 in tear fluid up-regulated in chronic condition[17]. Another study revealed that IL-6 was significantly related to the schirmer tear test. IL-6 levels can be used as indicators to determine the severity and efficacy of anti-inflammatory drugs for DED[18]. Using enzyme-linked immunosorbent assay in tear sample patients with DED that are reported IL-6 level is elevated[19,22]. Another study showed IL-6 level was significantly increased in evaporative DED and correlated with meibography and schirmer tear test[18].

**Interleukin 1**

The IL-1 family consist of three forms, namely IL-1a, IL-1b, and IL-1 receptor antagonist. The mechanism action of IL-1a and IL-1b activate an immune response that trigger activation to a cascade of inflammation by infiltration, the proliferation of lymphocyte and macrophage, and increasing chemokine production. In ocular surface, IL-1 has been related to corneal neovascularization, bullous keratopathy and reported increasing level in Sjogren Syndrome and ocular rosacea[19,20]. This study noted the pro-inflammatory cytokines IL-1 associated with the intensity of corneal defect epithelium and density of goblet cell [21]. IL-1 receptor antagonist is a form anti-inflammatory of interleukin-1, which has a role to be a natural homeostatic mechanism for preventing undesirable activation of IL-1 mediated inflammatory events on ocular surface[20,21] .

**Interferon-gamma**

Interferon-gamma (IFN-γ) is secreted mainly through lymphocyte T helper type 1, cells, cytotoxic T cells, and natural killer. IFN-γ have a role in the innate and adaptive immune response. In tear fluid, the increase IFN-γ level inducing conjunctival goblet cell loss and apoptosis of lacrimal gland cell. In the ocular surface, elevated IFN-γ in tear correlated with tear hyperosmolarity and corneal fluorescein. Another study revealed the IFN-γ level associated with TBUT[28]. In murine model, high IFN-γ expression correlates with severity of conjunctival pathology[23]

**Interleukin 8**

The chemokines IL-8 is a potent pro-inflammatory cytokine that are chemotactic for lymphocytes, neutrophils, and basophils. IL-8 significantly

increases in the tear of DED. The IL -8 contributes to the occurrence of dry eye disease through infiltration and activation of T lymphocytes, leading to damage lacrimal gland and ocular surface[18].

**Tumor necrosis factor alfa**

Tumor necrosis factor alfa (TNF-a) is a potent mediator inflammatory produced mainly through macrophages during the acute phase of inflammation. The primary role is responsible for the regulation immune system and selectively cytotoxic, leading to necrosis or apoptosis[24]. TNF-a significantly raised in DED and correlated with MMP activation and limit fibrosis. Multiple studies have concluded that TNF-a related to decreased tear production, disease severity, and correlation with OSDI score and schirmer tear test [8,24-26]. In the dry eye mouse model, TNF-a, IL-1, IL-6 level were detected in the tear fluid and conjunctival epithelial[27].

## Conclusion

In summary, the identification of molecules in tear fluid as biomarker provide evidence that inflammation is a key feature of the pathophysiology of DED. For dry eye disease, IL-6, TNF-a, IL-8, IL-1 by number studies to consistently reflect disease severity and strong correlation with schirmer tear test and another test for the ocular surface.

**Conflict of Interest:** Nil

**Ethical Clearance:** Nil

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# Serum Preptin Level in Iraqi Beta Major Thalassemic Patients

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## Abstract

Background Beta thalassemia syndromes are a set of hereditary blood disorders marked by a deficiency of beta-globin chain synthesis, result in decrease hemoglobin in red blood cells, anemia, and a reduced RBC production. Iron overload is a common finding in chronically transfused beta thalassemia major patients with possible effect on beta cell function and secretion. This study aimed to assess preptin level in the serum in beta major thalassemic patients, in order to indicate the effect of oxidative stress on preptin secretion. And explain preptin effect on bone cells. Subject and methods; A case-control study that was performed in the Ibn Albaladi Hospital (during the period from 1st of September 2020 to the end of January 2021. It included 48 beta major thalassemic patients and 36 subject as healthy control. Information was taken from each subject including age, diseases. Subjects with any cardiovascular diseases, hyperemesis gravidram, liver diseases, kidney diseases, bone disease, diabetes mellitus, and patients take corticosteroid as well as patients in childhood were excluded in this study. The biomarkers studied were: fasting serum preptin, insulin were assessed. Serum preptin and insulin were measured by ELISA technique. Results; The mean values of (Preptin, Insulin) in patients group were less than control group. There was a moderate direct significant correlation $P<0.01$ between preptin and insulin. Conclusion; The mean value of serum preptin was less in thalassemic major group than control group. And direct correlated with insulin level which is also reduced in thalassemic patients.

***Keywords:*** *Preptin, insulin, thalassemic patients*

## Introduction

Thalassemia is one of the most common diseases in the world, which is causes the rapid destruction of erythrocytes, and in order to retain red blood cells, patients need to undergo daily blood transfusions. Regular blood transfusions, however, cause iron overload, which can lead to complications such as heart disease, diabetes, osteoporosis, and kidney disorders(1).

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Thalassemia is graded into two classes based on the two polypeptide chains: 1. Beta ($\beta$) –thalassemia. 2. Alpha ($\alpha$) - thalassemia. The globin chain is affected or the abnormal hemoglobin involved in beta-thalassemia is beta-globin gen. While in alpha-thalassemia, the alpha-globin gene affects the globin chain. Beta thalassemia can be categorized into three categories clinically: A. Thalassemia major, B. Thalassemia intermedia, C. Thalassemia minor or trait. Thalassemia major means the patient has serious anemia and may require blood transfusions for the remainder of their lives. Thalassemia intermedia is a type of anemia that causes mild to moderate anemia and necessitates blood transfusions on occasion. Patients with thalassemia minor, typically do not

require blood transfusions and appear healthy[2].

Thalassemia minor usually goes unnoticed and has no symptoms. If it does, mild anemia is the most common symptom. Extreme hemolytic anemia is normal in thalassemia major. The symptoms of thalassemia major can consist of:

• Fatigue • Inability to thrive • Jaundice • Spleen and liver enlargement

• Abnormalities of the bones, especially those of the face and pale skin[3].

The key determinant of thalassemia patients' survival is daily blood transfusion. Normal blood transfusions, on the other hand, can lead to severe complications, which can increase thalassemia patients' morbidity and mortality. Most of these complications are: -Iron deficiency. -Infections transmitted via blood transfusions, especially hepatitis B virus (HBV) and hepatitis C virus (HCV). -Thrombotic occlusions of the cerebral, portal, retinal, and coronary circulations have been identified in patients with excess iron as a result of other factors such as arterial stiffness and endothelial dysfunction[4]. Multiple endocrine dysfunctions are common in TM patients, including: Hypogonadism, Growth failure, Diabetes, Hypothyroidism, Hypoparathyroidism, less frequently, hypoadrenalism[5].

Patients with TM are seriously anemic and must undergo blood transfusions for the remainder of their lives. Iron builds up in different organs in patients due to frequent blood transfusions[6]. However, TM induces long-term extravascular hemolysis, which increases intestinal iron absorption while decreasing iron bioavailability. This phenomenon, when combined with several blood transfusions over time, could result in iron overload and an increase in the amount of iron ions in the body[7]. As a result, ROS production will be encouraged. Oxidative stress is caused by a change in the balance between ROS and the antioxidant protection mechanism, which promotes ROS[8]. Excess ROS react immediately with other molecules, causing many organelles, especially the membrane, to malfunction in the cell, resulting in cytotoxicity and organ failure[9]. In the visceral organs (primarily the heart, liver, and endocrine glands), iron deposition as well as oxidative stress cause tissue damage and, finally, organ dysfunction or failure[10].

Preptin, an oligopeptide secreted by pancreatic beta-cells, is involved in glycometabolism and bone metabolism. Preptin is a 34-amino-acid peptide hormone discovered in 2001 that is co-secreted with insulin and amylin from secretory granules derived from cultured murine beta-cells and corresponds to Asp69-Leu102 of the proinsulin-like growth factor II E-peptide[11]. Proteases cleave preptin at the 21st phenylalanine amino acid residue. The truncated preptin peptide (preptin 1–16) that results from this cleavage has no effect on insulin secretion. However, full-length (34-amino-acid) preptin enhances glucose-mediated insulin secretion physiologically[12]. Preptin increases osteoblast replication while decreasing apoptosis. Preptin administration increases bone area and mineralizing surface. As a consequence, preptin plays a role in bone anabolism and contributes to bone mass preservation in hyperinsulinemia disorders like obesity[13]. The (1-16) N-terminal fragment of preptin is responsible for its bone function. This peptide promotes osteogenesis by phosphorylating p42/ 44 mitogen-activated protein kinases via a G protein coupled receptor[14]. Preptin levels in the blood are reduced in osteoporosis and osteopenia patients, and they are attributed to bone mineral densities BMD. As a result, preptin plays a role in osteoporosis pathogenesis, most likely by bone development rather than bone resorption[13].

Osteopenia and osteoporosis are common causes of morbidity in thalassemia patients of both genders. The pathogenesis of osteoporosis in TM is complex, and it varies from the pathogenesis of nontransfused patients' bone deformities, which are triggered by inadequate haemopoiesis and progressive marrow expansion. The loss of bone mass in TM has been related to a variety of factors. Ineffective haemopoiesis

with progressive marrow expansion, direct iron toxic effects on osteoblasts, impaired sexual maturation, growth hormone (GH) and insulin growth factor (IGF)-1 deficiency, parathyroid gland dysfunction, diabetes, hypothyroidism, and also liver disease, have been identified as possible causes of thalassemia-induced osteoporosis[15].

## Method

Specimens were collected during the period from 1st of September 2020 to the end of January 2021. A total of eighty four subjects included in this study were divided into two groups: The first group (patients) forty eight subjects have beta major thalassemia as a thalassemic group their age from 18 and above. The second group (healthy control) thirty six subjects (not thalassemic) as a control group their age (18 – 35 years). Thalassemia participants were selected from Ibn Albaladi Hospital.

Subjects with any cardiovascular diseases, hyper emesis gravidram, liver diseases, kidney diseases, bone disease, diabetes mellitus, and patients take corticosteroid as well as patients in childhood were excluded in this study.

## Statistics

Continues data were Described as median and interquartile ranges, mean± standard error of the mean (SEM). Student's t- test was used to analyze and compare between the means of the markers and variables between the patients and control. Pearson correlation was performed to test significant correlation among the parameters. Alpha level for statistical significance was set top < 0.05. Statistical Microsoft excel software version 2019 and IBM SPSS Statistics 26.0 software (IBM SPSS Inc., Chicago, IL) were used for statistical analysis. Graph Pad prism version 8 was used for standard curve fitting.

## Results

The mean values of (Preptin, Insulin) in patients group were less than control group.

**Table (1) Descriptive statistics and comparison of means of the study parameters between thalassemic and control groups**

| | Statistic | n | Median (Interquartile range) | Mean± Standard error of the mean | P value t-test |
|---|---|---|---|---|---|
| Preptin pg/ml | Patients | 48 | 61.13 (46.84-74.53) | 69.86± 6.98 | < 0.001 |
| | Control | 35 | 131.56 (83.89-164.4) | 124.36± 8.52 | |
| Insulin mU/L | Patients | 48 | 3.19 (2.56-4.86) | 4.06± 0.31 | < 0.001 |
| | Control | 35 | 8.34 (4.97-9.83) | 7.98± 0.71 | |

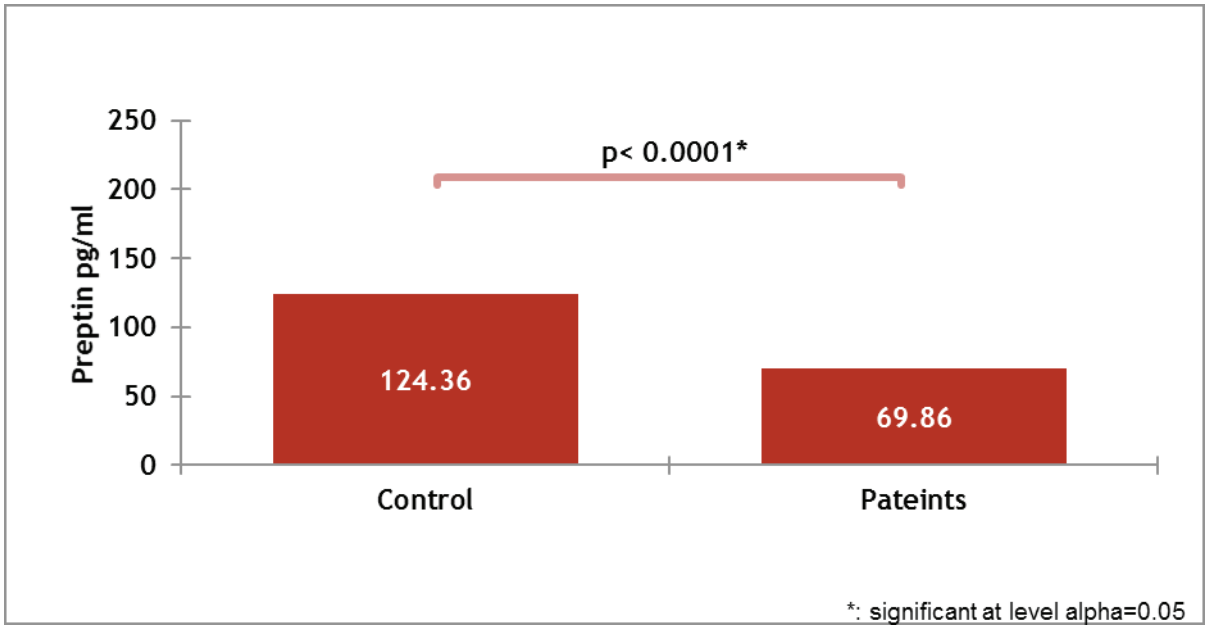


**Figure 3-1 Bar chart compering means of serum preptin between patients and control groups using student's t-test. Significant P-value is set at alpha = 0.05**

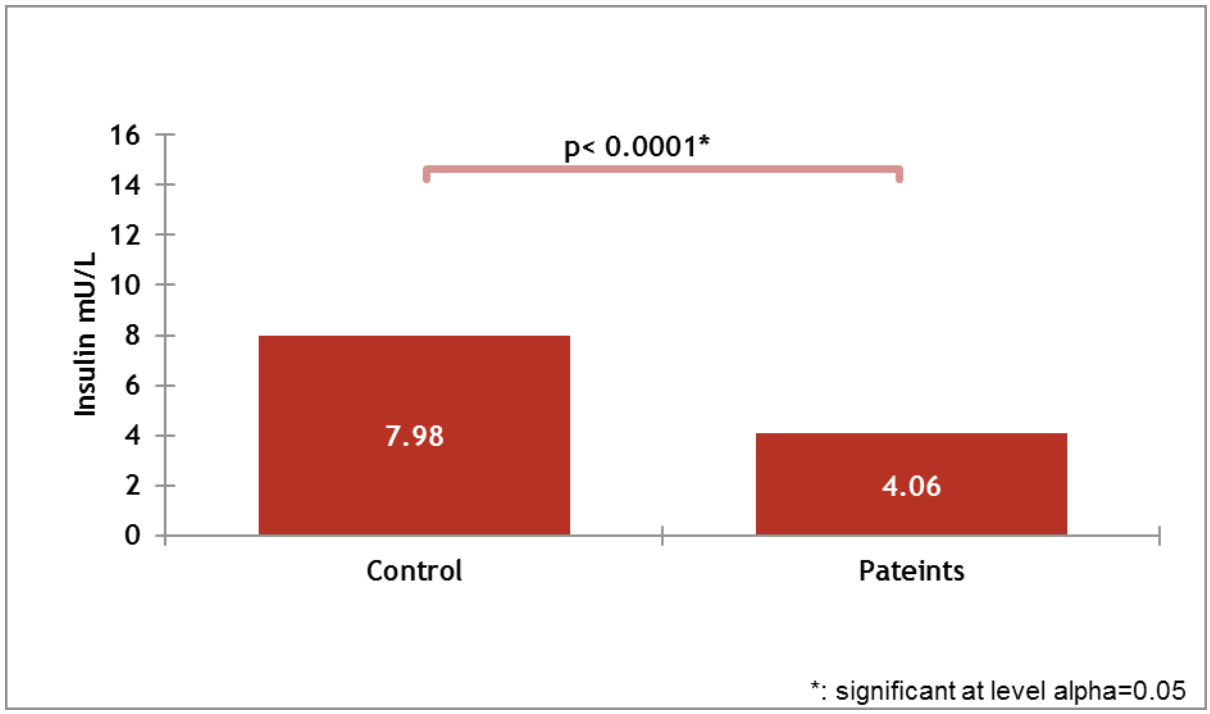


**Figure 3-2 Bar chart compering means of serum Insulin between patients and control groups using student's t-test. Significant P-value is set at alpha = 0.05**

**Table 2 Pearson Correlation in the patients group. Presenting the correlation coefficients and p-values for serum Preptin and insulin.**

| | | Preptin | Insulin |
|---|---|---|---|
| | | | |
| Preptin | r | | 0.36 |
| | p | | 0.01 |
| Insulin | r | 0.36 | |
| | p | 0.01 | |

There is a moderate direct significant correlation P<0.01 between preptin and insulin in patients group.

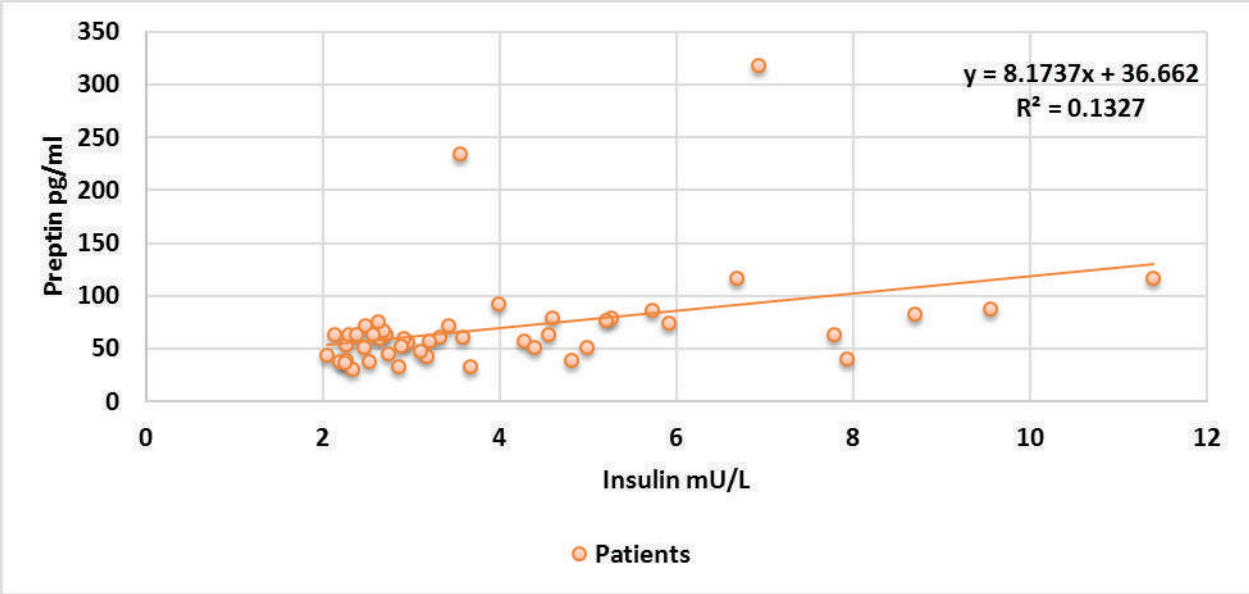


**Figure 3-3 Scatter plot showing the correlation between (Preptin pg/ml & Insulin mU/L) in the patient's group, coefficient equation, model fit R2 and regression line plotted to ease the interpretation**

## Discussion

Beta thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β-globin chain synthesis [16]. There is damage and premature destruction of RBC precursors, causing ineffective erythropoiesis leading to severe anemia and compromised oxygen transport. In some patients, death would result without chronic blood transfusions. Laboratory abnormalities include microcytic anemia with abnormally shaped RBCs and abnormal Hb electrophoresis [17]. A Preptin hormone serum level was decreased significantly high in patients group than control group. But from other previous studies that concerned with serum ferritin, iron overload and oxidative stress in thalassemia major can explain that the increase of serum ferritin and iron overload occur from chronic blood transfusion result in oxygen reactive species generation and oxidative stress that affect pancreatic beta cells leading to secretion dysfunction [9, 10, 18]. Insulin serum level also showed a significant decrease in patients group than control group. This result agreed with previous study that represent insulin deficiency and insulin resistance in thalassemia major as a complication of disease in patients with hyper transfusion due to the high

serum ferritin and iron overload on different body tissue which cause pancreas and liver impairment as well as impact pancreatic islet cells [19]. Reactive oxygen species caused exclusively toxic effects and were associated with pathologies. Because of their high reactivity, ROS react with all types of biological molecules. Thus, high and sustained concentrations of ROS can cause damage to many cellular and extracellular constituents, including DNA, proteins, lipids, carbohydrates, and nucleic acids, often inducing irreversible functional alterations or even complete destruction [20]. As a result of these effect of oxidative stress, most of thalassemic patients may have functional decline in most of systemic glands especially pancreas. And these results have been proved by measuring some of pancreatic enzyme preptin and insulin which had indicated beta cells status [11,21]. Furthermore, there was also direct significant correlation relationship between preptin and insulin observed in correlation (Table 1 and 2). This agreed with other previous study that explained the secretion of preptin peptide from beta cell. Preptin was co-secreted with insulin and also sometimes enhance insulin secretion. So this result was indicator of beta cell status and represented the beta cell dysfunction in thalassemic group [12,22].

## Conclusion

The mean value of serum preptin was less in thalassemic major group than control group. And direct correlated with insulin level which is also reduced in thalassemic patients. However, preptin also indirectly correlated with ferritin level but it was not significant.

### Recommendation

1. Further studies about thalassemia major patients and osteoporosis needed that represent more clearly mechanisms that cause osteoporosis.

2. Expanding this study for explain the mechanism of preptin hormone on bone cells in thalassemic patients with osteoporosis in different age and gender using DEXA, vitamin D3, parathyroid hormone and other clinical measurements determine the degree of osteoporosis.

3. More studies about preptin hormone and it's correlation with insulin in thalassemic patients with diabetes mellitus type 1.

4. Genetic studies involve B-thalassemia and B-cell destruction could be involved in upcoming studies.

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** Not required

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# Effect of KI on SDF Treated Cavities

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## Abstract

**Background:** Dental caries is the most frequent chronic disease worldwide. However, Dental caries can be prevented or arrested. Recently interest in the use of silver diamine fluoride (SDF) has been growing. (SDF) is a solution containing ionic silver, fluoride, and ammonia that arrests the progress of carious lesions and prevents the development of future caries. In Vitro studies demonstrated that SDF increases the pH of biofilm, reduces dentin demineralization, and has antimicrobial action against cariogenic bacteria. But it produces a lesion darker (brown to black) than the original, which is the major criticism of the material. To counter the staining, many studies have tested SDF treatment immediately followed with a saturated solution of potassium iodide.

**Material and Methods**: A vitro study done in Qassim University, with sample of 30 extracted premolars, divided into 3 groups, (Group A): received silver diamine fluoride, followed by application of Potassium iodide. (Group B): cavity preparation 1 mm in enamel then Received silver diamine fluoride followed by application of Potassium Iodide. And (Group C): cavity preparation 1 mm beyond dentoenamel junction then Received silver diamine fluoride followed by application of Potassium Iodide.

**Result:** There was a statistically significant differences between three groups on color measurement p= (0.020), this means that the prevalence for group c-based change color teeth compared to group A and B.

**Conclusion**: SDF + KI treatment showed a low intensity staining in superficial and deep cavities. The intensity of staining decreased significantly in dentin prepared cavities.

***Key words:*** *silver diamine fluoride - Potassium Iodide- Enamel -Dentin – Dark stain.*

## Introduction

Dental caries is the most severe chronic condition worldwide. The drill and fill technique of dental caries demand well trained dentists and equipment [(1)].

Some cases of patients that have been severely compromised and underserved leave carious lesions without treatment. However, it is possible to avoid or arrest dental caries [(2)]. Recently concern has been rising in the use of silver diamine fluoride (SDF). In 2015, US Food and Drug Administration approved (SDF) as a treatment for dentinal sensitivity .SDF solution was recently approved as a caries-arresting medicament, this solution is containing ionic silver, fluoride, and ammonia that arrests the progress of caries process and prevents the development of future

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caries [3,4].

Many studies confirmed that SDF increases the pH of biofilm, reduces dentin demineralization, and showed antimicrobial action against cariogenic bacteria. However, it produces a lesion darker (brown to black) than the original, which is the major criticism of the material [5-7].

To counter the staining, many researchers have tested SDF treatment immediately followed with a saturated solution of potassium iodide [8].

It has been approved by studies that Treatments of teeth with KI solution after SDF treatment significantly reduced the discoloration caused by SDF. In this research, we tested SDF application followed by KI effectiveness in different cavity depths.

## Material and Methods

Thirty human premolars, extracted for periodontal problem, were collected free of caries, previous restorations or stains.

The premolars teeth surfaces were hand scaled to remove any remaining soft tissue and were stored in a 0.1% thymol solution at 4 °C before use.

Each individual premolar was mounted in (Shiva product dental stone plaster, Maharashtra-India) ensuring visible entire crown.

The premolar teeth were divided into 3, first group (Group A): received silver diamine fluoride, followed by application of Potassium iodide.

The second group (Group B) were prepared for a class I glass ionomer restoration, 1 mm into Enamel, with parallel walls and a stain-free dentinoenamel junction. Silver diamine fluoride followed by application of Potassium iodide were applied to the prepared teeth. The reaction products washed away and dried with oil-free compressed air and filled with glass ionomer restoration.

Third group (Group C): were prepared for a class I glass ionomer restoration, 1 mm into dentin, with parallel walls. Silver diamine fluoride followed by application of Potassium iodide were applied to the prepared teeth. The reaction products washed away and dried with oil-free compressed air and filled with glass ionomer restoration.

Class I cavity preparation in groups B and C prepared using a high speed 330 carbide bur. SDF, Fagamin - 38% Silver Diamine Fluoride- Tedequim) was applied to prepared cavities a small brush followed by application KI liquid, J. CROW'S.

Lugol's sol- distilled water 85% potassium Iodide 5% - J. Crow Company, using separate brush.

The formed white precipitation was washed away with water. Self-cure Glass ionomer restoration (Harvard Ionoglas Fill Extra OptiCaps, shade A2) was applied to the prepared cavities. The cavity was washed with water and dried with cotton pellet. Harvard Iono glass conditioner (20% polyacrylic acid)) applied using cotton pellet for 20 seconds to remove the smear layer. Cavity rinsed with the water and dried Gently. Mixing the omegalas capsule for 10 seconds and a cavity filling within 15 seconds after end of mix. Then Harvard Ionocoat LC applied and light cure (using halogen curing light (3 M ESPE, St Paul, USA) with a light output of 600 mW / cm2) for 20 seconds. All specimens were preserved in artificial saliva.

Specimens were photographed, using, Camera (iPhone max 12 MP, f/1.8, 26mm ½.55", 1.4µm, dual pixel PDAF, OIS) immediately after treatment and every week for four weeks.

The color of each tooth was measured using Nix Pro color sensor (Canada).

The color sensor provides its own calibrated light source using industry standard 45/0° measurement and color readouts in CIELAB (is a color space defined by the International Commission on Illumination) and

calculate the color difference in Dellta-E2000.

The color of each tooth was recorded immediately after treatment and one weekly four weeks later, The Nix color sensor was applied to collect color change data. ΔE and ΔL records of each group were measured and compared between each of the groups.

### Statistical Analysis

Analysis of the data was carried out using SPSS, version 24 statistical program for descriptive and analytical statistics to detect difference between groups, the significant was considered when the p-value is less than 0.05 ($p < 0.05$).

Two well-known tests of normality were used, the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test.

## Results

Thirty specimens were treated with silver diamine fluoride followed by KI.

Figure (1) shows each group example photos of the teeth immediately and after preparation, every week, for one-month post-treatment.

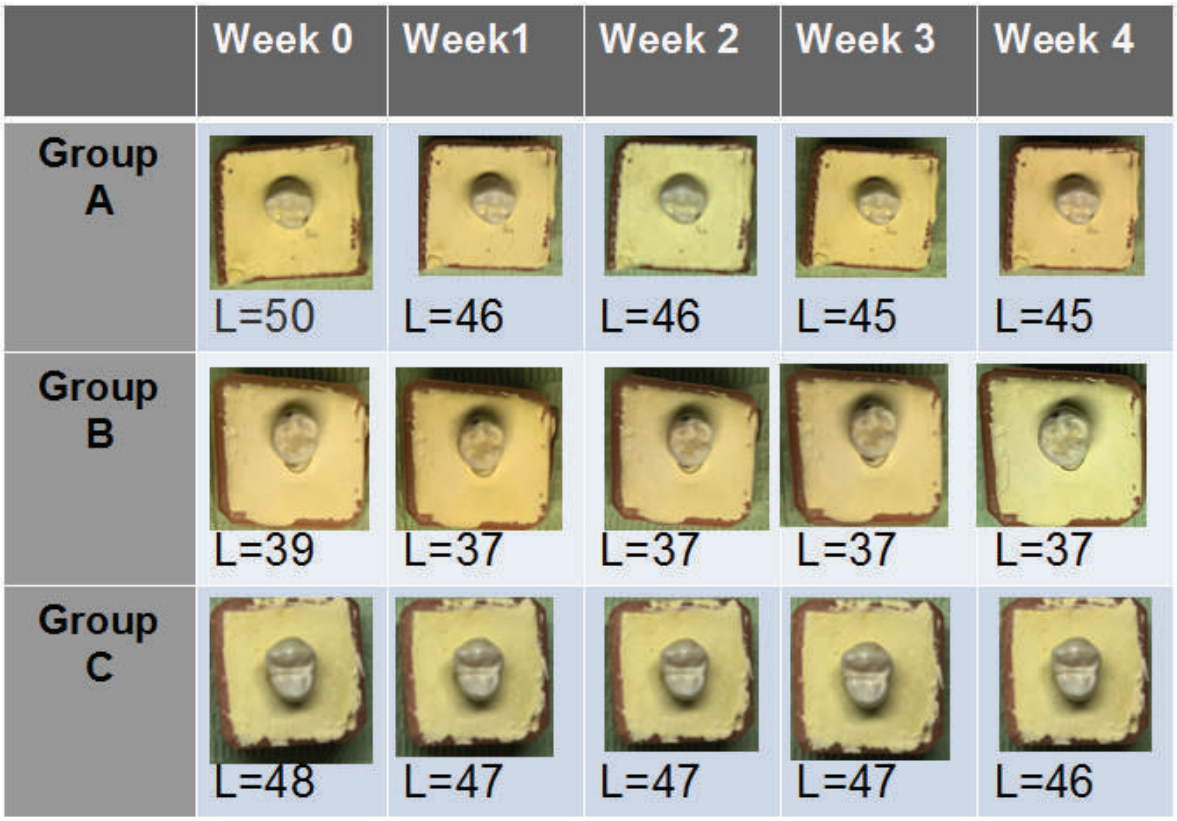


**Fig. (1): Each group example photos**

After the four weeks, the weekly color measurements for groups A, B and C were compared to determine the effectiveness of Potassium application on teeth color receiving SDF in different cavity depth.

It was found for group A that "No change in color" was the highest 60 %, whilst "Change in color not visible" and "Change in color is visible" were 30% and 10% consecutive on week 1. "Change in color not visible" on Week 2 and week 3 recorded the highest proportion equally 60% and on week4 50% fig (2).

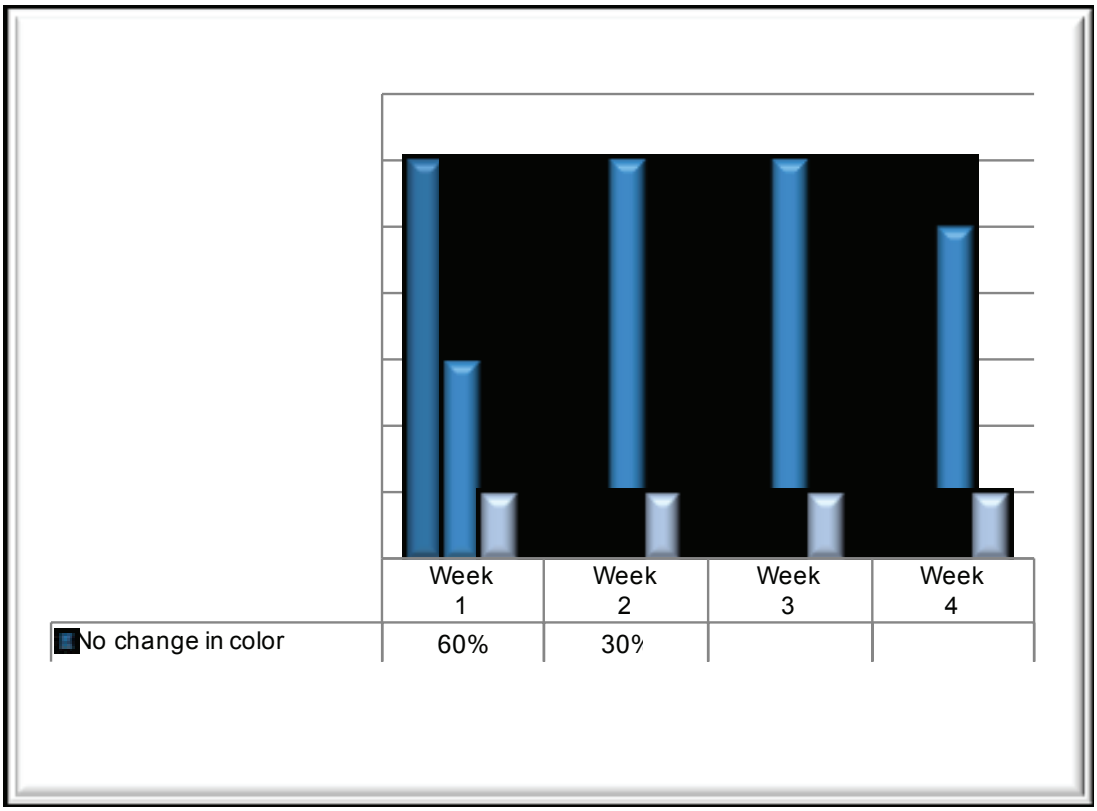


**Fig (2): Chart Shows the color change in group A.**

In group B it was found that "No change in color" was the highest percentage on week1 and on week 2, 60 % and 50% respectively. "No change in color" on Week 2 and on week 3 recorded the highest proportion 50% and 60% consecutive fig. (3)

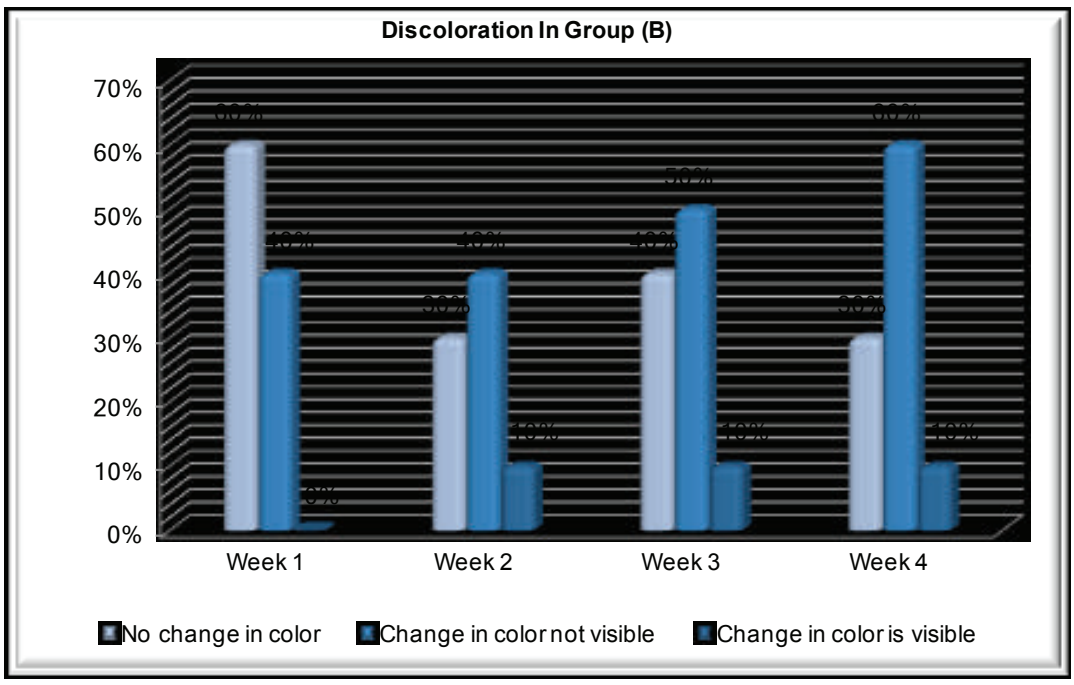


**Fig (3): Chart shows the color change in group B.**

In group C, it was found that "No change in color" was the highest percentage on all four weeks (week1 90%, while week 2, week3 and week4 equally 70% fig (4).

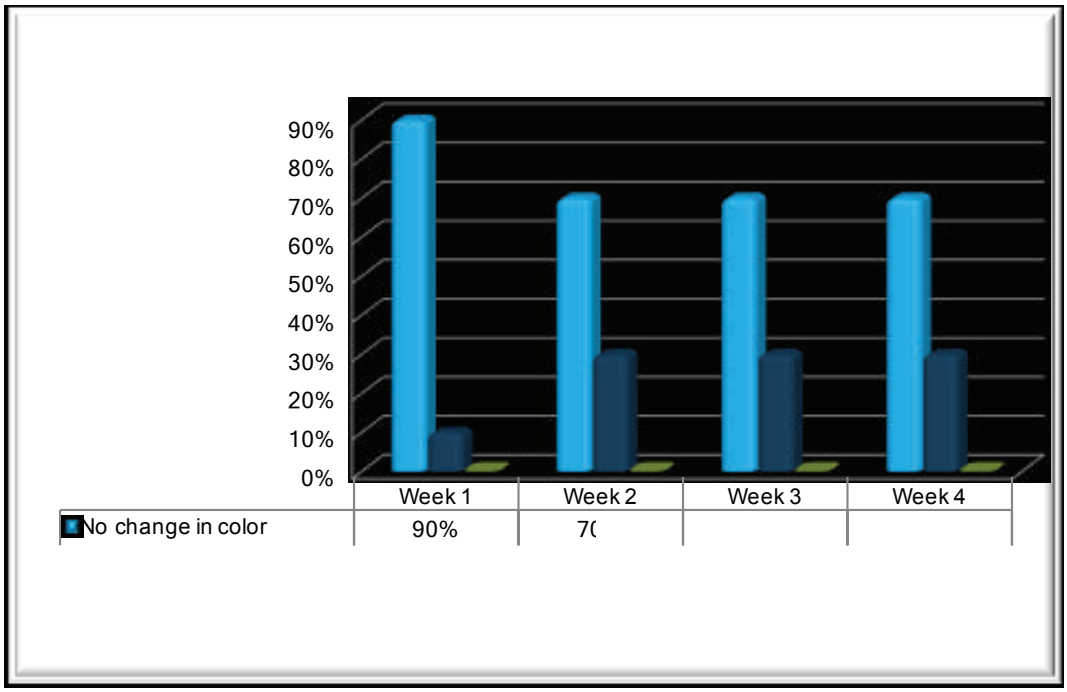


**Fig 4: Chart shows the color change in group C.**

The Distribution of Color measurements score of the three groups showed that there is no change in color result difference, in group c in four weeks, that had a 1 mm beyond Dentioenaml junction cavity and receive sodium diamine fluoride followed by application of Potassium iodide, so Potassium application on teeth receiving SDF in differences cavity depth is effective table 1.

**Table 1: Distribution of Color measurements score on group (A), (B) and (C) among 4 weeks**

| Week | Color measurements | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No change in color result difference (1-3) | | | Change in color not visible (4-6) | | | Change in color is visible (7-10) | | |
| | A | B | C | A | B | C | A | B | C |
| Weak1 | _ | -- | 10teeth | 10teeth - | 10teeth | -- | -- | -- | -- |
| Weak2 | -- | -- | 10teeth | -- | 10teeth | -- | 10teeth | -- | -- |
| Weak3 | -- | -- | 10teeth | -- | -- | -- | 10teeth | 10teeth | -- |
| Weak4 | -- | -- | 10teeth | -- | -- | -- | 10teeth | 10teeth | -- |

**Testing for Normality Distribution:**

Two well-known tests of normality were used, the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test. The Shapiro-Wilk Test is more appropriate for small sample sizes (< 50 samples.) if the Sig. value of the Shapiro-Wilk Test is greater than 0.05, the data is normal. If it is below 0.05, the data significantly deviate from a normal distribution table 2.

**Table 2. Presents the results from two tests of normality, namely the Kolmogorov-Smirnov Test and the Shapiro-Wilk Test.**

| | Kolmogorov-Smirnov b | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | **Statistic** | **Df** | **Sig** | **Statistic** | **Df** | **Sig** |
| Week1 | .360 | 10 | .001 | .731 | 10 | .002 |
| Week2 | .324 | 10 | .004 | .794 | 10 | .012 |
| Week3 | .324 | 10 | .004 | .794 | 10 | .012 |
| Week4 | .272 | 10 | .036 | .802 | 10 | .015 |
| a. | VAR00003 is constant. it has been omitted | | | | | |
| b. | Lilliefors Significance Correction | | | | | |

**Test differences between teeth groups:**

discoloration of teeth on the tested three groups evaluated using the Chi-square test ($\alpha$ =0.05), that was indicated for qualitative data, for a non-matching comparison between two groups.

**Table 3. shows that there are statistically significant differences between three groups on color measurement p= (0.020), this means that the prevalence for group c-based change color teeth compared to group A and C.**

| Test Statistics[a,b] | |
|---|---|
| | VAR00013 |
| Chi-Square | 7.873 |
| Df | 2 |
| Asymp. Sig. | .020* |
| a. Kruskal Wallis Test | |
| b. Grouping Variable: VAR00012 | |

## Discussion

(SDF) is a solution containing ionic silver, fluoride, and ammonia which

arrests the progression of carious lesions and prevents potential caries from developing [9,10].

The free fluoride ions are required for enamel and dentin remineralization; however, the silver precipitate is the main cause of the black stain of the treated dental tissues [11].

A positive approach to solving this problem is the immediate application of the KI solution after SDF treatment. As KI reacts with free silver ions, forming a creamy yellowish silver iodide precipitate that can be washed away and eliminates the black staining caused by SDF application [8].

The effectiveness of SDF in arresting caries was not affected, or minimally affected by the application of KI potassium. Many studies found that SDF dental tissues staining potential is modified by application of KI and that KI had little to no darkening ([12,13].

Our color analysis results showed that tested cavities prepared in dentin had lesser SDF darkening effect than tested cavities papered on enamel surface.

The teeth having no or in enamel cavities had more potential for discoloration on the margin of cavity or the surface.

These findings may be explained as

The KI solution is suggested to be able to react with SDF to form a bright yellow solid compound (silver iodide) [14] and this reaction may minimize the excess free silver ions that result in black staining [15].

While the bright yellow precipitates may be seen after KI application and washed away, slight darkening of tooth external tooth surfaces in this study could still be observed with the SDF + KI treatment to teeth surface or in enamel prepared cavities surfaces.

Also, this darkening increased and could be eye visible week by week.

Silver iodide is considered highly photosensitive and, by exposure to light, can dissociate into metallic silver and iodine, Hence, darkening still occurred on enamel margins.

Li et al reported immediate application of KI after SDF had nonsignificant effect on reducing the characteristic black stain of SDF [16]. SDF= KI treated enamel lesions turned yellow immediately after application of KI, but after 30 months the color of arrested lesions was stained similarly in case of the treatment with SDF or SDF-KI.

Application of a KI can delay the staining process but eventually the arrested enamel lesion becomes dark.

## Conclusion

SDF + KI treatment showed a low intensity staining in superficial and deep cavities. The intensity of staining decreased significantly with the increase of prepared cavity depth with the highest staining intensity on surface enamel treatment.

We recommend SDF + KI treatment especially in cavities with caries recurrence.

List of Abbreviations

SDF: Silver diamine fluoride

KI: Potassium Iodide

**Conflict of Interests: None**

**Ethics**. Qassim university scientific research ethics committee approval with the reference number: F2018-3004.

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# The Cytotoxic Effect of Iraqi *Rumex Acetosella* against Breast and Esophagus Cancer Cells

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## Abstract

**Background:** cell lines derived from cancer cells are frequently used in research, including use as a model to understand cancer and to identify potential new treatments. **The aim** of this article has been prepared to evaluate the anticancer effect of *Rumex Acetosella* that has been studied for their anticancer activity on esophagus and breast cancer cell line. **Method**: AMJ13 (new breast cancer *cell line* (*AMJ13*) has been established from an Iraqi breast cancer patient and SK-GT-4 cells (Human esophageal adenocarcinoma *cell line*) were treated with whole methanolic extract of the leaves of *Rumex acetosella.* the MTT assay to determine the anticancer activity was done using 96-well plates where cell lines were seeded at 1 × 104cells/well after 24 hrs.m the cells were treated with tested compounds at different concentrations. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The absorbency was determined on a microplate reader at 492 nm. **Conclusion**: Plant extract from *Rumex Acetosella* showed particularly strong anticancer capabilities since it inhibited actual tumor progression in a breast adenocarcinoma mouse model. Our results suggest that whole plant extracts are promising anticancer reagents.

***Keywords****: Medicinal Plants, Phytochemicals, Natural Compounds, anticancer Activity.*

## Introduction

The International Agency for Research on Cancer stated that the incidence of prevalence and mortality from different types of cancer for 184 countries were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide [1]. By 2030, it is expected that there will be 26 million newly diagnosed cancer cases and 17 million cancer deaths [2]. Meanwhile, although considerable focus and effort, cancer still an aggressive killer over the world. In addition, during the last years, new and novel anticancer agents in use clinically have not succeeded in curing the malignant conditions despite the cost and time for their development. Therefore, there is a continuous demanding to develop new, effective, and affordable chemotherapeutic drugs [3]. Herbal products have received increasing attention

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over the last 30 years for their substantial anticancer potential [4,5]. In parallel, there is increasing evidence for the potential of plant-derived compounds as suppressor of several stages of tumorigenesis and associated inflammatory processes. Approximately 60% of drugs currently are used for cancer therapy isolated from natural products [6].

Currently, more than 3000 plants worldwide have been investigated to reveal anti-cancer features. Globally, the plant-derived products for cancer treatment are from 10% to 45% and can reach up to 50% in Asiatic patients [7–9].

Breast cancer is one of the major common cancer types in women worldwide, accounting for approximately 570,000 deaths in 2015. Over 1.5 million females (25% of all women with cancer) are diagnosed with breast cancer yearly throughout the world. Moreover, Breast cancer is a heterogeneous disease on the molecular level and most malignant cancer in woman. In the past 10–15 years, treatment concepts have been evolved to consider this heterogeneity into account, with the more focus on biologically- directed products and less aggressive treatment to reduce the adverse effects [10.11].

In The united states of America, it is estimated that 30% of most new cancer patients among women are breast cancer in 2017 [12]. Importantly, breast cancer is metastatic cancer and can commonly transfer to other organs such as the bone, liver, brain and lungs which known on their incurability. Interestingly, early diagnosis of the disease can lead to promising prognosis and a significant survival rate [13]. Moreover, in north American, the 5-year relative survival rate of breast cancer is up to 80% due to the early detection of this disease [13].

On the other hand, there're several risk factors such as sex, family history, estrogen, ageing, gene mutations, and unhealthy lifestyle, which can increase the possibility of inducing breast cancer [14]. Most breast cancers happen in women and the number of cases is 100 folds greater than that in men [12]. Although the incidence rate of breast cancer in America increases year after year, the mortality rate decreases due to the wide early screenings and developed medical therapies [14].

Esophageal cancer is considered a serious malignancy regarding its prognosis and death rate. Approximately more than 400000 death cases worldwide in 2005 [15]. Esophageal carcinoma is the 8$^{th}$ common type of cancer, and the 6$^{th}$ most common cause of cancer-related deaths worldwide in developing countries, which account for more than 80% of total cases and deaths [16]. Over 490000 newly diagnosed cases of esophageal cancer were discovered in 2005 only [16]. In contrast, several other kinds of cancer are expected to reduce in incidence in the next 10 years by 2025, however, the prevalence of esophageal cancer is expected to elevated by 140% [5]. According to the National Cancer Institute in USA, esophageal cancer cases are around 17990 new cases and 15210 deaths in 2013 [17]. Regarding the Prognosis in esophageal cancer is majorly based on local invasion as well as spread to regional and distant tissues within the body. Esophageal cancer is known with its aggressiveness, spreading by a various of pathways including lymphatic spread, direct extension, and hematogenous metastasis. Because of lacking of serosa in the esophageal wall plays an important role in the local spreading of esophageal cancer. As no anatomical barrier, the initial tumor can extend quickly into the adjacent organ of the thorax and neck including the thyroid gland, lung, trachea, larynx, pericardium, aorta, and diaphragm [18].

The Rumex species, belonging to the Polygonaceae family, including about 200 species widely-distributed worldwide. Moreover, the name Rumex comes from the word for dart, alluding to the shape of the leaves in Latin [19]. Many researches have been reported in different ethnobotanicals and ethnopharmacological literature the traditional uses of Rumex species [20]. In some areas, the leaves of Rumex species (e.g., *R. acetosa, R. tuberous, R. acetosella, R. abyssinicus, R. Crispus, R. sanguineus, and R. thyrsiflorus, R.*

*vesicarius*) are used as foods, mainly in the forms of sauces sour soups (usually with milk), and salads [21].

*Rumex acetosella* is known as red sorrel, field sorrel, sheep's sorrel and sour weed, is a flowering plant species in the buckwheat family Polygonaceae. R. acetosella is belong to native of Europe, the Middle East, Russia, and northern Africa. Also, R. acetosella is naturalized in New Zealand, and the southern tip of Africa, western South America, Iceland, and the USA. R. acetosella is characterized by green arrowhead-shaped leaves and red-tinted deeply ridged stems, and it sprouts from an aggressive and spreading rhizome and its flowers emerge from a tall, upright stem while flowers are maroon in color in female plant [22].

Sorrel is an important part as it is considered as a nutritional powerhouse, providing significant amounts of substantial micronutrients, including vitamin A, a fat-soluble vitamin that helping human to maintain healthy vision, reproductive health, skin, immune function, and growth. Also, vitamin C, an important antioxidant that helps your body to resist infection [23].

Furthermore, one American formula is known essiac which is a common anti-cancer treatment. The whole plant is used in the fresh status as refrigerant, diaphoretic, and diuretic. Moreover, tea makes from the leaves is utilized in the treatment of scurvy, fevers, and inflammation. While, the juice of the leaves is useful in the treatment of urinary and kidney diseases [24-26]. leaf poultice is also applied to different cancers, cysts, etc, while the folk usage for cancer. Additionally, tea produced from the roots is astringent which can be used to treat excessive menstrual bleeding and diarrhea [27]. A one-cup of sorrel provides about 4 grams of fiber to maintain cholesterol levels, regular bowel movements, and blood sugar. Fiber can also be used in certain health conditions including cancer, type 2 diabetes and obesity [27].

Sorrel is also sometimes useful for medicinal purposes such as sheep's sorrel which is a primary ingredient in Essiac tea, which is a herbal tea that was rumored to treat breast cancer and prevent other diseases including HIV/AIDS and diabetes [27]. Moreover, Essiac tea, containing R. acetosella, is investigated for its action to scavenge reactive oxygen species and for its mechanism on DNA damage. This formula is used in homeopathic cancer treatment and also to treat a variety of diverse allergies, osteoporosis, and hypertension. In vitro, Essiac tea has been shown to suppress cells proliferation and to improve differentiation in human prostate cancer cell lines [28,29].

Finally, many phytochemical compounds have also been isolated from R. acetosella which include Anthraquinones (emodin, Chrysophanol, physcion, emodin-8-O-β-D-glucopyranoside barbaloin, sennosides, and rhein ) [30] , phenolic compound(gallic acid and p- coumaric), flavonoids (luteolin 7-O-glucoside, apigenin-C-glycosides quercetin derivatives, significant amounts of kaempferol derivatives, as well as myricetin and methylated derivatives of flavonols are present), stilbenoids and terpenoids [31-33].

So, in this study, it will be investigated anticancer activity of the leaves of *Rumex acetosella* naturally abundant tree found in every city in Iraq.

## Material and Methods

### Plant material collection.

The leaves of *Rumex acetosella* were collected from the peripheral of Sulaymaniyah of Kalo bazyan in March 2019. The plant was identified and authenticated by Prof. Dr. Sukaena Abass /Department of Biology /College of Sciences/ University of Baghdad. Leaves were washed thoroughly, dried under shade, and ground in a mechanical grinder to a fine powder.

### Method of work

The air-dried powder of the leaves is weighted then defatted with N-hexane to get rid of chlorophyll and waxy material and then extracted by maceration with methanol for 72hr then the extract is combined and dried by a rotary evaporator the dry extract is

weighted and then used for anticancer activity.

**Chemicals and Reagents**

**Table 1: chemical and reagent used**

| No. | Items | Company | Country |
|---|---|---|---|
| 1 | Trypsin/EDTA | Capricorn | Germany |
| 2 | DMSO | Santacruz Biotechnology | USA |
| 3 | RPMI 1640 | Capricorn | Germany |
| 4 | MTT stain | Bio-World | USA |
| 5 | Fetal bovine serum | Capricorn | Germany |

**1-EDTA (Ethylenediaminetetraacetic acid)** acts as a metal chelator, which is added to **trypsin** solutions to enhance activity. **EDTA** is added to remove the calcium and magnesium from the cell surface which allows **trypsin** to hydrolyze specific peptide bonds. The principal reason for using the **EDTA** along with **trypsin** is to remove cell to cell adhesion

2-The suggested mechanism for **DMSO** (Dimethyl Sulfoxide)

cytotoxicity is the effect on the physical properties of the phospholipids in membranes. As an amphipathic solvent, **DMSO** can interact with the plasma membrane allowing pores formation, which contributes to decrease membrane selectivity and increases **cell** permeability

**3-RPMI 1640**, also known as **RPMI medium**, is a growth medium used in cell culture.

4-**Molecular Targeted Therapies** (**MTT assay**) is used to measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity. ... The darker the solution, the greater the number of viable, metabolically active cells. This non-radioactive, colorimetric **assay** system using **MTT** was first described by Mosmann,

5-**Fetal bovine serum (FBS**) is a byproduct of harvesting cattle for the meatpacking industry—it's used extensively by both academic and industrial researchers as a supplement to the basal growth medium in cell culture applications. FBS is the liquid portion that remains after blood is drawn from the bovine fetus coagulates

### Instruments

**Table 2: instruments used.**

| No. | Item | Company | Country |
|---|---|---|---|
| 1 | CO2 incubator | Cypress Diagnostics | Belgium |
| 2 | Microtiter reader | Gennex Lab | USA |
| 3 | Laminar flow hood | K & K Scientific Supplier | Korea |
| 4 | Micropipette | Cypress Diagnostics | Belgium |
| 5 | Cell culture plates | Santa Cruz Biotechnology | USA |

### Maintenance of cell cultures

AMJ13 (new breast cancer ***cell line*** (***AMJ13***) has been established from an Iraqi breast cancer patient. It is considered unique because it is the first for an Iraqi population) and SK-GT-4 cells (Human esophageal adenocarcinoma *cell line*) were maintained in RPMI-1640 supplemented with 10% Fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37 °C [34,35].

### Cytotoxicity Assays

To determine the cytotoxic effect of *Rumex Acetosella*, the MTT assay was done using 96-well plates [36,37]. Cell lines were seeded at 1 × 104cells/ well. After 24 hrs. or a confluent monolayer was achieved, cells were treated with tested compounds at different concentrations. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT, and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µL of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking [38]. The absorbency was determined on a microplate reader at 492 nm; the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation [39,40].

Inhibition rate = A- B/A*100

where A is the optical density of control, and B is the optical density of the samples [41].

To visualize the shape of the cells under an inverted microscope, the cell was seeded into 24-well micro-titration plates at a density of 1×105 cells mL−1 and incubated for 24 h at 37 °C. Then, cells were exposed to *Rumex Acetosella* at IC50 concentration for 24hr. After the exposure time, the plates were stained with crystal violet stain and incubated at 37 °C for 10–15 min [39]. The stain was washed off gently with tap water until the dye was completely removed. The cells were observed under an inverted microscope at 100× magnification and the images were captured with a digital camera attached to the microscope [42,43].

## Statistical Analysis

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6 [44]. The values were presented as the mean ± SD of triplicate measurements and P value $< 0.05$ considered significant [45].

## Results and Discussion

In this study, the cytotoxic effect of *Rumex Acetosella* against cancer cells was evaluated. The antitumor activity of the *Rumex Acetosella* was assessed by studying their ability to inhibit the proliferation of cancer cells through cancer cells line of esophagus and breast. The results of this research are showed that highly significant cytotoxic activity against the human cancer cell lines as showed in the Figures below (1,2). These data suggest that there is an ability of *Rumex Acetosella* to suppress the growth of cancer cell lines and importantly, this effect is concentration-dependent manner (Fig. 1,2). The previous result is coordinated with traditionally used plant in folk medicine for long time as natural remedies with significant therapeutic effects in many areas including prevention of antimicrobial, cardiovascular diseases, anti-inflammatory, and anticancer activity. Also, the emergence of resistance to cancer chemotherapy and its substantial adverse effect has forced researchers toward natural products of plant and marine origin.

Although many compounds isolated from plants are being rigorously tested for their anticancer properties, it is becoming clear that the beneficial effects of whole plants are due to its complex interplay of the mixture of compounds which present in the whole plant (additive/synergistic and/or antagonistic) rather than single constituent alone [46, 47].

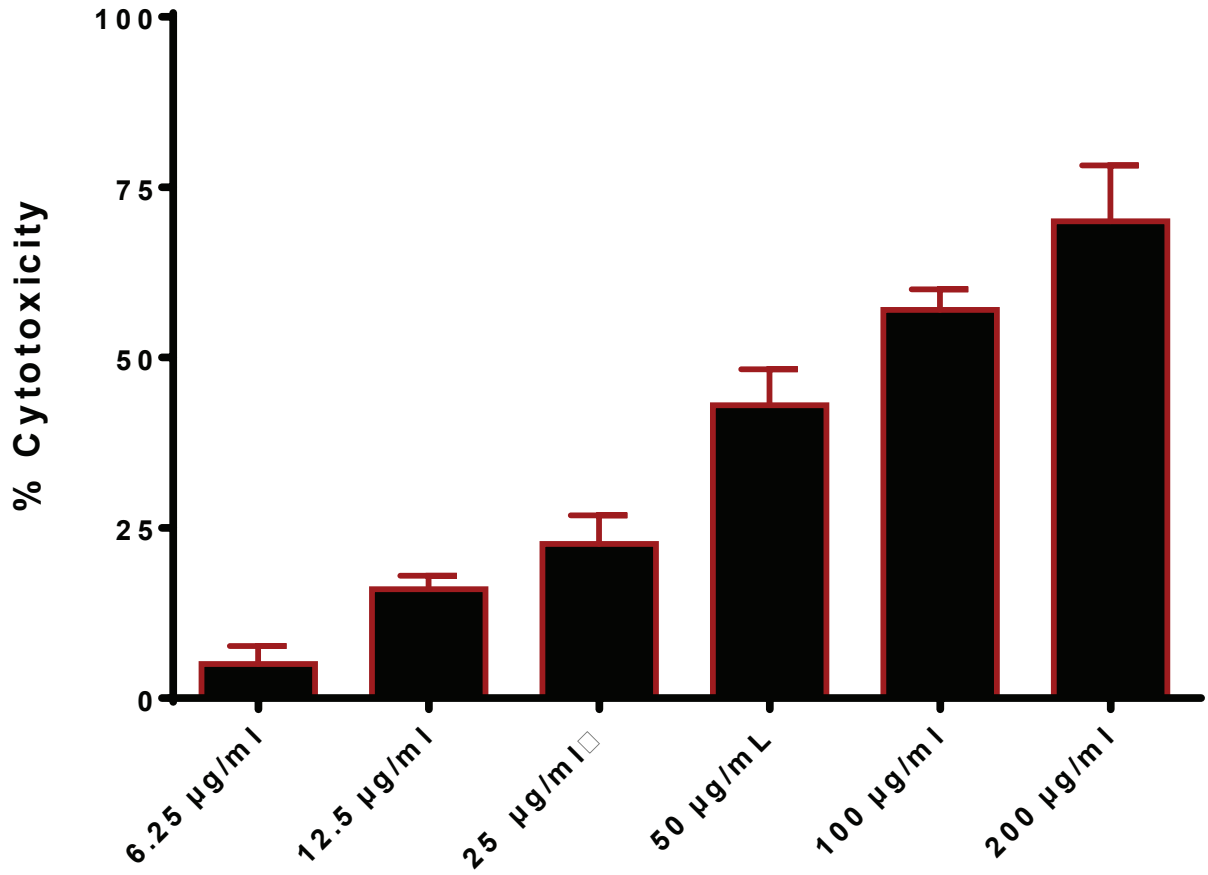


**Figure 1: Cytotoxic effect of *Rumex Acetosella* in SK-GT-4 cells. IC50= 42.62 µg/ml**

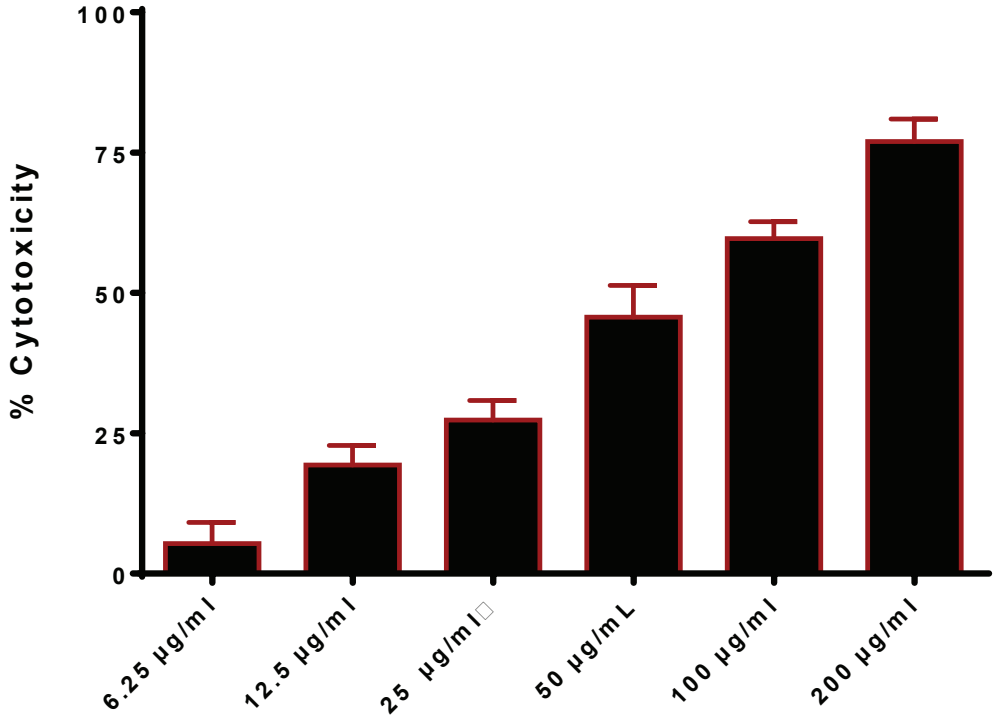


**Figure 2: Cytotoxic effect of *Rumex Acetosella* in AMJ13 cells. IC50= 29.33 µg/ml**

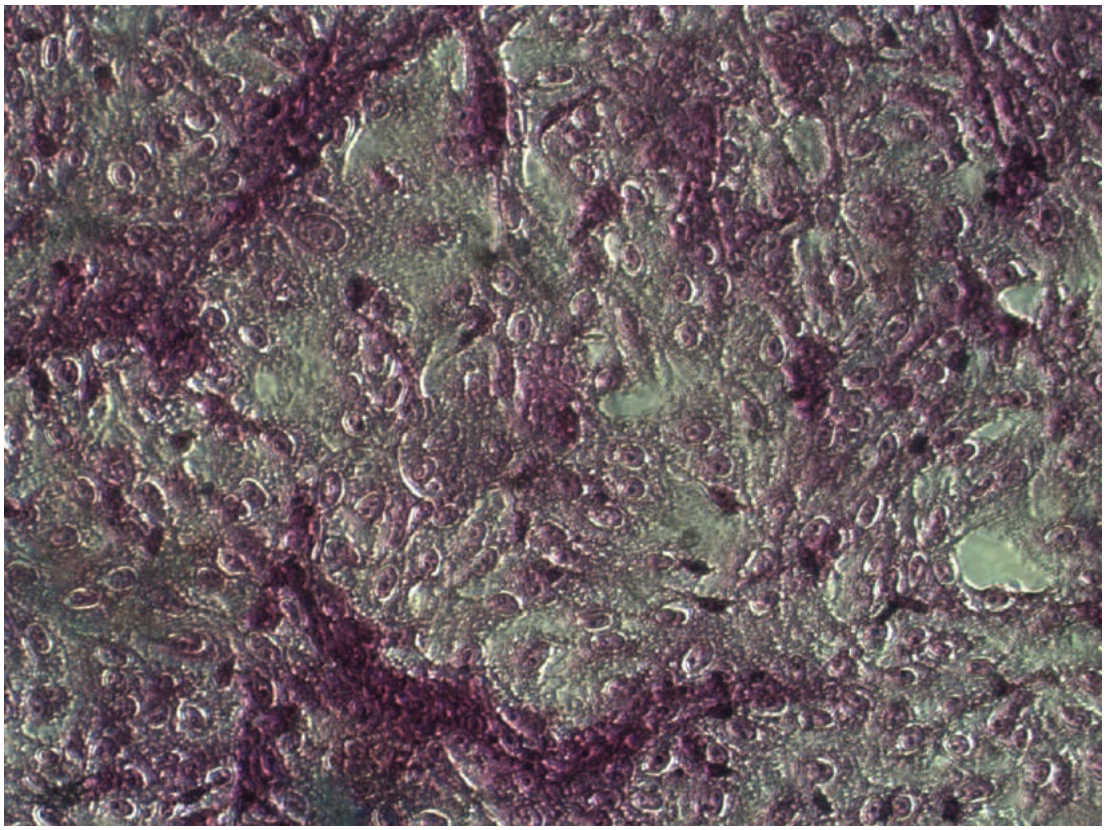

**Figure 3: Control untreated SK-GT-4 cells**

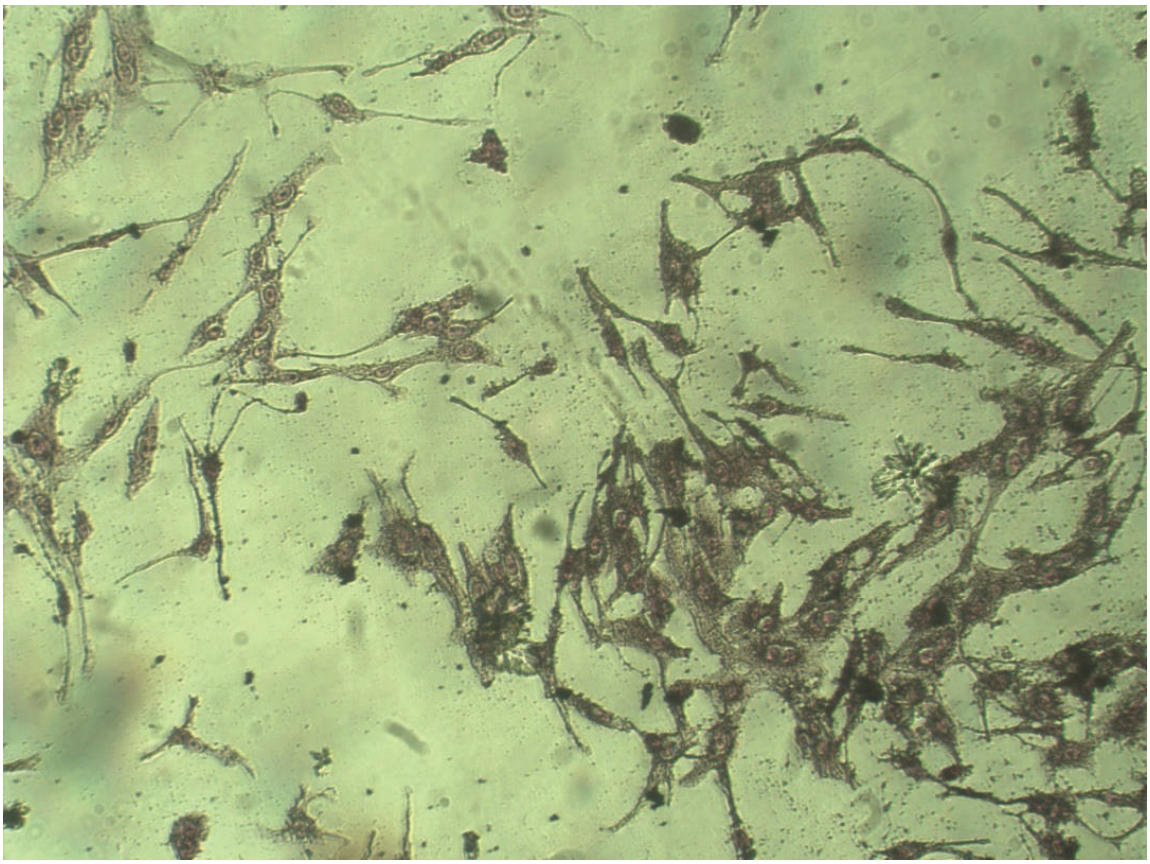

**Figure 4: Morphological changes in SK-GT-4 cells after treated with *Rumex Acetosella* extract**

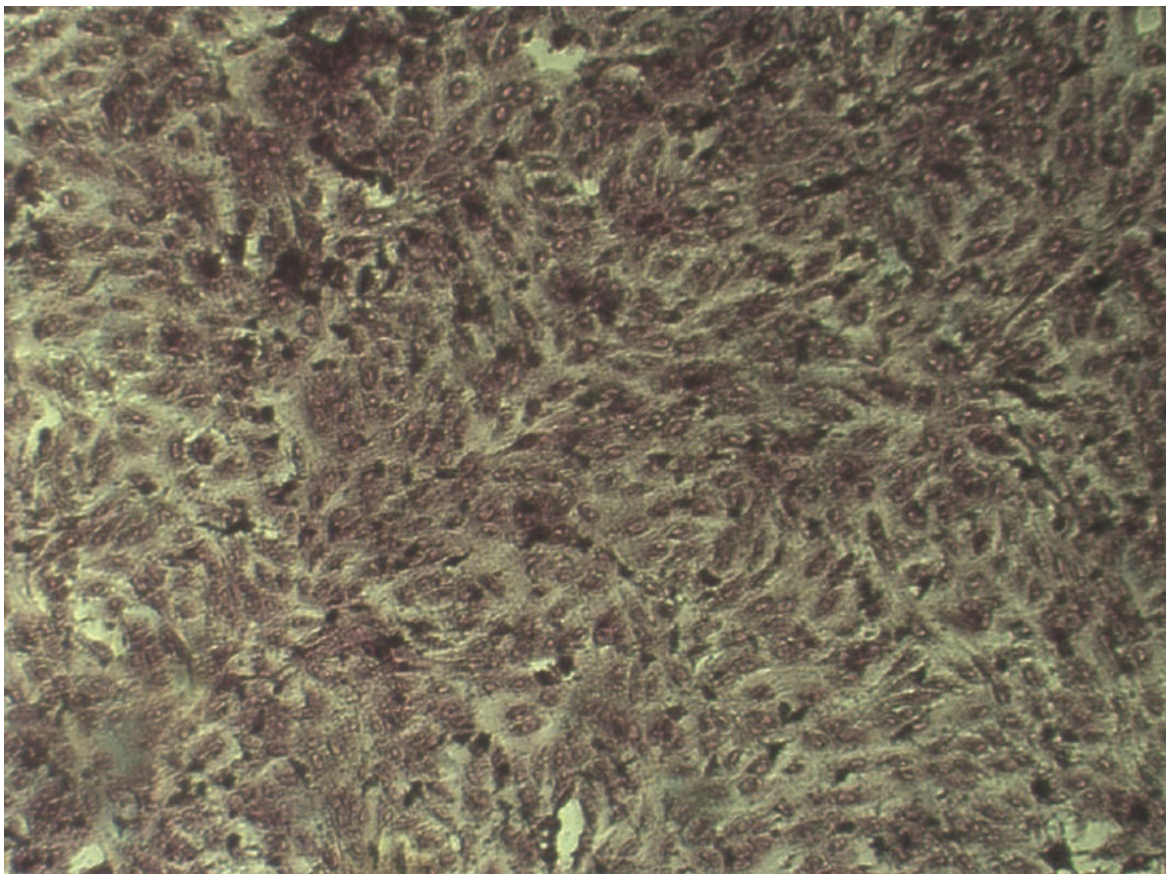

**Figure 5: Control untreated AMJ13 cells**

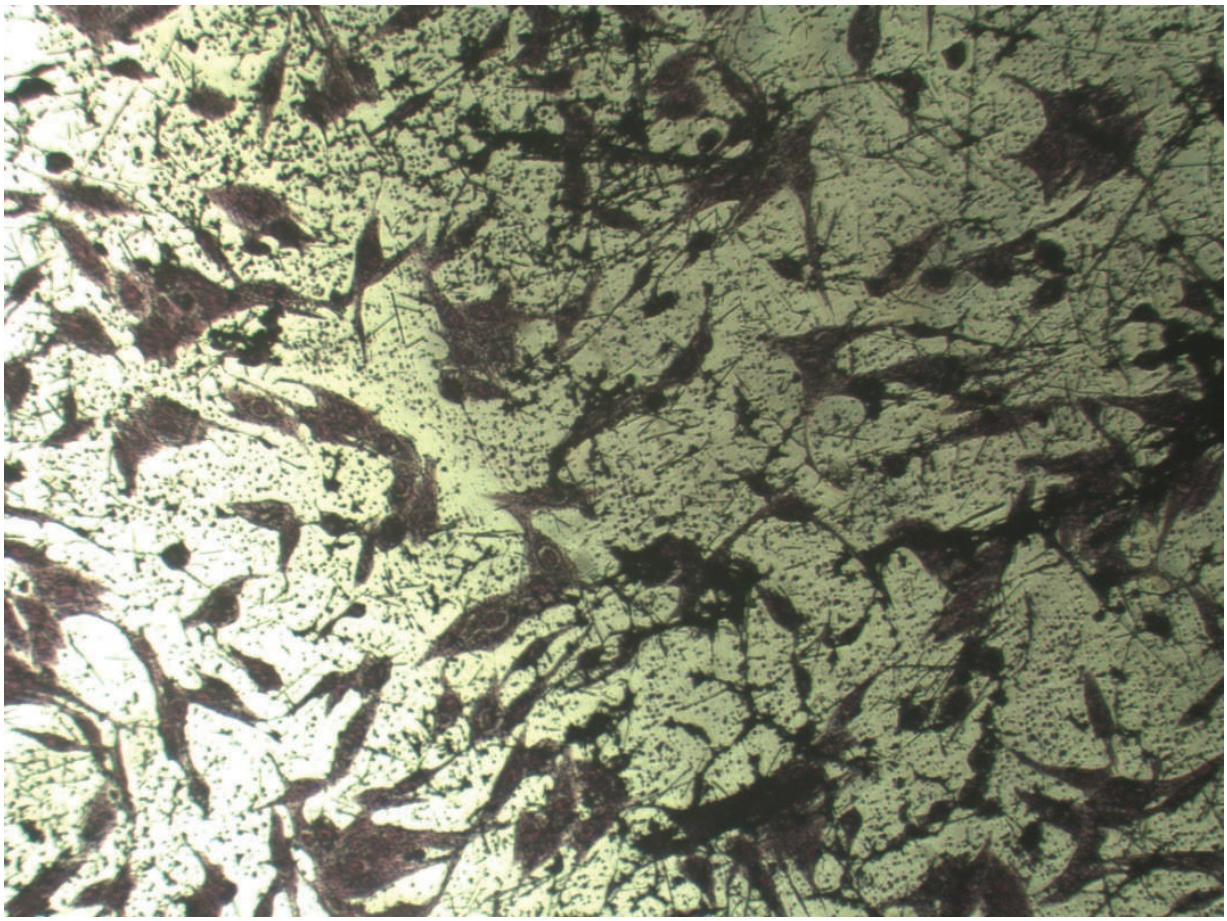

**Figure 6: Morphological changes in AMJ13 cells after treated with *Rumex Acetosella* extract.**

## Conclusions

Whole-cell extracts (methanolic extract) from *Rumex Acetosella*, plants indigenous to the coastal plain and desert areas of Iraq, exhibited dose and time-dependent killing capabilities on various human-derived hematological and solid tumor cell lines and primary cultures established from patients 'biopsies. The killing activity was specific toward tumor cells, as the plant extracts did not affect primary cultures of healthy cells line. Cell death caused by the whole plant extracts was via apoptosis. Plant extract from *Rumex Acetosella* showed particularly strong anticancer capabilities since it inhibited actual tumor progression in a both breast and esophagus cancer cell line. Our results suggest that whole plant extracts are promising anticancer reagents.

**Conflict of Interests:** The authors declare that there is no conflict of interests regarding the publication of this paper

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**Ethical Clearance:** According to ethical committee of college of pharmacy/ University of Baghdad

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# A Molecular Study of the *Microsporum Canis* and *Trichophyton Mentagrophytes* Associated Fungal Infection: Athlete's Foot among Farmers

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## Abstract

Athlete's foot (*Tinea pedis*) and toe nails infection (onychomycosis) are disease conditions caused by dermatophytes; both diseases are prevalent in adults, especially in farmers who often wear robber shoes during farming. Proper treatment of dermatophytes related skin diseases needs a proper understanding of susceptibility of the causative agents to the intended treatment option. This knowledge can only be derived from proper identification and characterization of the related fungi. In the present study, both traditional and molecular identification approaches were applied on cultured samples for detection and identification of *Tinea pedis*. From the PCR analysis, *Microsporum canis* and *Trichophyton mentagrophytes* were identified as the two commonest species implicated in Tinea pedis basing on their DNA banding patterns. From the fifty two identified dermatophyte isolates via the conventional identification methods, only 45 isolates were confirmed via molecular approach, with 25 isolated being confirmed as *M. canis* while 20 isolates were *T. mentagrophytes*. The same was confirmed with real time PCR quantification.

***Keywords:*** *Tinea pedis,* A*thlete's foot, M. canis, T. mentagrophytes, Ringworm.*

## Introduction

Dermatophytes are the commonest cause of superficial mycoses in human; the lesions of dermatophyte infection are characterized by desquamation, alopecia, circular disposition, and erythma of the edges [(1)] Among the infections caused by dermatophytes are athlete's foot, ringworm, and jockey itch; these infections result from direct contact with either the hyphae or spores of any Microsporum, Epidermophyton, or Trichophyton. Typically, the Trichophyton species (such as *T. mentagrophytes & T. rubrum*) and *M. canis* are commonly implicated in hair, nails and skin infections. Candidiasis and tinea infection remain the commonest forms of skin fungal infections; they are common in all regions of the globe and their incidence has continued to increase. [(2, 3).] Dermatophytes-related superficial infections can occur in both healthy and immune-compromised persons [(4)]; they also affect a wide range of mammals but not commonly found in birds. Dogs and cats are often infected with fungal dermatophytosis, including the cattle [(5)]. The very reason for the high incidence of dermatophytosis, is it is more contagiousness and difficult to identify along with costly treatment. This worms are easily transmitted to humans, especially farmers owing to their close contact with such infected

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animals. [6] The main lesions of dermatophytosis are alopecia, crusting, and erythema. These dermatophytes usually feed on keratin as such called as keratinophilic pathogenic fungi. These pathogens invade the epidermal stratum corneum and the related keratinized tissues (e.g., skin, hair, nails, fur) [6, 7]

Farmers were considered the best subjects for this study because the working conditions in the farm encourage the emergence of antropophilic fungal infections. Being that farmers spend long working days in the humid farming conditions, they are required to wear rubber footwear during such long periods as they continuously contact with organic matter. When compared to other professions, farmers are more exposed to pathogenic fungi; they can be infected by pathogenic fungi present in the soil or zoonotically [8, 9]; hence, dermatophyte-related infection, such as tinea pedis is commonly prevalent among farmers. Other conditions that can be caused by dermatophytosis complex include interspace fissuring, leukokeratosis, and hyperkeratosis. [10]

Taxonomically, these fungi belong to *Trichophyton* and *Microsporum*, where individual species which propagate by sexual methods are placed into *Arthroderma* of the *Ascomycota*. These are zoophilic, soil-adapted and mostly anthropophilic. [11]

The most commonly seen dermatophyte species within animals are *T. verrucosum, M. canis, T. mentagrophytes, M. gypseum, and M. nanum.* [12] Normally, routine laboratory diagnosis of dermatophytosis requires a direct examination of the clinical samples under a microscope, followed by in-vitro culturing of the sample. [13] A quick microscopic method of identifying fungal elements is direct emulsification of the clinical samples in 10% KOH solution; however, the sensitivity and specificity of this approach remains an issue as both depends on the skills and experience of the analyst. As per S Jarraud,et al. (2013) [14], the KOH smear approach can only approach a sensitivity level of 73.3% (CI: 66.3 to 79.5%) while the culture approach can achieve a sensitivity level of 41.7% (34.6 to 49.1%). Although the culture method is somewhat specific, it is a time-consuming approach [15], taking up to 4 weeks to achieve the final diagnosis. Furthermore, the polymorphism of dermatophytes couls make morphological identification misleading [16] Hence, PCR-based techniques are devised to reduce the diagnostic accruable process and achieve good results with a reasonable level of specificity and sensitivity in comparison to the conventional methods. [15, 17]

There are numerous PCR-based techniques, such as Random Amplification of Polymorphic genomic DNA (RAPD) [18], PCR fingerprinting [19] , Restriction Fragment Length Polymorphism (RFLP) [20] and real-time RTPCR [21] which are widely used to screen the dermatophytes *in vitro*. Moreover, TRFLP (PCR-terminal restriction fragment length polymorphism) [22] , nested PCR, and PCR-ELISA [23] are some of the other advanced techniques that have been found useful in identifying dermatophytes. But still there is a need to design a sensitive molecular-based approach to easily handle large amounts of clinical isolate samples within a short time. But the major hindrance to the cause is lack of a consistent method to extract fungal DNA from the clinical specimens. The aim of the current study is to use PCR to detect Tinea pedis using a method as described by [24] BL Wickes, (2018) for onychomycosis identification among suspected farmers. The process involves extracting fungal DNA and electrophoresing the PCR products.

*M. canis* is among the common causes of the simplex interdigital types of infections among the general populace, while *T. mentagrophytes* is implicated in inflammatory/vesicular Tinea pedis which is typically characterized by tense, hard vesicles on the mid-anterior plantar surface. [10] The vesicular lesions present diameters of about 1 to 5 mm and settle deep within the epidermis, while chronic moccasin or hyperkeratotic tinea pedis due to *T. mentagrophytes* infection exhibits chronic plantar erythema that ranges from slight scaling to severe hyperkeratosis.

Dry hyperkeratotic scaling normally affects the entire plantar surface and could extend to the lateral foot, with the foot's dorsal surface normally unaffected. Meanwhile, there could be manifestation of thick hyperkeratotic scales with fissures. [13, 25, 26] Mild to severe pruritus may be observed and painful fissures may be experienced while walking. This is normally encountered in patients with low immune status, such as those living with diabetics. [27] Both anthropophilic Microsporum and Trichophyton species are implicated in ulcerative tinea pedis; this condition is characterized by vesiculopustular lesions, erosions and ulcers that spreads rapidly; it is often associated with a secondary bacterial infection. The characteristic lesions usually have maceration and scaling border, normally starting in the 3rd to 4th interdigital spaces and progressing to the lateral dorsum, plantar surface, and sometimes to the entire sole. [10, 28]

## Materials and Methods

Clinical samples were procured from microbiology diagnostic laboratory at Baghdad Training Hospital. Samples from fifty two patients suspected of ringworm infection were initially examined by the macro- and micromorphology.

**Microscopic analysis:** Samples for fungal microscopic identification, piece of acetate tape was gently touched on the wound surface and carefully applied onto a glass slide with a drop of methylene blue stain and two to three drops of the KOH+DMSO. [29] The mount used for microscopy was equal volumes of 10% KOH and 40% Dimethyl sulphoxide (DMSO). A clean cover slip was placed on the sample and squashed to prevent any air bubbles from forming. 'The slides were then examined under low power (10X) and high power (40X) for the presence of hyphae and arthroconidia.

**Molecular analysis:** Clinical samples are procured from the microbiology diagnostic laboratory at Baghdad Training Hospital. Samples from 52 patients suspected of ringworm infection were initially examined via direct microscopy, followed by culturing on Sabouraud dextrose agar (SDA was supplemented with 0.05g/L chloramphenicol and 0.4g/L cycloheximide) at 30°C for 14days. Pure cultures were derived based on macro- and micro-morphology analysis. The pure cultures were used for the DNA extraction process from the isolation regions as described by [30].

**DNA extraction:** The clinical isolates were allowed to grow in 2ml of Sabouraud liquid medium with cycloheximide and chloramphenicol (HiMedia, India) and incubated in a shaker incubator for 8 days at 27°C. Following incubation, the cells were centrifuged at maximum speed, and the pellet obtained was re-suspended in 500µl of lysis buffer (400mM Tris-HCl [pH 8.0], 60mM EDTA [pH 8.0], 150 mM NaCl, 1% sodium dodecyl sulfate) for 10min RT. To the suspension, 150µl of potassium acetate (pH 4.8) was centrifuged at 12,000 × *g* for 1min following vortexing. The supernatant obtained was transferred to a fresh tube and the DNA pelleted with equal volumes of isopropyl alcohol was later stored in 50µl of TE (10mM Tris, 1mM EDTA) buffer.

**Multiplex PCR amplification:** PCR primers were designed using the primer 3 software, and used for amplifying Chitin synthase1 of dermatophytes. panDerm_F (5'GAAGAAGATTGTCGTTTGCATCGTCTC3') and panDerm_R (5'CTCGAGGTCAAAAGCACGCCAGAG3') were used in this study. Multiplex PCR mixtures consisted of (2x PCR Mix [HiMedia, India], Taq DNA polymerase 0.1U/µL, MgCl2 4.5mM, dNTPs (dATP, dCTP, dGTP, dTTP) 0.5mM of each dNTP). The total volume of the reaction mixture was 15µL with primer of 0.1µL (both FW and RV) and 2µL of DNA was used for the reaction. The amplification was carried out thermocycler ( Eppendorf make) with time-temperature profile for the PCR as follows: Initial denaturation at 95 °C for 3min, followed by 45s at 94 °C, 45s at 55 °C, and 45s at 72 °C for about 35cycles. Following amplification, the PCR products were run a 3% agarose gel. The PCR products would be

approximately 361 and 643bp for *T. mentagrophytes and M. canis* respectively.

**RNA extraction:** Total RNA was extracted according to the protocol as described by [31] TRIzol-mediated method was adopted so as to attain good quality RNA for qRT-PCR analyses. Each sample (*M.canis* and *T. mentagrophytes)* from the culture plates were placed into 2ml microfuge tubes with single 3mm tungsten bead. Following which the tubes were freeze into liquid nitrogen for 1min. following incubation, the microfuge tubes were placed in homogenizer and ground for 3min at 25-30Hz/s. To the extraction, 1ml of TRIzol was added and vortexed briefly for 5sec. The contents were then incubated for 5 min at RT, and centrifuged for 10min at 12,000 × *g* at 2–8°C. About 0.2ml of chloroform was added and tubes were vigorously vortexed for 15sec in a shaker. Following vortexing, the contents were centrifuge for 15min at 12,000 × *g* at 2–8 °C. The upper aqueous phase was transferred to a new 2ml vial, and added with 0.25ml of isopropanol. The contents were mixed thoroughly and incubated for 10min at RT, followed by centrifuge 10min at 12,000 × *g* at 2–8°C. The pellet obtained was washed with 1ml of 70% ethanol and centrifuged for 5min at 7500 × *g* at 2–8°C. The pellet obtained was air-dried and re-suspended in 20–50µl of RNase-free water.

**cDNA synthesis:** The cDNA synthesis was done with RT PCR kit using Superscript TMII Reverse Transcriptase, 200U/µl (HiMedia). About 2µg of the total RNA obtained was used along with random primers and 1µl of RT enzyme. The contents were incubated at 25°C for 10 min and then by incubating the contents at 70°C for 45min. This cDNA was used in real time PCR.

**Real-time PCR:** Primers used for the traditional PCR were used for the real time PCR also. The real-time PCR assay was then performed according to Salam Abbas *et al* (2019) [32] using SYBR Green (HiMedia). The primers of 600nM and 1µl of the cDNA was used with a total volume of 12.5µl. The assay was done in duplicates alongside with negative control. Housekeeping gene β-actin (β-act) [Accesion no: XM_002845542] was used in the study.

**Expression of pander F members:** Real-time PCR was carried on the samples in the Corbett Research cycler (Bio-Rad). The panDerm_F primers (both FW and RV) of 600nM concentration was used in the program. 1.1µl of RNA was used for about 40 cycles at 94°C for 45s, 62°C for 50s, and with an elongation at 71°C for 55s. Beta actin primers (FW: CTCCTGAGGCTCTCTTCC; RV: GTAGTACCGCCGGACATG; Product length 142bp] was used (Ciesielska, A, 2018) [33] was also amplified for a comparative analysis of the expression. The comparative analysis was done by ΔΔCt method. The Ct values thus obtained for the desired gene was normalized to its housekeeping gene.

## Results:

All samples were first processed via direct microscopy and culturing prior to multiplex PCR analysis. The direct microscopic evaluation using KOH was performed on all the 52 subjects with physical manifestation of Athlete's foot. However, 19 clinical samples turned out negative upon culturing while the rest of the samples were positive. *M. canis* was identified as the main causative agent, accounting for 25 (80.5%) of the positive cases, while *T. mentagrophytes* was implicated in 20 positive cases. The data in figure 1 showed that the PCR process improved the detection rate of dermatophytes by about 21.1 %.

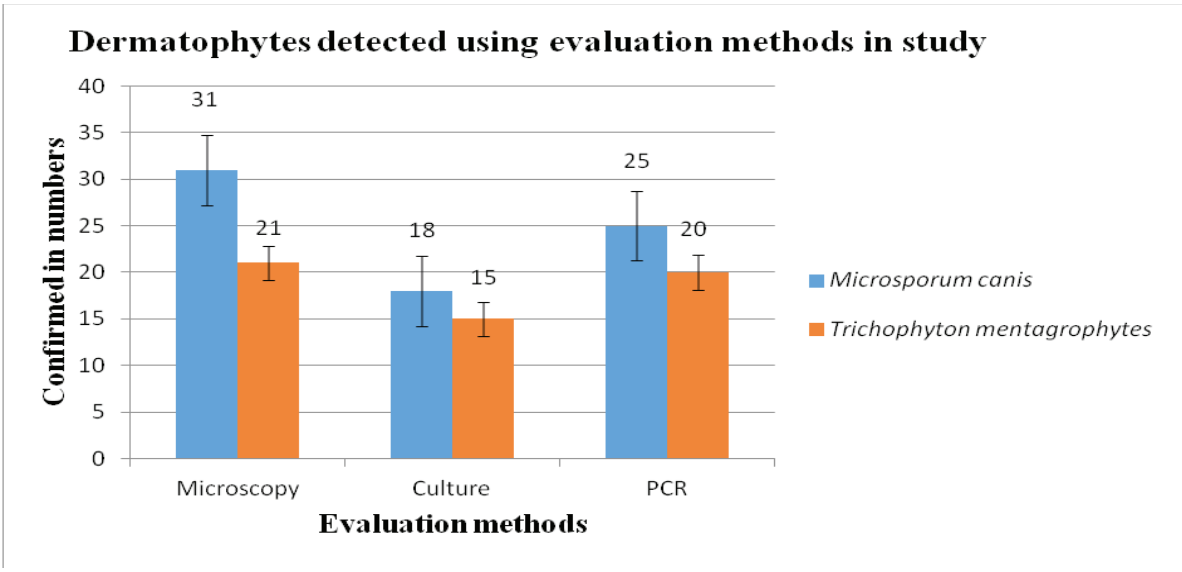


**Figure 1: Graph showing the confirmed Dermatophytes in numbers using the three methods of evaluation Microscopy observation, culturing and PCR.**

The clinical isolates were differentiated based on their gross colonial and microscopic presentations. *T. mentagrophytes* produces powdery to downy colonies while *M. canis* produces powdery to granular colonies. Owing to the inconsistency in the cultural characteristics of the fungi, further testing was performed to establish their identity. Both *T. mentagrophytes* and *M. canis* can hydrolyse urea but only *T. mentagrophytes* can produce yellow diffusible pigments on Littman Oxgall agar and this differentiates it from *M. canis*.

**Table 1: Classification of lesion changing patterns resulting in the detection of dermatophytes scraping skin samples**

| Fungal causative agents | Dermatophytes lesion patterns. | No. | Percentage |
|---|---|---|---|
| Microsporum canis | Inflammatory or vesicular (vesiculobullous) | 10 | 32.2% |
| | Chronic hyperkeratotic (moccasin) | 18 | 58% |
| | Ulcerative Tinea pedis | 3 | 9.7% |
| Trichophyton mentagrophytes | Inflammatory or vesicular (vesiculobullous) | 3 | 14.2% |
| | Chronic hyperkeratotic (moccasin) | 17 | 80.9 % |
| | Ulcerative Tinea pedis | 1 | 4.7 |
| Total | | 52 | 100% |

Dermatophytosis simplex lesions typically manifests between the 4th and 5th digits and commonly presents with itching, malodour, and burning as observed in the 52 patients included in this study. The most common clinical type was chronic hyperkeratotic (moccasin) is seen in 18 (58%) and 17 (80.9%) of patients infected with *M. canis* and *T. mentagrophytes,* respectively. This syndrome is common in immunocompromised persons, such as diabetic patients and normally involves *Trichophyton* species [34](Leung et al., 2020). (See Table 1).

**Microscopic analysis:** Samples obtained from the infection sites were viewed under low (10X) and high power (40X) for screening the hyphae and arthroconidia with methylene blue. From the staining, it was found that 31 of them are *M.canis* and 21 of them are *T. mentagrophytes* out of the 52 samples isolated [Figure 2].

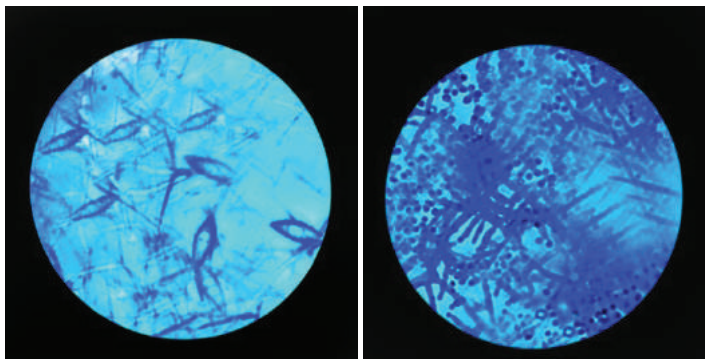

**Figure 2: Images as viewed under 40X power, Left:** *M. canis*; **Right:** *T. mentagrophytes.*

**PCR amplification:** The results indicate that the PCR could be the most accurate one to detect dermatophyte-specific DNA from the microbes (*M. canis and T. mentagrophytes*) in the study. All samples were first processed via direct microscopy and culturing prior to multiplex PCR analysis. On running the products on 3% agarose gel, it was found that specified bands were obtained at 361 and 643bp for *T. mentagrophytes and M. canis* respectively. This confirms the presence of the genes. However, some bands seems to be varying in intensity for both the genes used in the study [Figure 3, 4].

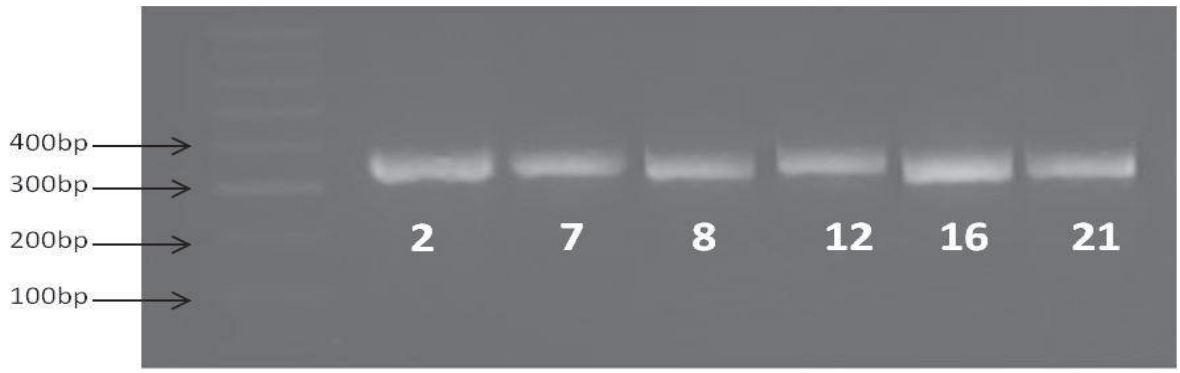


**Figure 3: 3% agarose gel showing the amplified bands at approximately 361bp. Molecular marker of 100bp was used in the study. Bands of few PCR products of the mentioned sample numbers were 2, 7, 8, 12, 16 and 21.**

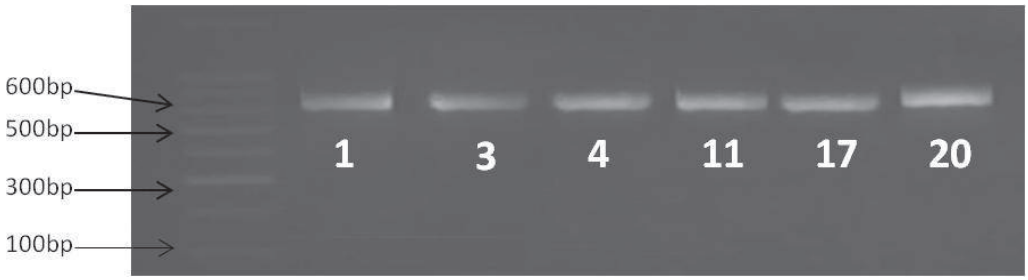


**Figure 4: 3% agarose gel showing the amplified bands at approximately 643bp. Molecular marker of 100bp was used in the study. Bands of few PCR products of the mentioned sample numbers were 1, 3, 4, 11, 17 and 20.**

**Real time PCR of gene members:** The results obtained which are normalized to its housekeeping gene (GAPDH), reduces the differences between the samples. The level of mRNA expression of the gene for both the samples was separately studied. The sample with the lower ΔΔCt values was chosen to be the calibrator so the samples were compared with that calibrator. The Ct values of beta actin was found to be 11. The ct values for the gene of *T. mentagrophytes and M. canis* were found to be 16 and 17 respectively. Based on the Ct values, the Ct values of the samples (treatment with the drug) were normalized to the housekeeping gene (beta actin). The beta actin expression was considered to be 100% in the study. From the calculated 2−ΔΔCt values, it was found equal expression for both the samples. In *T. mentagrophytes* the gene was expressed 64 times, and in *M. canis* it was expressed 32 times.

## Discussion

Till the last 2 to 3 decades, microscopy and culture methods on SDA were alone used for diagnosis of dermatophytes [35]. Sometimes, due to little sporulation, identification of dermatophytes becomes impossible or difficult in a lab setting. Such problems are addressed by isolating the DNA and sequencing with aligning to the available fungal databases.

But still such methods are time-consuming and very expensive as such we proposed a method to show good detection following the traditional methods. Even though we could end up with 100% detection of dermatophyte DNA but still the assay needs to be better optimized. And moreover, we need to use different reagents than those which are previously used [36] . And also, the gold standard methods could only permit the correct identification, if at all carried by a qualified mycologist who is having a sound knowledge on the morphological features of dermatophytes micro- and macroconidia. In addition to this, presence of chlamydospores also need to be addressed. In the present context, the detection was quite possible within 5hr of time (excluding the culturing time) and moreover, these results are objective and do not need an experienced investigator.

In addition to the above pros, this method uses a small amount of sample. And very few diagnostic laboratories round the world, use such modern molecular assays like PCR due to lack of a proper diagnostic algorithm [37]. However, the method of fungal DNA extraction previously proposed brings is simple, accurate and cost effective for diagnosing dermatophytosis from animals. This could be used as a standard method in the coming years for veterinary diagnosis.

This study explored the evolution, spread and reproduction of two major fungi (*M. canis and T. mentagrophytes*) implicated in tinea pedis. The medical practitioners will be well-guided in managing *Tinea pedis* if provided with the accurate clinical data that detailed the appropriate diagnosis and correct identification of the causative agents. These suggest that habits, such as wearing of rubber foot

wears and nylon socks, as well as practice of animal husbandry, could be the most important determinants of the frequency of superficial fungal infections and their aetiological agents among farmers and forestry workers. The outcome of this study confirmed the screening of dermatophyte-specific DNA within the clinical samples from farmers. Adaptation of this assay could pave ways to design a much more reliable method to detect the dermatophytes-specific DNA directly from the patients in less time.

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**Ethical Clearance**- No Need

**Source of Funding-** this done by myself and our colloquies

**Conflict of Interest** – the Authors have declared, **No Conflict of Interest**

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# Features of Teleroentgenographic Indicators of the Position of the Teeth and the Profile of the Soft Tissues of the Face in Adolescents with Different Profiles and Types of Faces According to Schwarz A.M.

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## Abstract

One of the most relevant areas of research in biomedical anthropology is the establishment of morphometric parameters of the human body, individual organs and structures of the body, in particular cephalometric indicators and indicators of the jaw-facial apparatus. Such research is important from the point of view of practical medicine, first of all improvement of methods of identification of the person in forensic medicine and for creation of a normative database for diagnostics of dental deformities and improvement of consequences of surgical, orthopedic and orthodontic interventions. One of the most accurate and effective methods for determining cephalometric parameters is the method of lateral teleroentgenography. The object: to establish teleroentgenographic indicators that characterize the position of teeth and the profile of facial soft tissues and determine their sexual characteristics in Ukrainian young men and young women with orthognathic occlusion depending on the profiles and types of faces according to Schwarz A. M. Conclusions: the results prove the need to consider not only age, but also sex, type and profile of the face for an individualized approach in determining the normative teleroentgenographic indicators of human, are important in forensic medicine to improve the efficiency of identification and in practical dentistry to assess the condition of the dental apparatus and ensure effective therapeutic and aesthetic results of orthopedic and orthodontic dentistry.

***Key words:*** *forensic odontology, identification, cephalometry, Schwarz method.*

## Introduction

One of the most relevant areas of research in biomedical anthropology is the establishment of morphometric parameters of the human body in general and individual organs and structures of the body in particular. This fully applies to both cephalometric indicators and indicators of the jaw-facial apparatus. Such research is important from the point of view of practical medicine, first of all improvement of methods of identification of the person in forensic medicine. [5, 15, 16]

The definition of biometric indicators for assessing the role of constitutional factors in the development of diseases is indisputable and increasingly relevant;

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in dentistry, to create a regulatory database for the diagnosis of dental-jaw deformities and improve the consequences of surgical, orthopedic and orthodontic interventions; sports medicine – first of all, to determine the most promising people in certain sports. [2, 19, 23] One of the most accurate and effective methods of determining cephalometric parameters is the method of lateral teleroentgenography, which allows the analysis and determination of not only qualitative characteristics but also quantitative indicators of both bone structures of the head and soft tissues of the face.[1]

*The aim of the work* is to establish teleroentgenographic indicators that characterize the position of teeth and the profile of facial soft tissues and determine their sexual characteristics in Ukrainian young men and young women with orthognathic occlusion depending on the profiles and types of faces according to Schwarz A. M.

## Materials and Methods

We analyzed lateral teleroentgenograms of young people in Ukraine – 49 young men (YM) aged 17 to 21 years and 76 young women (YW) aged 16 to 20 years with a physiological bite as close as possible to orthognathic. Both YM and YW were divided into 6 separate study groups depending on the profile or type of person according to Schwarz A. M. [24, 25]

Teleroentgenographic study was performed on a dental cone-beam tomograph Veraviewepocs 3D Morita (Japan) followed by analysis using a licensed program OnyxCeph³ ☐, a version of 3DPro (Germany), developed for image analysis in dentistry.

Cephalometric points were determined according to the recommendations of Phulari B. S. [18] and Doroshenko S. I., Kulginsky E. A. [7]

Teleroentgenographic indicators of the position of the teeth and the profile of the soft tissues of the face were determined according to the method of Schwarz A. M. [24, 25] (Fig. 1).

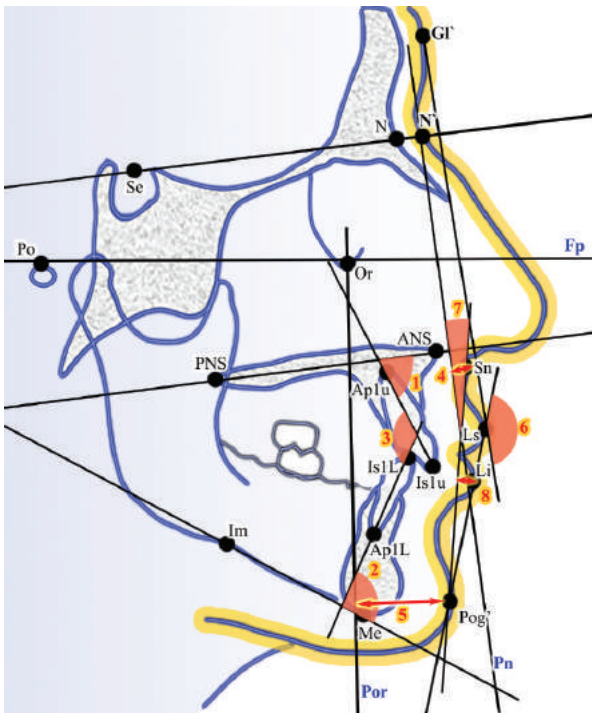


**Fig. 1. Teleroentgenographic indicators of the position of the teeth and the profile of the soft tissues of the face according to the method of Schwarz A. M.: 1 – angle Max1-SpP S-arz; 2 – angle Mand1-MP Schwars; 3 – angle II; 4 – distance Sn-Pn; 5 – Pog'-Por distance; 6 – angle Gl'LsPog'; 7 – angle SnPog'-Pn; 8 – distance Li-SnPog'.**

Statistical mathematical processing of the research results was performed in the licensed package "Statistica 6.0" using non-parametric methods of evaluation of the obtained results. The reliability of the difference between the values between the independent quantitative values was determined using the U-test of Mann-Whitney.

## Results

When comparing the values of the angle Max1-SpP S-arz in YM found higher values of this indicator: in the group with the first profile of the face (1PF), compared with the second (2PF) and third profiles (3PF) (respectively, $p<0.05$ and $p<0.01$); in representatives with the first (1TF) and second type (2TF) of face compared with the third type (3TF) (respectively, $p<0.001$; $p<0.05$) (Table 1). This figure is higher in YW 2TF than in YW 1TF ($p<0.05$) (see Table 1). The angle of Max1-SpP S-arz is higher in YM 1TF ($p<0.05$) and 1PF ($p=0.092$) compared to YW with the corresponding profile or face type (see Table 1).

**Table 1. Teleroentgenographic indicators by the method of Schwarz A.M., which characterize the position of the teeth in YM and YW with different profiles, or with different face types.**

| Groups | Young men | | | Young women | | | p |
|---|---|---|---|---|---|---|---|
| | (M±σ) | p!-! | | (M±σ) | p!-! | | |
| Max1-SpP S-arz (°) | | | | | | | |
| Profile 1 | 71,70±5,02 | p1-2 | <0,05 | 69,57±5,76 | p1-2 | >0,05 | =0,092 |
| Profile 2 | 67,44±4,80 | p1-3 | <0,01 | 68,60±4,15 | p1-3 | >0,05 | >0,05 |
| Profile 3 | 66,71±5,42 | p2-3 | >0,05 | 67,75±6,64 | p2-3 | >0,05 | >0,05 |
| Type 1 | 72,85±3,60 | p1-2 | >0,05 | 68,70±5,44 | p1-2 | >0,05 | <0,05 |
| Type 2 | 70,22±5,19 | p1-3 | <0,001 | 69,71±5,65 | p1-3 | >0,05 | >0,05 |
| Type 3 | 65,50±5,06 | p2-3 | <0,05 | 68,14±6,22 | p2-3 | >0,05 | >0,05 |
| Mand1-MP Shwars (°) | | | | | | | |
| Profile 1 | 83,96±6,26 | p1-2 | >0,05 | 84,59±5,46 | p1-2 | >0,05 | >0,05 |
| Profile 2 | 84,22±8,01 | p1-3 | >0,05 | 86,93±7,20 | p1-3 | <0,05 | >0,05 |
| Profile 3 | 85,35±8,82 | p2-3 | >0,05 | 87,96±7,93 | p2-3 | >0,05 | >0,05 |
| Type 1 | 89,62±4,89 | p1-2 | <0,05 | 86,39±4,76 | p1-2 | >0,05 | =0,081 |
| Type 2 | 84,67±7,24 | p1-3 | <0,001 | 88,00±6,81 | p1-3 | >0,05 | >0,05 |
| Type 3 | 80,61±7,06 | p2-3 | >0,05 | 84,34±7,74 | p2-3 | =0,056 | >0,05 |
| II (°) | | | | | | | |
| Profile 1 | 133,1±5,4 | p1-2 | >0,05 | 129,7±8,2 | p1-2 | =0,096 | =0,054 |
| Profile 2 | 132,2±7,8 | p1-3 | >0,05 | 133,4±7,2 | p1-3 | <0,05 | >0,05 |
| Profile 3 | 134,8±10,4 | p2-3 | >0,05 | 134,3±8,7 | p2-3 | >0,05 | >0,05 |
| Type 1 | 137,6±5,2 | p1-2 | =0,097 | 131,3±8,1 | p1-2 | >0,05 | <0,05 |
| Type 2 | 134,8±8,4 | p1-3 | <0,01 | 134,0±7,7 | p1-3 | >0,05 | >0,05 |
| Type 3 | 129,3±6,9 | p2-3 | <0,05 | 130,6±9,0 | p2-3 | =0,083 | >0,05 |

In YW 3PF, the value of the angle Mand1-MP Shwars is greater than in YW 1PF ($p<0.05$); and in YW 2TF – than in YW 3TF ($p=0.056$) (see Table 1). The Mand1-MP Shwars angle in YM 1TF is greater than in YM 2TF and 3TF (respectively, $p<0.05$ and $p<0.001$) (see Table 1). The magnitude of the Mand1-MP Shwars angle is greater ($p=0.081$) in YM with 1TF than in YW with the corresponding face type (see Table 1).

Angle II in YW 3PF and 2PF is greater than in YW 1PF (respectively $p<0.05$ and $p=0.096$); and YW 2TF – larger than YW 3TF ($p=0.083$) (see Table 1). The value of the angle II have higher values: in YM 1TF and 2TF than in YM with 3TF (respectively, $p<0.01$ and $p<0.05$), as well as in YM 1TF than in YM 2TF ($p=0.097$) (see table. 1). Angle II values were found to be higher in YM 1TF ($p<0.05$) and 1PF ($p=0.054$) than in YW with the corresponding type or facial profile (see Table 1).

In YM 3TF and 2TF the distance Sn-Pn is greater than in YM 1TF (respectively $p<0.01$ and $p=0.093$) (Table 2). In YW 3TF and 2TF, the Sn-Pn distance is greater than in YW 1TF and 2TF (respectively, $p<0.001$ and $p<0.01$); also the value of this indicator is higher in YW 2TF than in YW 1TF ($p=0.085$) (see Table 2). YM 1PF and 2TF have a greater Sn-Pn distance ($p<0.05$ in both cases) than YW with the corresponding profile or face type; also in YM with 3TF – higher than in YW 3TF ($p=0.054$), and in YM 1TF – higher than in YW 1TF ($p=0.084$) (see Table 2).

**Table 2. Teleroentgenographic parameters of soft tissues of the face by the method of Schwarz A.M. in YM and YW with different profiles, or with different face types.**

| Groups | Young men | | | Young women | | | p |
|---|---|---|---|---|---|---|---|
| | (M±σ) | $p_{!-!}$ | | (M±σ) | $p_{!-!}$ | | |
| Sn-Pn (мм) | | | | | | | |
| Profile 1 | 12,70±3,56 | $p_{1-2}$ | >0,05 | 10,30±3,47 | $p_{1-2}$ | >0,05 | **<0,05** |
| Profile 2 | 12,78±4,12 | $p_{1-3}$ | >0,05 | 10,60±3,54 | $p_{1-3}$ | >0,05 | >0,05 |
| Profile 3 | 12,47±4,93 | $p_{2-3}$ | >0,05 | 9,875±3,366 | $p_{2-3}$ | >0,05 | >0,05 |
| Type 1 | 10,23±2,65 | $p_{1-2}$ | **=0,093** | 8,044±3,735 | $p_{1-2}$ | =0,085 | =0,084 |
| Type 2 | 12,22±3,80 | $p_{1-3}$ | **<0,01** | 9,917±3,020 | $p_{1-3}$ | **<0,001** | **<0,05** |
| Type 3 | 14,78±4,29 | $p_{2-3}$ | >0,05 | 12,21±2,18 | $p_{2-3}$ | **<0,01** | **=0,054** |
| Pog'-Por (мм) | | | | | | | |
| Profile 1 | 20,17±5,19 | $p_{1-2}$ | **<0,05** | 19,30±4,67 | $p_{1-2}$ | **<0,01** | >0,05 |
| Profile 2 | 24,89±3,55 | $p_{1-3}$ | **<0,001** | 22,40±3,62 | $p_{1-3}$ | **<0,001** | >0,05 |
| Profile 3 | 29,24±7,70 | $p_{2-3}$ | >0,05 | 25,46±4,56 | $p_{2-3}$ | **<0,05** | >0,05 |
| Type 1 | 21,23±5,80 | $p_{1-2}$ | >0,05 | 20,22±4,94 | $p_{1-2}$ | >0,05 | >0,05 |
| Type 2 | 23,17±8,34 | $p_{1-3}$ | **<0,01** | 21,79±5,62 | $p_{1-3}$ | **<0,05** | >0,05 |
| Type 3 | 27,33±5,71 | $p_{2-3}$ | **<0,05** | 23,21±4,72 | $p_{2-3}$ | >0,05 | **<0,05** |

***Cont...*** **Table 2. Teleroentgenographic parameters of soft tissues of the face by the method of Schwarz A.M. in YM and YW with different profiles, or with different face types.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| GI'LsPog' (°) | | | | | | | |
| Profile 1 | 157,9±5,8 | $p_{1-2}$ | **<0,05** | 160,8±4,4 | $p_{1-2}$ | **<0,01** | **<0,05** |
| Profile 2 | 162,9±4,5 | $p_{1-3}$ | **<0,001** | 165,7±4,5 | $p_{1-3}$ | **<0,001** | >0,05 |
| Profile 3 | 167,4±5,4 | $p_{2-3}$ | **<0,05** | 169,3±6,1 | $p_{2-3}$ | **<0,05** | >0,05 |
| Type 1 | 163,8±7,2 | $p_{1-2}$ | >0,05 | 166,0±6,1 | $p_{1-2}$ | >0,05 | >0,05 |
| Type 2 | 161,8±7,1 | $p_{1-3}$ | >0,05 | 166,2±6,3 | $p_{1-3}$ | **<0,05** | **=0,060** |
| Type 3 | 161,2±6,5 | $p_{2-3}$ | >0,05 | 161,8±5,5 | $p_{2-3}$ | **<0,05** | >0,05 |
| SnPog'-Pn (°) | | | | | | | |
| Profile 1 | 11,26±4,52 | $p_{1-2}$ | **<0,01** | 9,027±2,930 | $p_{1-2}$ | **<0,001** | **=0,068** |
| Profile 2 | 6,000±2,646 | $p_{1-3}$ | **<0,001** | 5,933±2,282 | $p_{1-3}$ | **<0,001** | >0,05 |
| Profile 3 | 4,235±2,611 | $p_{2-3}$ | >0,05 | 3,250±2,541 | $p_{2-3}$ | **<0,01** | >0,05 |
| Type 1 | 8,923±5,204 | $p_{1-2}$ | >0,05 | 6,435±3,369 | $p_{1-2}$ | >0,05 | >0,05 |
| Type 2 | 8,333±5,841 | $p_{1-3}$ | >0,05 | 6,625±4,116 | $p_{1-3}$ | >0,05 | >0,05 |
| Type 3 | 6,611±3,292 | $p_{2-3}$ | >0,05 | 6,690±3,704 | $p_{2-3}$ | >0,05 | >0,05 |
| Li-SnPog' (мм) | | | | | | | |
| Profile 1 | 2,391±1,803 | $p_{1-2}$ | >0,05 | 2,568±1,923 | $p_{1-2}$ | **<0,01** | >0,05 |
| Profile 2 | 1,889±1,691 | $p_{1-3}$ | >0,05 | 0,933±1,534 | $p_{1-3}$ | **<0,05** | >0,05 |
| Profile 3 | 1,529±1,908 | $p_{2-3}$ | >0,05 | 1,125±2,007 | $p_{2-3}$ | >0,05 | >0,05 |
| Type 1 | 1,846±2,154 | $p_{1-2}$ | >0,05 | 1,783±2,315 | $p_{1-2}$ | >0,05 | >0,05 |
| Type 2 | 1,889±1,641 | $p_{1-3}$ | >0,05 | 1,458±2,000 | $p_{1-3}$ | >0,05 | >0,05 |
| Type 3 | 2,222±1,833 | $p_{2-3}$ | >0,05 | 2,069±1,771 | $p_{2-3}$ | >0,05 | >0,05 |

The Pog'-Por distance is greater in YM 2PF and 3PF than in YM 1PF ($p<0.05$ and $p<0.01$, respectively); and in YM 3TF is greater than in YM 1TF and 2TF (respectively, $p<0.01$ and $p<0.05$) (see Table 2). This figure is higher in YW 2PF and 3PF than in YW 1PF (respectively, $p<0.01$ and $p<0.001$), and in YW 3PF – higher than in YW 2PF ($p<0.05$); also, the Pog'-Por distance in YW 3TF is greater than in YW 1TF ($p<0.05$) (see Table 2). Pog'-Por distance was higher in YM 3TF than in YW 3TF ($p<0.05$) (see Table 2).

GI'LsPog' angle values were found to be higher in YM 3PF than in YM 2PF ($p<0.05$) and 1PF ($p<0.001$) and in YM 2PF than in YM 1PF ($p<0.05$) (see Table 2). YW 3PF have a greater GI'LsPog' angle than YW 2PF ($p<0.05$) and 1PF ($p<0.001$); and in YW 2PF – than in YW 1PF ($p<0,01$); the value of this indicator in YW 1TF and 2TF is greater than in YW 3TF ($p<0.05$ in both cases) (see Table 2). GI'LsPog' angle values were found to be higher in YW 1PF than in

YM 1PF ($p<0.05$) and the tendency to a higher value of this indicator was found in YW 2TF than in YM 2TF ($p=0.060$) (see Table 2).

The SnPog'-Pn angle in YM 1PF is greater than in YM 2PF and 3PF ($p<0.01$ and $p<0.001$, respectively) (see Table 2). In YW with different facial profiles, the SnPog'-Pn angle is greater in individuals with 1PF than in YW with 2PF and 3PF ($p<0.001$ in both cases) and greater in YW with 2PF than in YW 3PF ($p<0.01$) (see Table 2). There was also a tendency for higher values of the SnPog'-Pn angle ($p=0.068$) in YM 1PF, compared with YW 1PF (see Table 2).

The Li-SnPog' distance is only greater in YW 1PF than in YW 2PF ($p<0.01$) and 3PF ($p<0.05$) (see Table 2).

## Discussion

Numerous author's methods of cephalometric analysis of teleroentgenograms are used in different countries of the world, the most common and recognized of which are the methods of Steiner, Harvold, Tweed's, McNamara, Schwarz, Ricketts, Bjork, Jaraback, Burstone, etc. The urgent need for such an analysis at present is to determine the features of morphometric parameters of the head and its individual structures in certain groups of people. After all, according to numerous researchers, these indicators can differ significantly in people of different sexes, races, ethnicities, ages, populations, geographies of residence, different craniotypes and face types. [3, 4, 11, 17, 20, 21] Taking these features into account will greatly facilitate the identification of the deceased.

As a result of our study, we obtained and analyzed the position of teeth and soft tissue profile of the face in adolescents with different profiles and types of face according to Schwarz, which can usually change, adjust according to normative, appropriate values during the most common dental manipulations in orthodontic and orthopedic practice.

The values of the angles Max1-SpP S-arz and SnPog'-Pn were found to be significantly higher in the YM 1PF than in YM with 2PF and 3PF; and in YW 1PF, the values of the SnPog'-Pn angle and the Li-SnPog' distance are significantly larger than in the YW 2PF and 3PF.

YM 2PF had significantly higher values of Pog'-Por distance and GI'LsPog' angle than YM 1PF; and YW 2PF had significantly greater Pog'-Por and GI'LsPog' angles than YW 1PF and SnPog'-Pn angle than YW 3PF.

YM 3PF had significantly higher Pog'-Por and GI'LsPog' values than YM 1PF and GI'LsPog' angle than YM 2PF; and YW 3PF have significantly higher values: Mand1-MP Shwars, II and GI'LsPog' angles than YW 1PF; GI'LsPog' angle than YW 2PF and Pog'-Por distance than YW 1PF and 2PF.

YM 1TF had significantly higher values of Mand1-MP Shwars and II angles than YM 2TF and angles Max1-SpP S-arz and Mand1-MP Shwars than YM 3TF; and YW 1TF had significantly higher GI'LsPog' values than YW 3TF.

YM 2TF had significantly higher values of Max1-SpP S-arz and II angles than YM 3TF; and YW 2TF had significantly higher GI'LsPog' angle values and a tendency to have a higher Mand1-MP Shwars angle value than YW 3TF.

YM 3TF had significantly higher values of Sn-Pn distance and Pog'-Por distance than YM 1TF and Pog'-Por distance than YM 2TF; and YW 3TF had significantly higher Sn-Pn and Pog'-Por distances than YW 1TF and Sn-Pn distances than YW 2TF.

Almost all researchers from different countries note the presence of sex differences in both cephalometric and gnatometric teleroentgenography indicators, which are usually greater in males, which can be explained by usually higher values in males as anthropometric indicators of the body in general and head size in particular. [6, 12, 13, 22]. At the same

time, no significant sex differences in cephalometric parameters were found in Jordanians. [10]

In our study, we found pronounced signs of sexual dimorphism in certain gnatometric parameters – mostly higher values in YM than in YW of the same profile or the same face type.

It should be noted that all differences between YM and YW with the same facial profiles are set for people 1PF. Thus, YM 1PF have significantly higher values of Sn-Pn distance and a tendency to greater values of angles II and SnPog'-Pn. Instead, YW 1PF have a significantly larger GI'LsPog' angle.

There are many differences between YM and YW with the same face types. YM 1TF have significantly higher values of the angle Max1-SpP S-arz and angle II, YM 2TF – significantly greater values of the distance Sn-Pn, and YM 3TF – significantly greater distance Pog'-Por and the tendency to greater value of the distance Sn-Pn; YW 2TF tend to have a larger GI'LsPog' angle.

Thus, we established and determined the differences of teleroentgenographic indicators of the position of teeth and characteristics of soft tissues of the face in YM and YW of Ukraine with orthognathic occlusion depending on the profile and type of face by the method of Schwarz and revealed pronounced manifestations of sexual dimorphism between adolescents with different profiles and types (more pronounced) of the face according to Schwarz.

## Conclusions

The obtained results prove the need to take into account not only age but also sex, type and profile of the face for an individualized approach in determining normative teleroentgenographic indicators of man, which is important in forensic medicine to improve the identification of persons and in practical dentistry to assess the dental apparatus and aesthetic results of interventions in orthopedic and orthodontic dentistry.

**Ethical Clearance:** Ethical clearance was obtained from the 'Ethics Committee' of the Institution prior to the start of the study.

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# Study of Genetic Variation of the gene *NOS3* and Cadmium Concentrations in a Sample of Iraqi Patients with Essential Hypertension

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## Abstract

High blood pressure is defined as a systolic blood pressure of 140 mm Hg or more, or a diastolic blood pressure of 90 mm Hg or more. Blood pressure is the force exerted by the circulation of blood on the walls of the body's arteries, which are the main blood vessels in the body. High blood pressure is classified into normal, first stage, second stage, or third stage. Risk factors include lifestyle factors, environmental factors, and genetics. There are two types of high blood pressure, essential hypertension and non-essential hypertension. samples were collected, representing 50 samples from welding laborers patients with high blood pressure, in addition to 50 healthy samples. This study was conducted with the aim of studying the relationship between genetic variation of some genes and environmental factors associated with basic blood pressure in Iraqi patients. The studies were conducted in the laboratories of the Institute of Genetic Engineering - University of Baghdad, as well as in the research laboratories of the Ministry of Science and Technology. Total genomic DNA was extracted using a special kit (Geneaid) from fresh unfrozen blood samples, and then normal polymerase chain reaction and PCR-RFLP were used to detect mutations in the gene *NOS3* rs1799983 T>G (Glu 298 Asp) using primers and specialized severing enzyme is *MboI*. The results of the wild-type TT of *NOS3* gene showed a bundle (206 bp), while the heterozygous TG genotypes showed (206, 119 and 87 bp), respectively, and for the mutant GG genotypes (119 and 87 bp). Nitrogenous base sequence analysis (Sequencing) was conducted for both infected and healthy samples. The results of the study showed that the incidence of hypertension in the age group (20-65) years was higher in males than in females, reaching (60% and 40%), respectively. The study showed that stress, smoking and exposure to pollution all have a clear and dangerous effect on the percentage of people with high blood pressure. The results also showed that mutations between patients and healthy subjects in the *NOS3* gene for wild-type TT is (60%) *versus* (40%), respectively, at a significant level ($p<0.01$), while for the heterozygous TG it is (28)% *versus* (32%) found not significant. As for the GG homozygous mutant, the results were (12%) *versus* (28%) found to be significant at ($p<0.05$) and these results indicate that genetic variation (T>G) may be a risk factor (TT) for high blood pressure. The results of the *NOS3* gene also showed the relationship between the TT genotype of the *NOS3* gene and the cadmium concentration between patients and healthy subjects for the wild-type TT is $4.82 \pm 0.09$ *versus* $0.388 \pm 0.025$ with a level ($p<0.01$) while for the heterozygous TG is $4.96 \pm 0.10$ *versus* $0.371 \pm 0.035$ with a level ($p<0.01$). The homozygous mutant GG is $5.02 \pm 0.08$ *versus* $0.356 \pm 0.025$ with a level ($p<0.01$). These results indicate that the high level of cadmium in welding laborers has an effect on high blood pressure.

***Keyword:*** *NOS3 gene, NOS3rs1799983 T>G, Glu 298 Asp, SNP*

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## Introduction

Elevated blood pressure is linked to an increased risk of cardiovascular disease (CVD) in a linear manner. Starting with a blood pressure of 115/75

mm Hg, every 20 mm Hg increase in systolic blood pressure (SBP) and/or a 10 mm Hg increase in diastolic blood pressure (DBP) doubles the risk of death from stroke, heart disease, or other vascular illness [1]. Increases in SBP have the strongest relationship to cardiovascular (CV) illness, although other blood pressure components, such as DBP, pulse pressure, blood pressure variability, and mean arterial blood pressure, have also been connected to CVD [2]. Depending on the etiology, there are two forms of hypertension: 'primary' (sometimes known as 'essential') hypertension and 'secondary' hypertension. The most common type of hypertension is essential hypertension, which affects 90-95 percent of hypertensive individuals [3]. Nitric Oxide Synthase (*NOS3*), a gene on chromosome 7q35-7q36 that covers around 23 kilobases of the genome, is an intriguing potential gene for essential hypertension (EH). Endothelial synthase (eNOS) activity suppression reduces the production of nitric oxide (NO) in blood arteries. Nitric oxide (NO) is an important relaxing element in the human body that serves a variety of physiological functions. Primary hypertension has a multifaceted and convoluted etiology. Genes play a significant role. Sedentary lifestyle, stress, smoking, obesity, salt (sodium) sensitivity, and alcohol consumption are all significant environmental influences [4,5].

## Materials and Methods

Genomic DNA was extracted using *gSYNC* Genomic DNA Extraction Kit (Geneaid/ Taiwan). PCR was performed using *Accu Power* PCRPreMix (Bioneer, South Korea) and specific primers Table (1). The programmed was a 5-minute denaturation of 94 °C, 35 cycles consisting of 94 °C denaturation 1minute, annealing 60 °C 1 minute, elongation 72 °C 1 minute and final elongation 72 °C, 7 minutes.

**Table (1): sequences of primers used in this study*NOS3* gene (6).**

| Primers | Sequences 5 →´3 ´ | Band size/bp |
|---|---|---|
| Forward | CAT GAG CGT CAG CCC CAG AAC | 206 |
| Reverse | AGT CAA TCC CTT TGG TGC TCA C | 206 |

### RFLP-PCR

PCR product was digested using restriction enzyme *MboI*. The reaction was done as showed in Table (2).

**Table (2): *MboI*Restriction enzyme, *N0S3* gene.**

| Component | Quantity (µl) |
|---|---|
| PCR product | 10 |
| Buffer enzyme | 2 |
| Enzyme | 1 |
| D.W | 2 |
| Total volume | 15 |

Incubate for 3hr 65(˚C)

## Results and Discussion

The targeted fragment was amplified using PCR, which was done using unique primers. In the exon8 of the *NOS3* gene, the fragment size amplified flanking the T894G SNP was 206 bp. as shown in Figure(1A).

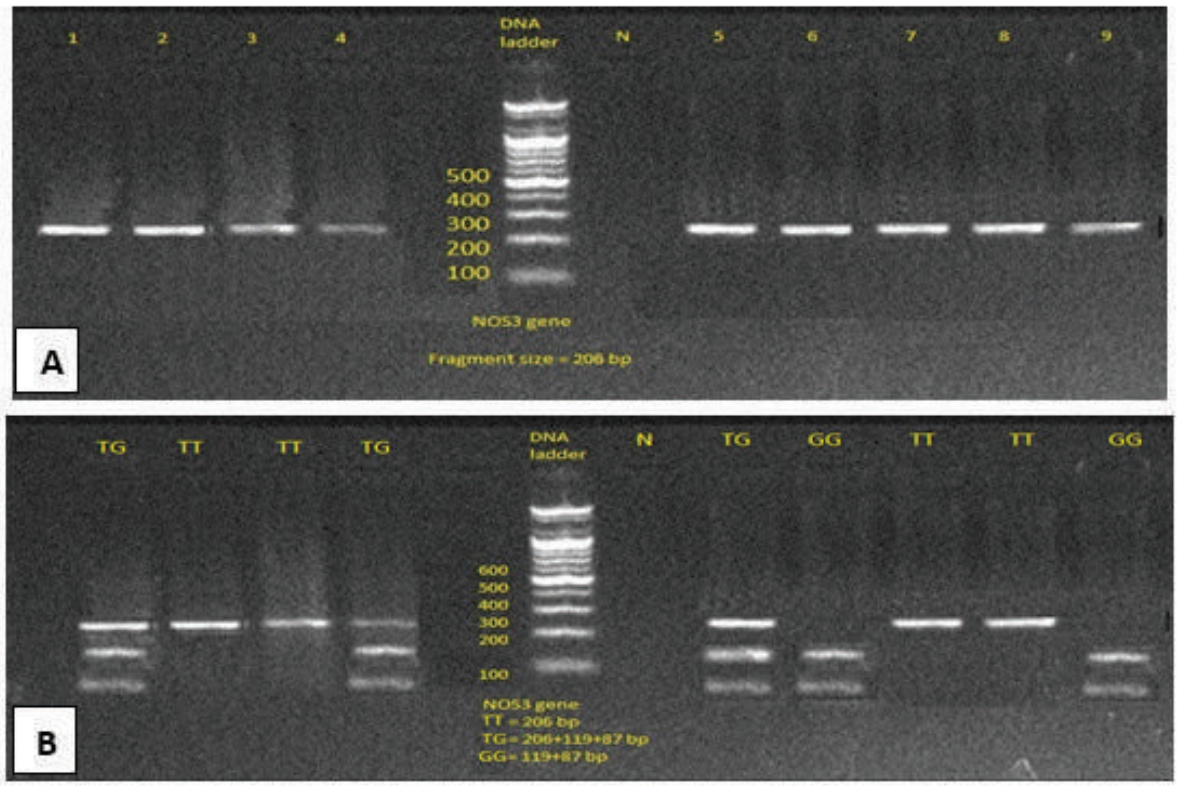


**Figure 1**

**A: PCR product (206bp) of T894G SNP visualized under UV light after staining with ethidium bromide . The electrophoresis was on 2% agarose gel at 70 volt for 1hour. DNA ladder=100bp; N= negative control.**

**B: PCR-RFLP analysis of the*MboI* digest of the PCR product that contains position T894G of the *NOS3* gene separated on a 2%agarose gel . DNA ladder= 100 bp , N= negative control , TT= wild-type ; TG= heterozygous ; GG= homozygous mutant.**

The polymorphism in exon 8 of the *NOS3* gene was identified using PCR-RFLP and the *MboI* restriction enzyme. The *MboI* enzyme had one restriction site in the targeted fragment, and the studied SNP T894G was located within the enzyme target sequence. (↓GATC CTAG ↑).

PCR fragments containing thymine (T) were left uncut (206 bp), while fragments containing guanine (G) were cut into two fragments (119 and 87 bp ) (Figure 1B). The tymine (T) genotype carriers had one fragment (206bp), whereas the (TG) genotype carriers (heterozygous) had three fragments (206,119, and 87 bp), and the (GG) genotype carriers (homozygous mutant) had two fragments (119 and 87 bp) as shown in Figure (1B). Because of a single nucleotide transformation in the two strands of DNA, both sequences of *MboI*restriction sites in the DNA of the target fragment had shifted.

The genotypes and allele frequency distributions of the T894G SNP in the *NOS3* gene in hypertensive patients and apparently healthy controls were compared are shown in Table (3). The TT genotype represent a risk factor for the incidence of essential hypertension (EH) (60% *versus* 40% for EH patients and control subjects, respectively, OR=1.263, $\chi^2$=7.250, p<0.01), whereas, GG genotype represent a protective factor against essential hypertension (EH) incidence (28% *versus* 12% for control subjects and EH patients, respectively , OR=0.877,$\chi^2$=5.381, p<0.05) . There is a G allele-related protective factor against EH in Iraqi patients (60% *versus* 40% for control and patients, respectively, OR=1.263, p<0.01).

**Table(3): The Distribution and allele frequency of *NOS3* gene in control and patients groups .**

| Genotype (*NOS3*) | Control (N= 50) No. (%) | Patients(N= 50) No. (%) | O.R. | Chi-Square ($\chi^2$) |
|---|---|---|---|---|
| TT: Wild | 20 (40.00%) | 30(60.00%) | 1.263 | 7.250 ** |
| TG: Hetro. | 16 (32.00%) | 14 (28.00%) | 0.385 | 1.095 NS |
| GG: Mutant | 14 (28.00%) | 6 (12.00%) | 0.877 | 5.381 * |
| GG+TG | 30 (60.00%) | 20 (40.00) | 1.263 | 7.260 ** |
| **Allele** | **Frequency** | | | |
| T | 0.56 | 0.74 | -- | -- |
| G | 0.44 | 0.26 | -- | -- |
| * (P≤0.05), ** (P≤0.01). | | | | |

As shown form table(4) there were no significant differences among genotypes of rs1799983 in *NOS3* gene as related with blood cadmium (cd) levels in apparently healthy subjects and hypertensive welding laborers. Cadmium (cd) levels were significantly (p<0.01) higher in hypertensive welding laborers than in apparently healthy subjects with respect to genotype of rs1799983 in *NOS3* gene ( 4.82, 4.96 and 5.02 *versus* 0.388 , 0.371 and 0.356 ppb , respectively ) .

**Table (4): Relationship between genotype of *NOS3* gene and concentration of cadmium**

| Genotype | Mean ± SE of cadmium | | P-value |
|---|---|---|---|
| | Control group | Patients group | |
| TT | 0.388 ± 0.025 | 4.82 ± 0.09 | 0.0001 ** |
| TG | 0.371 ± 0.035 | 4.96 ± 0.10 | 0.0001 ** |
| GG | 0.356 ± 0.025 | 5.02 ± 0.08 | 0.0001 ** |
| LSD value | 0.083 NS | 0.254 NS | --- |
| P-value | 0.744 | 0.262 | |
| ** (P≤0.01), NS: Non-Significant. | | | |

As related with blood cadmium levels , the results in the present study found increase in the blood cadmium concentration of welding laborers were in accordance with the study of [7] who found significant increase in the blood cadmium concentrations of workers in Baiji thermal power station [8] found an increase in blood cadmium levels of battery workers , generator operators, oil workers , traffic affiliates and bus drivers in Mosul city and the levels increased with exposure duration due to its accumulation. Also, [9] found an increase in blood cadmium levels of workers in fertilizer industry. The results of [6] showed Cadmium can affect polymorphisms in the *NOS3* gene, resulting in a reduction in nitric oxide (NO) production and an increase in systolic and diastolic blood pressure. Cadmium is a heavy metal that is classified as class II B in the periodic table. With ligands containing the elements O, S, and N, this metal reacts quickly. Cadmium is harmful to the body because it interacts with ligands that are essential for normal body function. Some of the ligands present in living cells that can form complex bonds with metals like Cadmium include OH, - COO-, -OPO3H-, -C = O, -SH, -S-S-, -NH2 and NH. Cd exposure also causes an increase in free radicals and oxidative stress in the body, as shown by lower super oxide dismutase (SOD) and Glutathione peroxidase levels (GPx) [10]. When gene polymorphisms are present, human vulnerability to heavy metal exposure increases [11]. According to the report, gene polymorphism is a significant factor influencing human exposure to toxic substances like cadmium (Cd). In research on people with cardiovascular disorders (hypertension, coronopathies, myocardial infarction, atherosclerosis), a high concentration of cadmium was discovered in the blood of patients with these diseases [12,13]. The results of the present study were disagreed with the previous results of [14]who found that the Asp variant of the Glu 298 Asp polymorphism was associated with hypertension , while in the present study the Asp variant was a protective factor for essential hypertension . Since the wild type genotype (TT genotype) of Glu 298 Asp polymorphism was a risk factor for essential hypertension in this study and also we found a protective effect of Asp variant for essential hypertension in this study which dissimilar the preveous studies, This implies that a large cohort study is needed to determine the existence of any essential hypertension-polymorphism connection. [15] In the Ukrainian population, researchers discovered a connection between the Glu 298 Asp polymorphism and essential hypertension. [16] In a Caucasian population, researchers discovered a connection between the Glu 298 Asp polymorphism and essential hypertension. There was no connection between Glu 298 Asp and essential hypertension in other studies [17,18]. [19] They found no connection between the Glu 298 Asp allele polymorphism in the *eNOS3* gene and hypertension in the Javanese ethnic group, and they blamed this on the limited sample size. [20] The T 894 G mutation causes a conformational alteration in the *eNOS* protein, causing the helix to tighten. [21] Indicated that the G allele is absent in an African population from Mali, and that the hypertensive group has a higher G allele frequency, despite the fact that the G allele is regarded as a protective allele in this sample. The G allele has been linked to resistance to conventional therapy, according to a report[22,23].

**DNA Sequencing**

In Figure (2) show the matching and the peaks of sequencing for fragment in *NOS3* gene that flanking rs1799983 SNP (g.12965 T>G) and appear G instead of T in the position.

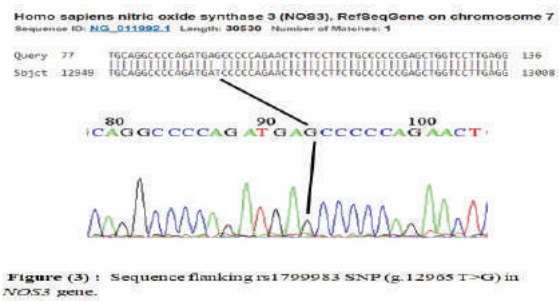


**Figure (3) : Sequence flanking rs1799983 SNP (g.12965 T>G) in *NOS3* gene.**

**Figure 2: Sequence flanking rs179998 SNP (g.12965 T<G) in NOS3 gene.**

## Conclusions

High blood pressure is a common disease nowadays, especially in the age groups (20-65). The results showed that gene (*NOS3*) had a role in determining the incidence of high blood pressure disease. Blood samples taken from workers in the field of welding laborers showed the presence of a high concentration of cadmium in the blood, which is one of the main causes of high blood pressure in the *NOS3* gene.

**Conflict of Interest**: None

**Funding**: Self

**Ethical Clearance**: Not required

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# Environmental Analysis of Massive Mask Waste due to the Covid-19 Pandemic in Indonesia

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## Abstract

The Covid-19 pandemic has a big impact. The requirement to use masks to prevent the spread of the virus increases the amount of mask waste. This study aims to analyze the environment due to the accumulation of mask waste during the Covid-19 pandemic in Indonesia. This research uses the literature study method from various literature references and qualitative analysis. Medical waste that is not managed with proper procedures has the potential to transmit the virus to other people. In addition, the pile of mask waste is also bad for the environment. Various appropriate designs and technologies are made for more appropriate and environmentally friendly management of mask waste.

***Keywords:*** *Covid-19, Environmental analysis, Indonesia, Mask Waste*

## Introduction

In the current state of the Covid-19 pandemic, the public is strongly encouraged and required to comply with health protocols recommended by the government. One of the health protocols that must be done is to use a mask. The proper use of masks has been regulated by the Ministry of Health of the Republic of Indonesia. In circular number of Indonesian Ministry of Health, the recommended masks are of two groups, namely medical masks and masks made of cloth. The medical masks used by medical personnel are in the form of surgical masks and N-95 masks, while masks for all those who have activities outside the home are in the form of 3-layered cloth masks[(1)].

Even though there are recommendations to use cloth masks, there are still many people out there who still use disposable masks. Medical masks are considered more effective in preventing Covid-19 transmission. But there is another problem that lurks from using disposable masks, namely the waste that pollutes the environment. The use of disposable masks will result in an accumulation of mask waste. In addition, disposable mask waste can be one of the causes of the spread of the virus if the waste is not treated properly[(2)].

The use of disposable masks by Indonesians has led to an increase in the amount of mask waste during the Covid-19 pandemic. During the Covid-19 academy, the need for masks increased rapidly, especially disposable masks. The Ministry of Environment and Forestry noted that the increase in medical waste during the Covid-19 pandemic ranged from 30% to 50%. Total waste until October 2020 reached 1,6662.75 tons based on reports from 34 provinces[(3)]. Meanwhile, the current quantity of medical waste in Indonesia has increased by 30% from 296 tons per day before the pandemic and after

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the pandemic to 382 tons per days[4].

In the Jakarta area (the capital of Indonesia) it is known that there are 1.54 tons of mask waste from April 2020 to December 2020. If this problem is not immediately addressed, the mask wastes will not only cause environmental problems but also health problems. Therefore, before the masks are thrown away, it would be nice if the community first processed the medical masks they had used[4].

In processing non-medical mask waste in the community, it is also necessary to follow directions from the ministry of health. According to the Ministry of Health (2020) masks that have been used can be a medium for transmitting viruses and agents that cause disease, and of course, this becomes very dangerous[5]. Piles of mask waste can pollute river water because Indonesian people still like to throw garbage in the river. Worse, waste classified as dangerous can cause damage to the environment and the living ecosystems of living things around it (humans, animals, and plants). Following up on these problems, this study aims to analyze the environmental impact of the unmanaged collision of mask waste during the Covid-19 pandemic.

## Materials and Methods

This research uses a qualitative approach. The data is sourced from secondary data in the form of reports from ministries, agencies, health and environmental organizations in Indonesia. The analysis was carried out qualitatively. The qualitative analysis in question is an in-depth analysis of the data and events that occur, the problem in this research is to mask waste during the pandemic in Indonesia.

## Results and Discussion

At this pandemic, many environmental medicine experts and environmental experts warned of an increase in infectious waste amid the Covid-19 pandemic. Based on the Chinese case study, there was an addition of mask medical waste from 4,902.8 to 6,066 tons per day. The same thing happened in Indonesia along with the addition of positive cases of Covid-19.

Before the pandemic, the Ministry of Environment and Forestry estimated the national waste generation amount to reach 64 million tons per year if using the assumption that 0.7 kg of waste produced per person per day is assumed. Waste generation has increased after a pandemic, especially the amount of medical waste. The amount of medical waste has increased by 30-50 percent with the total infectious waste until October 15, 2021, reaching 1,662.75 tons. According to data from the Ministry of Environment and Forestry, the amount of Covid-19 waste generated from March 2020 to February 2021 was 6,417.95 tons.

Not only nationally and in the national capital, the generation of mask waste in various big cities, small cities, and regions in Indonesia has also increased. In Surabaya, one of the big cities in Indonesia which is known for its clean waste management and urban planning. In February 2021, the mask waste collected at nine reduce, reuse, and recycle collective landfills in Surabaya reached 581 kilograms. Meanwhile, at the beginning of the recording of mask waste in August 2020, the amount collected was 120 kilograms.

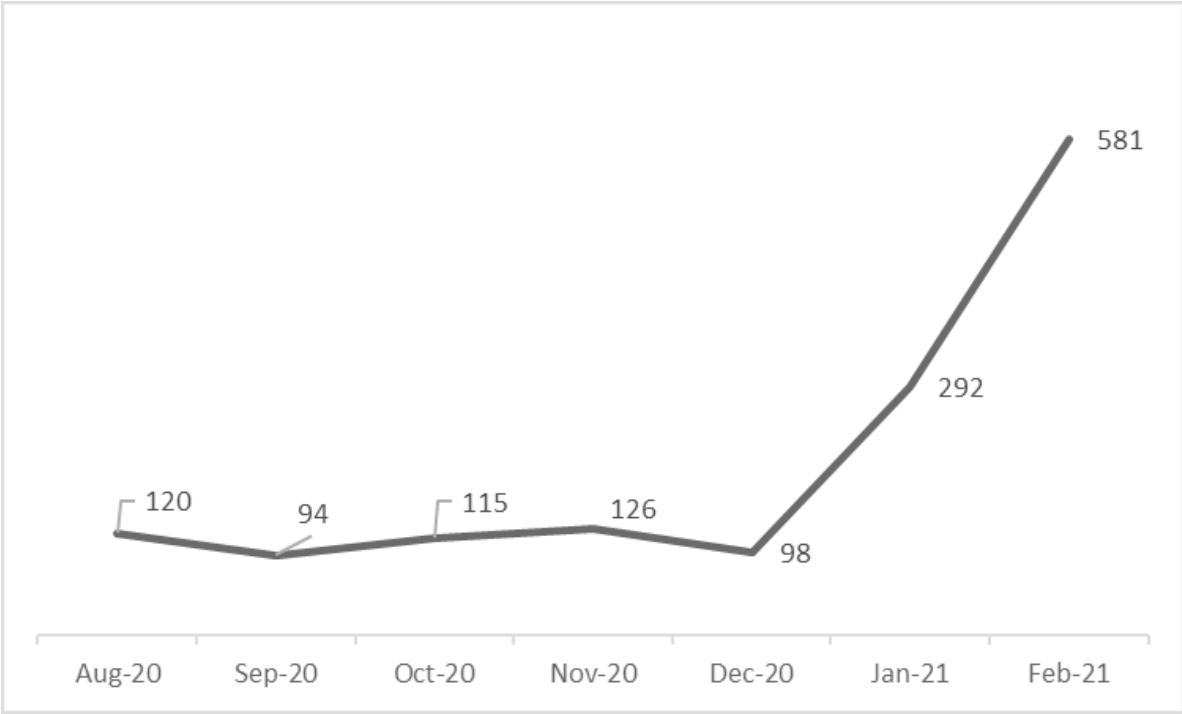


**Figure 1. Graph of the Amount of Medical Waste at collective landfills Surabaya**

Worldwide Wildlife Fund (WWF) reported concerns about the wrong disposal of mask waste, saying: "If only 1 percent of masks were disposed of improperly, this would result in 10 million mask per month which scattered in the environment. Considering that the weight of each mask is about 4 grams, this would require dispersion of more than 40 thousand kilograms of plastic in nature.

Masks of waste are disposed of carelessly in public garbage dumps, rivers, and other places in intact condition without being treated first. The environmental impact will be caused widely. In addition, the dangers of medical waste not only have an impact on the environment but also have the potential to spread viruses. Masks can be used to prevent us from infecting others. But masks have the potential to infect if we throw masks used in the trash and even in places where there is no safety. As we know, the spread of the coronavirus through the droplets of infected people. Not only when we talk to infected people, but droplets that stick to the surface of an object can also be a medium of transmission. "If we touch the surface of an object and the hands stick to the nose, eyes, or mouth, the coronavirus will stick to the surface of our respiratory tract through the interaction of the protein spike that interacts with the ACE 2 receptors in the breath.

Both medical disposable masks and ordinary masks must pass through. washing process with detergent or disinfectant to get rid of viruses. Especially for disposable masks, after going through the disinfection process, they need to be destroyed by cutting or tearing all parts including the hook straps.

Mask waste needs to get special treatment from the officer. processing before being destroyed. For example, mask waste must be separated separately. Masks must also be cut. Then soaked with disinfectant liquid. Management of medical mask waste is a big challenge, so it needs support from all sectors. treatment capacity and emerging waste piles, distribution of treatment facilities, coordination between agencies, and the role of local government and financing issues.

The World Health Organization (WHO) and Public Health England (PHE) have issued recommendations on the management of PPE waste which includes medical masks to be put in two-layer yellow plastic bags which are then stored for 72 hours in temporary storage/shelters before being disposed of at the facility. final stage waste treatment. With such treatment, it is hoped that the virus that may be present in PPE will die before being taken to the final stage of the waste treatment facility. In addition, it is recommended that medical mask waste not be burned because it is feared that it will become waste of hazardous and toxic materials which can pollute water, soil, and air[6].

The Ministry of Environment and Forestry has also regulates the management of disposable mask waste from household waste[7]. In addition, the Indonesian Ministry of Health also provides guidelines for managing disposable mask waste used by ordinary people[8]. In processing medical waste, the procedure has been regulated in the Minister of Environment Regulation concerning Procedures and Technical Requirements for Management of Hazardous and Toxic Waste from Health Facilities. The COVID-19 Handling Task Force through the Waste Sub-Sector is making appropriate policies for community COVID-19 waste management involving the Ministry of Environment and the Ministry of Health. The Task Force itself has so far provided 5 incinerators to 5 provinces in Indonesia and helped manage waste in several major hospitals in DKI Jakarta.

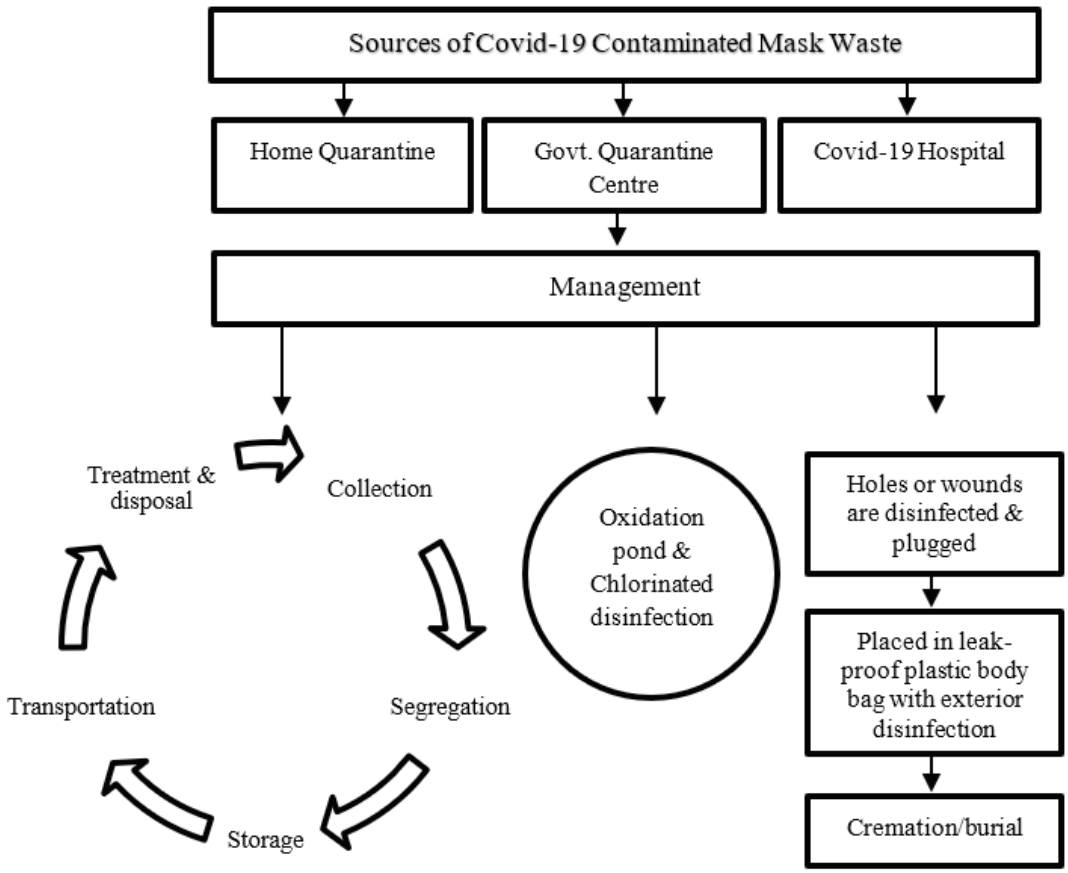


**Figure 2. Schematic flow diagram for safe management of COVID-19 waste**

Covid-19 waste treatment is most effective with the use of chemicals. Previously, waste was separated from

its source category. Based on the source, it is divided into five, namely home quarantine, government quarantine center, covid-19 hospital, wastewater from medical centers, homes and quarantine, covid-19 bodies[9]. Then all waste based on the five sources is managed. Waste management is divided into three parts. The initial part is the waste collection, waste separation, storage, waste transportation, and finally waste processing and disposal. The second part is the chlorinated oxidation and disinfection pool. The last part is making holes or wounds disinfected and plugged in, then placed in a leak-proof plastic bag with exterior disinfection and finally cremation or burial[10].

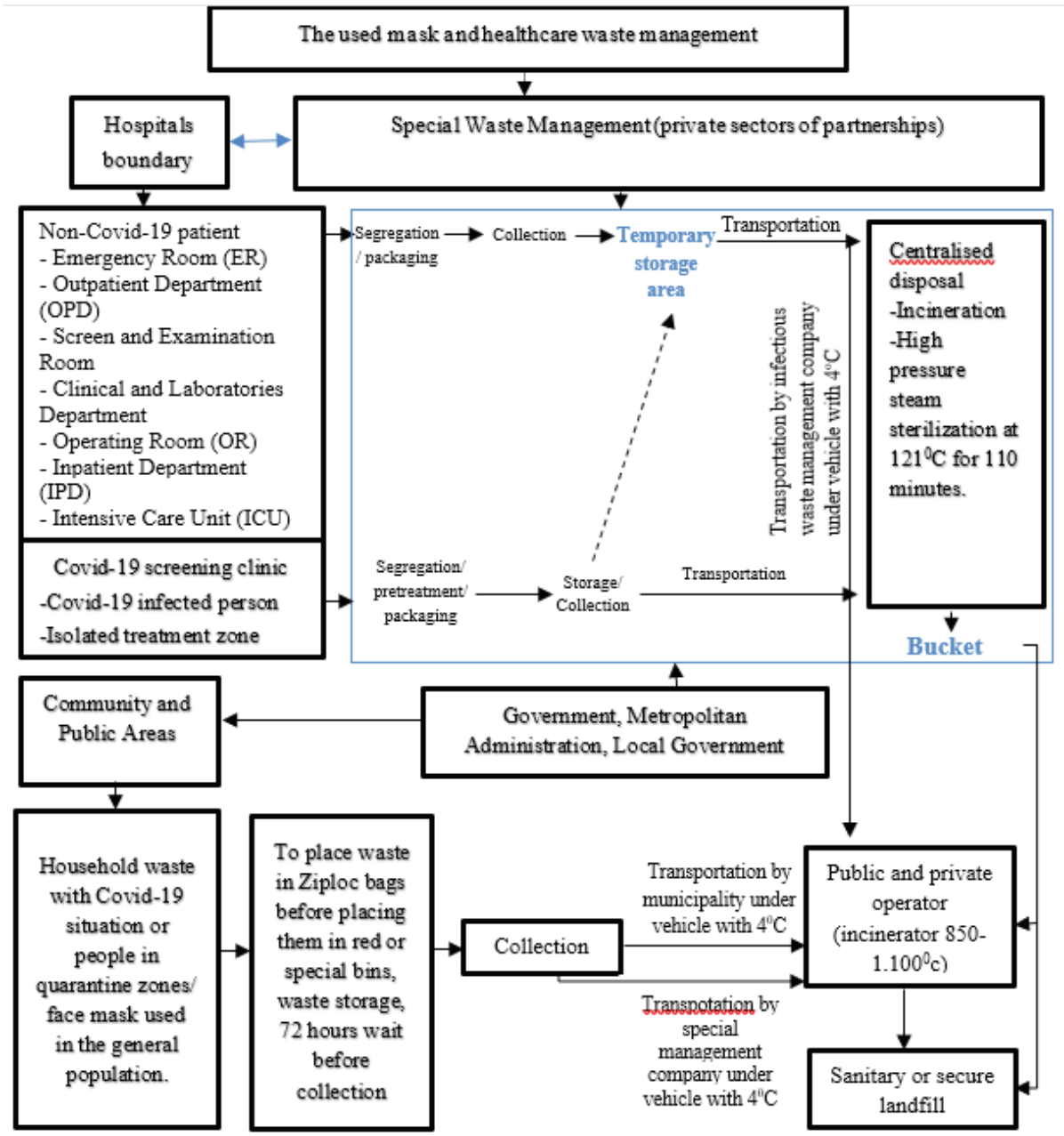


**Figure 3. Schematic Flow Chart of Medical and Household Waste Management During the COVID-19 Outbreak**

## Conclusion and Acknowledgement

**Management of mask waste management is carried out comprehensively using appropriate procedures to prevent environmental impacts. Various designs and innovations are made for maximum results.** Covid-19 waste treatment is most effective with the use of chemicals. Besides, The Task Force itself has so far provided 5 incinerators to 5 provinces in Indonesia and helped manage waste in several major hospitals in Jakarta.

**Ethical Clearance** : We have no ethical clearance to disclose. We have coordinated with the ethical clearance committee of the Research and Development Institute, Ministry of Health of the Republic of Indonesia. This research paper does not require ethical clearance. This research does not use human subjects or other living things. The data presented in this study is not in the form of data on living things, but in the form of modeling to develop science and promote environmental medicine.

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**Conflict of Interest**: We have no conflicts of interest to disclose.

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# Immunohistochemical Characterization of Hepatic Nuclear Factor 4 Alpha Expression in the Choroid Plexus of the lateral and 4th ventricles of adult Male Rat Brain

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## Abstract

The choroid plexus (CP) is present in brain ventricles. It is responsible for cerebrospinal fluid (CSF) secretion and various vital functions. Special proteins present in choroidal epithelium play important roles in CSF production and energy metabolism.

This study aims to compare between the lateral and fourth ventricles CPs using hepatocyte nuclear factor 4 alpha (HNF4α), metabolism marker, to evaluate the functional activity of this tissue in the two regions.

Ten adult male albino rats were used to study the histological features of the CPs and to study the functional activity by quantitative immunohistochemical labeling with HNF4α marker.

The CP of the fourth ventricle had more functional activity than the CP of the lateral ventricle. A quantitative assessment of HNF4α using Aperio ImageScope Software Analysis showed that the lateral ventricle CP mean positivity 0.264 ± 0.083 pixel/micron² while the fourth ventricle CP have mean positivity 0.297 ± 0.043 pixel/micron². The immunohistochemical expression of marker in the fourth ventricle CP were significantly, $P \leq 0.05$ higher than those in the lateral ventricle at $P \leq 0.05$. Immunohistochemical detection of metabolism marker went along with findings of other histological and biochemical studies to define the CP as a highly dynamic structure with regional variations forming a continuum of one entity tissue capable of functional adaptation according to body needs.

***Keywords:*** *Choroid Plexus, Lateral Ventricles, Fourth Ventricle, HNF4α*

## Introduction

The choroid plexuses (CPs) are leaf-like highly vascular structures [(1)]. Four CPs floating inside the ventricular cavities of the brain: one in each of the two lateral ventricles, one in the third, and one in the fourth ventricle [(2)].

The CPs are extensions of the ependymal lining of the ventricular walls and consist of a fenestrated vasculature core surrounded by a single layer of polarized cuboidal epithelium with an interstitial stromal layer of connective tissue rich in fibroblasts and cells of the immune system in between [(3)]. Adjacent CP epithelial cells are joined together by tight junctions to form the blood-cerebrospinal fluid (CSF) barrier. Together with adherens junctions, the tight junctions also ensure the apico-basal polarity of membrane proteins (for example, transporters) that

are critical for normal epithelial cell function [4].

The main known function of the CP epithelium is to produce CSF via passive filtration of peripheral blood across the choroidal capillary endothelium in the vasculature core followed by regulated active secretion across the single-layered epithelium [5].

Hepatocyte nuclear factor 4 alpha (HNF4α) may play a role in the transcriptional control of drug transporters. It is a member of the nuclear receptor superfamily that regulates a broad range of xenobiotic–metabolizing enzymes and thus regulating the metabolism in the CP [6]. The HNF4α gene can also be found in the liver, pancreas, intestine, brain and recently in epithelial cell of CPs [7].

## Materials and Methods

### Animals and tissue preparation:-

A sample of 10 adult male rats (*Rattus norvegicus albinus*). The animals aged 3-6 months, with 300 ± 50 g body weight, and were fed with standard pellet diet. Animals were euthanized with chloroform soaked cotton in an air tight chamber for 5 minutes, then the brains were removed from the skulls and fixed for 18 hours in 4% paraformaldehyde at room temperature (22°C).

The brains were cut in coronal planes rostral to the optic chiasma and caudal to the midbrain in order to obtain lateral and third ventricles specimens, while fourth ventricle samples were made by trimming the remaining caudal part of the brain (cerebellum and brainstem). The specimens were then left in the fixative for another 18 hours and finally transferred into commercial 70% methanol where they were kept until further processing. Paraffin blocks were made and 5 µm thickness sections were cut for immunohistochemical labelling [8].

### Immunohistochemistry Labeling:-

The Super Sensitive IHC for Detection Kit HNF4α antibody was found in CP by following all subsequent steps, which carried out at room temperature in a humidified chamber. Super Sensitive IHC Detection Kit was used. Sectioning at 5 µm were used and deparafinization, Incubate tissue in appropriate pretreatment or digestive enzyme for primary antibody and PBS/TBS wash 3 times for 2 minutes. Then incubate slide in Hydrogen Peroxide Blocking Reagent for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Apply Blocking Reagent and incubate for 5 minutes, PBS/TBS wash 3 times for 2 minutes. Apply primary antibody and incubate according to manufacturer's recommended protocol (overnight) incubation, PBS/TBS wash 3 times for 2 minutes. Apply HRP Polymer and incubate for 10 minutes, PBS/TBS wash 3 times for 2 minutes. Add 20µl of DAB Chromogen to 1 ml of DAB Substrate, mix by swirling and apply to tissue. Incubate for about 3-5 minutes, PBS/TBS wash 3 times for 2 minutes. Finally counter stain and cover slip using a permanent mounting media [9].

### Controls

For positive controls, adult male rat kidney sections were labelled for HNF4α in the same procedure, while for negative controls adult male rat brain and kidney sections were labelled in the same procedure except that primary antibodies of HNF4α were replaced by PBS.

### Immunohistochemical Reaction Assessment

For HNF4α marker, fourty field images of immunohistochemically labelled slides were captured from the lateral ventricle CP, and a similar number of fields were captured from the 4th ventricle CP. A LEICA DM 750 light microscope equipped with Digital Microscopic Camera 5 Mega pixel digital camera were used to capture the fields. Images were processed with Aperio ImageScope v.11 program for total positivity. Microsoft office Excel® 2013 program was used to describe the collected data by calculating the Descriptive Statistics and t-Test were used to compare between means in this study.

## Results

### Immunohistochemical Labeling of the Choroid Plexus

### Hepatocyte Nuclear Factor 4 Alpha (HNF4α)

Light microscopic examination of sections labeled with anti-HNF4α showed high reactivity in choroidal epithelium compared with other cells of brain tissue. There was no detectable difference between reactivity of lateral and fourth ventricles CPs. Ependyma showed weaker reactivity to HNF4α marker than the choroidal epithelium (Figures 1-2). Endothelium of choroidal vessels were highly reactive to HNF4α marker whereas blood cells inside these vessels were non-reactive with this marker (Figure 2).

### Controls

External positive and negative controls, and internal negative controls are seen in (Figure 3).

### Aperio ImageScope Software and Statistical Analyses

### Assessment of Anti-HNF4α Reactivity

Statistical analysis of anti-HNF4α reactivity in the lateral and fourth ventricles CPs gave mean values of 0.264 ± 0.083 pixel/micron² and 0.297 ± 0.043 pixel/micron², respectively, with a wider range of reaction intensity in the lateral ventricle CP than that in the fourth ventricle CP (Figure 4). Two-sample assuming equal variances t-Test revealed a statistically significant difference between these values ($p<0.05$) (Tables 1-2).

**Table 1 Descriptive statistics of HNF4α marker labeling in the lateral and fourth ventricles CPs.**

| Descriptive Statistics | Lateral ventricle CP HNF4α | Fourth ventricle CP HNF4α |
|---|---|---|
| Mean | 0.264 | 0.297 |
| Standard Error | 0.013 | 0.006 |
| Median | 0.258 | 0.292 |
| Mode | #N/A | #N/A |
| Standard Deviation | 0.083 | 0.043 |
| Range | 0.428 | 0.185 |
| Minimum | 0.102 | 0.208 |
| Maximum | 0.531 | 0.394 |
| Count | 40 | 40 |

**Table 2 Comparison of the total positivity of HNF4α marker in the lateral and fourth ventricle CP.**

| Variable | Lateral ventricle CP HNF4α | Fourth ventricle CP HNF4α |
|---|---|---|
| Mean | 0.264 | 0.297 |
| Variance | 0.007 | 0.001 |
| Observations | 40 | 40 |
| Df | 78 | |
| t Stat | -2.211 | |
| P(T<=t) two-tail | 0.029 | |
| t Critical two-tail | 1.990 | |

## Discussion

Previous studies on the CPs of the lateral, third and fourth ventricles considered them as one entity but some authors reported differences in activities of certain metabolic enzymes of the various CPs [10]. The immunohistochemical reactivity of HNF4α in the CPs of both lateral and fourth ventricles were estimated with Aperio ImageScope software that could detect the cells labeled with the specified marker and categorized them into three groups: strongly positive, positive, and weakly positive, while negative areas were those without any reactivity. That was applicable for choroidal epithelium, ventricular ependyma, and endothelial cells of choroidal vessels, but not blood cells within (Figures 1(B)-3).

Positively labeled cells were marked up with Aperio ImageScope software as brown, light brown and yellow colored, indicating strongly positive, positive and weakly positive, respectively (Figure 4). In this study, it was not possible to localize, with precision, HNF4α receptors in cells which were previously localized in the basolateral side of choroidal epithelium plasma membrane, though it was clear to identify HNF4α labeling as a granular stain occur in the cytoplasm. This might be due to the presence of aggregations of transporter proteins across the B-CSF-B like ABCC, ABCB1, ABCB4 and transthyretein. In addition, HNF4α was observed in the endothelium preventing backflow of metabolites to the blood as ABCC proteins play a protective role in choroidal epithelium and mediate basolateral efflux of conjugates resulting from CSF drugs metabolism into the blood while ABCB1 proteins are distributed in the apical side of endothelium [11, 12].

Analysis of HNF4α reactivity in the CPs showed statistically significant higher readings in the fourth ventricle CP compared to that of the lateral ventricle (Tables 1-2), indicating higher activity in the choroidal epithelium of the fourth ventricle. However, cells of the CP of the lateral ventricle showed wider range of HNF4α expression, possibly reflecting a diverse state of activity in that CP since it is spread over wide regions in the brain's ventricles when compared to the smaller size CP impacted in the fourth ventricle.

Expression of HNF4α regulates many proteins and metabolizing enzymes like the ATP binding cassette ABCB4 and ABCC1 in human and rat [12], and transthyretine which is one of the proteins present in the cytoplasm of CP cells [13] at the BCSFB. Demonstration of intracellular reaction of HNF4α by

binding, for example, with transthyretin in choroidal cells cytoplasm reflects its role in regulation of this protein activity. The presence of well-developed endoplasmic reticulum and Golgi apparatus in CPs makes their ability to secret this protein high [14]. Transthyretin is secreted specifically by the CP and not in other parts of brain and it binds with HNF4α to control drug transportation [15]. All the above mentioned proteins can be labeled with anti- HNF4α to give a cytoplasmic reaction which may highlight an assumption of drugs metabolizing and transporting enzymes to be more concentrated in the fourth ventricle CP than that of the lateral ventricle, with clinical and pharmacological implications [16].

In this study, expression of HNF4α was significantly higher in the fourth ventricle CP compared to that of the lateral ventricle suggesting that protein regulation and metabolic activity are more in fourth ventricle CP, which is in contrast to that reported by Al-Kafagi et al. [16] who suggested the regulation of drug transporters is more in lateral ventricle CP. This disagreement might be due to the lack of use of controls in their work, or it might be caused by the different experimental setting when their conclusions were drawn on a different species. In addition, this study contradicts other findings on certain drugs metabolism where the CPs of lateral and fourth ventricles were found to be of similar activity [17], however, it is understood that the different methodology applied might explain this discrepancy.

Ependymal cells lining the lateral and fourth ventricles showed reactivity to HNF4α marker (Figures 1(B) and 2(B)), albeit at lesser extent on qualitative assessment. The mere observation of the ependymal cells expressing less HNF4α marker than the choroidal epithelium, but higher than the adjacent white matter of the brain, needs to be analyzed quantitatively in a further extension from this study.

In addition to the ependymal lining, HNF4α labelling was also seen in endothelial lining of choroidal vessels (Figures 2(A)), however, assuming equal vascular density of both the lateral and fourth ventricles CPs, this labelling would not bias the results in this study, but further quantitative analysis of the vascular profile of the CPs is indicated.

In this study, the expression of HNF4α in choroidal cells of the fourth ventricle was higher than that of the lateral ventricle. Therefore, it might be expected to have abundant amounts of secreted proteins in the cytoplasm of choroidal cells, suggesting that the endoplasmic reticulum content of the fourth ventricle CP is higher compared with that of the lateral ventricle and consequently the metabolic rate is higher in the fourth ventricle CP, which agrees with previous studies [10].

## Conclusion

While carrying the same name as a CP, that part in the fourth ventricle proved distinct functional characteristics from that in the lateral ventricle despite the structural similarities of their cells. In terms of transport system, this study showed preponderance in favour of the fourth ventricle CP, as well as in terms of metabolic activity no matter whether this is related to internal protein synthesis and fluid secretion, or is related to external substance metabolism.

These findings might add to previous works that showed higher functional activity in the CP of the fourth ventricle compared to that of the lateral ventricle, however short of addressing the two regions as distinct entities. Rather, they form a continuum of tissue capable of functional adaptation according to the body needs.

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# Profile Study of Motorcyclists Victims in Road Traffic Accidents at Jaipur Region- An Observational Antemortem Study

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## Abstract

World Health Organization defined accidents as '' an unexpected, unplanned occurrence which may involve injury''[1].Only 28 countries, covering 7% of the world's population, have comprehensive road safety laws on all five key risk factors: drinking and driving, speeding, and failing to use helmets, seat-belts, and child restraints as per the global status report on Road Safety 2013 by World Health Organization[1]. India is undergoing major economic and demographic transition coupled with increasing urbanization and motorization. Injuries on roads, at homes, and in the workplace have increased due to lack of safety-related policies and programs. The health sector bears the maximum brunt in terms of provision of acute care, and short-term and long term rehabilitation service. This study describes profile of motorcyclists' victims in road traffic accidents observation done and they were compared with the previous studies.This study was conducted toanalyzethe sociodemographic profile of motorcycle crashes among total cases of RTA.During study period, a total number of 22618 patients were admitted to trauma centre, from which 10564 were road traffic accident cases, from which 25 % were two wheeler clashes.

***Keywords:*** *Road Traffic Accidents, Motorcyclist, Drinking & Drive.*

## Introduction

Motor vehicle crashes are the leading cause of death in adolescents and young adults[2,] and of the estimated 856000 road deaths occurring annually worldwide[3], 74% are in developing countries. Road Traffic Accident is the most common cause of death in developing countries. In India rapid urbanisation, industrialisation, population explosion and migration of people in past two decades has resulted in enormous growth in the field of road transportation. This has resulted in increasing amount of the road traffic leading to increased risk for occurrence of road traffic accidents.

Factors predisposing to Road Traffic Injuries are classified into Agent, Human and Environmental. Analysis of this Epidemiological Triad is crucial to develop and implement mechanisms for control and prevention of fatal injuries. The major causes of accidents are drunk driving, driving over the speed limit, not using helmets and seat belts, rash and negligent driving including overconfidence,

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carelessness and thoughtlessness, failure to maintain lanes, brake failures, mishaps due to bad road conditions and curvy roads, etc. Generally, the behaviour of the younger age group involved in rash driving and enhanced acceleration capacities of the vehicles are the other contributing factors. Traffic and non traffic collisions may result between vehicles, between vehicle and pedestrian, between vehicle and animal, or between a vehicle and a living/ non living architectural obstacle.Currently two wheelers are major component of road traffic, most people prefer motorised two wheelers for various reasons with travellers opting for a powered two wheelers as a cost efficient alternative to expensive and less frequent public transport systems, their fuel efficiency, convenience in form of operation and maintenance for short distance travel with one or two persons especially at the peak hours as a means of reducing or avoiding the effects of congestion etc. Lack of systematic data generation mechanisms both at the national and state level leads to limitation in designing appropriate intervention strategies to deal with the problem in the country.

Considering the preciousness of human lives, along with financial loss that occurs during treatment, loss of earning, and many times leading to functional disability, this study had been undertaken to observe the socidemographic profile of road accidents Involving motorcycles. An attempt was also made to probe into medicolegal aspects of these accidents so as to suggest remedial measures to traffic rules and law enforcing agencies to decrease the toll of these accidents and to minimising the morbidity and mortality statistics related to motorcycle accidents.

## Aims and Objectives

1.To determine the proportion of motorcycle crashes among total cases of RTA.

2. To observe demographic profiles of these cases.

## Material and Methods

It was a Hospital Based Descriptive Observational study and was done from 1st August, 2019 to 31st July, 2020 at SMS Hospital, Jaipur. Patients with history of Road accidents while riding motorcycles from Jaipur region are included.

**Inclusion criteria:**

1.Patients admitted to trauma centre with history of RTA while riding motorcycles during.

2.Consent given by the patient/attendant.

study period

The present study was conducted at Department of Forensic Medicine, SMS Hospital and Medical College, Jaipur during 1st August, 2019 to 31st July, 2020 on cases of motorcycle accidents admitted to Trauma Centre of SMS Hospital, Jaipur.

## Observation and Results

A total of 22618 Medico-legal Cases were admitted at the trauma Centre out of which 46.7% cases amongst them were cases of Road traffic accidents (RTA). Further, out of them, 816 cases of RTA (25%) injured in motorised two wheeler accidents including occupants of motorcycles, mopeds, scooters, Activa and pedestrians. 243 cases (29.8%) cases injured in motorised two wheeler accidents were fatal amongst these but majority of them were either brought dead or fatal within 24 hours. The occupants of two wheelers other than motorcycles and pedestrians, cases with ambiguous history and those who did not consent for the participation in the study were excluded and 100 cases were included in the study on first come and first serve basis. Majority of victims of motorcycle accidents in the present study were between 20-40 years of age (69%) which shows that the active population of the society was suffering most consequent to the menace of casualties on the roads while riding motorcycles ( Table no -1). Least number of victims was senior citizens followed by the persons between 40-60 years of age.( Table-2) The

observations are quite obvious as the active proportion of society is the most vulnerable to such events of mishaps on the roads owing to many reasons. Mean age of victims of motorcycle accidents in the present study was 29.848±236 years. This is an obvious observation, males being the majorly productive members of the Indian society are more involved with commuting from one place to another especially using two wheelers, motorcycles being the most commonly used two wheeled vehicle in the country. Although more common in rural settings, it is also commonly used in urban and sub-urban settings in recent times; almost wiping off mopeds and scooters from the Indian roads. 89% victims of motorcycle accidents were males and rest 11% were females. 69.7% males and 63.6% females were from 20-40 years age group the active and productive population of the society participating in tasks requiring commuting from one place to another and thus more vulnerable to arias accidents (Table-3). The next age group to suffer trauma due to motorcycle accidents in males was 0-20 years in comparison to 40-60 year old females which is well explained on basis of the gender wise activity statue of population of Indian society where young and adolescent boys start participating in family tasks and also start riding motorbikes whereas females of this age group are neither allowed to participate in outdoor family tasks nor encouraged to move out of houses, whereas the female population of 40-60 years is still engaged in societal and cultural chores actively thus more prone to road accidents.

78% victims of motorcycle accidents included in the present study were drivers or riders or fresh riders and rest 22% were pillion riders. Majority of victims in the study were first riders as in majority of the cases were riding alone. There were seven cases (8.9%) in which the pillion riders suffered minor injuries not requiring admission for the same.

A higher proportion of riders (76.9%) were victimised with motorcycle accidents in 21-40 years age group in comparison to 40.9% pillion riders of the same age group (Table-4).Whereas there were 31.9% pillion riders and 7.7% riders; and, 22.7% pillion riders and 11.2% riders respectively in 41-60 years and less than 20 years age groups. This reflects that the pillion riders of more than 40 years age were most affected. The age group was significantly related to the occupant status of the accident victims. All the motorcycle riders i.e. first riders were males. No female victim was injured while driving the motorcycle in the present study which is an obvious observation as females are rarely seen driving motorbikes in Jaipur and the trend has recently changed with the practice recently being picked up by very few young girls. Occupant status was significantly related to the gender( Table-5).

**Table 1: Proportion of motorcycle crashes among Road Traffic Accidents admitted at Trauma Center of SMS Hospital, Jaipur during study period**

| | No. | Percentage |
|---|---|---|
| Total no. of cases admitted to Trauma Center SMSH | 22618 | 100% |
| No. of cases of Road Traffic accidents among them | 10564 | 46.7% |
| No. of cases of Road Traffic accidents of Jaipur region | 3264 | 30.9% |
| No. of cases of motorised two wheeler crashes of Jaipur region | 816 | 25% |
| No. of fatalities among motorised two wheeler accidents | 243 | 29.8% |

**Table 2: Age-wise distribution of cases of motorcycle accidents**

n=100

| Age group (in yrs.) | Number of cases | Percentage |
|---|---|---|
| 0-20 | 16 | 16% |
| 21-40 | 69 | 69% |
| 41-60 | 13 | 13% |
| >60 | 02 | 2% |
| Total (%) | 100 | 100% |

**Table 3: Age and Gender -wise distribution of cases of motorcycle accidents**

n=100

| Age group (in yrs.) | Male (%) | Female (%) | Total (%) |
|---|---|---|---|
| 0-20 | 16 (17.9%) | 0 | 16 (16%) |
| 21-40 | 62 (69.7%) | 07 (63.6%) | 69 (69%) |
| 41-60 | 10 (11.2%) | 03 (27.3%) | 13 (13%) |
| >60 | 01(1.2%) | 01 (9.1%) | 02 (2%) |
| Total (%) | 89 (100%) | 11 (100%) | 100 (100%) |

**$X^2$ (5,N=100)= 5.1151, p value=0.163557; p>0.5 Not Significant**

**Table 4: Age-wise and occupant status wise distribution of cases of motorcycle accidents**

**n=100**

| Age group (in yrs.) | Rider (%) | Pillion Rider (%) | Total (%) |
|---|---|---|---|
| 0-20 | 11 (11.2%) | 05 (22.7%) | 16 (16%) |
| 21-40 | 60 (76.9%) | 09 (40.9%) | 69 69%) |
| 41-60 | 06 (7.7%) | 07 (31.9%) | 13 (13%) |
| >60 | 01 (1.2%) | 01 (4.5%) | 02 (2%) |
| Total (%) | 78 (100%) | 22 (100%) | 100 (100%) |

**$X^2$ (5,N=100)= 12.6203, p value=0.005534; p<0.05 Significant**

**Table 5: Occupant status and sex-wise distribution of cases of motorcycle accidents**

**n=100**

| Gender | Rider (%) | Pillion rider (%) | Total (%) |
|---|---|---|---|
| Male | 78 (100%) | 11 (50%) | 89 (89%) |
| Female | 0 | 11 (50%) | 11 (11%) |
| Total (%) | 78 (100%) | 22 (100%) | 100 (100%) |

**$X^2$ (3,N=100)= 38.5684, p value<0.001 Most Significant**

## Discussion

22618 Medicolegal Cases were admitted at the trauma Centre of SMS Hospital during the study period from 1st August, 2019 to 31st July, 2020. 46.7% cases (10,564) from amongst them were cases of Road traffic accidents (RTA). Further, out of them, 816 cases of RTA (25%) injured in motorised two wheeler accidents including occupants of motorcycles, mopeds, scooters, activa and pedestrians. 243 cases (29.8%) cases of motorised two wheeler accidents were fatal amongst these but majority of them were either brought dead or fatal within 24 hours. The above data reflects that approximately about half of the traumatic casualties requiring admissions to hospitals and emergency care result from road traffic accidents. Although, motorised vehicles have changed the face of the society making transportation easy and thus, saving much time for other productive tasks and making life comfortable, turning the world into a smaller place with enhanced accessibility even to much remote and interior places; yet, this facility becomes menace when mishandled and results in mishaps. With the advancement of technology in the automobile industry, the world has been blessed with high speed automatic vehicles in attractive designs and speed has become the symbol of today's society. Motorcycles have seen an upsurge in past few years abs replaced almost all other two wheelers. Young adults use them not only as a means of transportation but also as a sports equipment to gain fun from speeding, racing and stunts with an associated risk of traffic accidents. There are many factors that increase the risk of accidents like over-speeding, violation of traffic rules, bad roads, untrained drivers, faulty licensing, poorly maintained vehicles etc. Overall, road accidents are one of most common causes of untimely fatalities and also a preventable cause of mortality. Morbidity and mortality resulting from vehicular accidents, especially motorcycle accidents is increasing day by day and must be monitored regularly to observe the pattern of injuries resulting from the changing trends of vehicles and traffic sense. An alarming rise in fatalities of motorcyclists compelled us to plan this study with this purpose to elaborate upon the pattern of injuries suffered in these cases to recommend ways for preventing accidents as well as to suggest measures to prevent the proportion of mortalities. The present study revealed that 25% cases of road accidents in Jaipur region resulted from motorcycle crashes and 29.8% of them resulted in fatality. This is a significant proportion in the era of COVID-19 pandemic full of lockdowns and restrictions on travel.

The present study reported that the maximum number of victims of motorcycle accidents were in their third and fourth decades of life, the active population of the society. 16% victims were less than twenty years of age and 15% were of more than forty years. The results show that the most affected age group was 20-40 years which is well known to be the most vulnerable age group for unnatural incidents resulting in trauma. 89% of the admissions due to

motorcycle accidents were of males. Jain A, et al (2009)[4]also observed same results 81 % male were the victims and maximum deaths occurred in age groups of 18-44 years, 77%, Sharma BR, et al (2007)[5] also found same results. Ogunlusi JD and Nathaniel C (2011)[6] said thatmales (M) were 127 while females (F) were 9, with M:F ratio of 14.1:1.0. It is due to thatobvious as men are more actively engaged in outdoor works in comparison to women who are more involved with household chores as per sociocultural norms of Indian society. Thus, the most commonly affected population of the study comprises of the most productive sections of the society thus resulting in exponential consequences of victimisation by not just causing physical harm to the victims but also resulting in socioeconomic setback to their families.

In the present study, 78% of the accident victims were riders and rest 22% were pillion riders. Comparison of occupant status to the affected age group, it was observed that, 76.9% riders were of 21-40 years age in comparison to 40.9% pillion riders; whereas, 36.4% pillion riders were more than forty years of age in comparison to 8.9% riders. Children, adolescents and young adult pillion riders contributed twice as much than the riders in the same age groups. **Chichom-Mefire A, et al (2015)[7]**results said that 405 motorcycle crashes were out of total 621 injury victims This distribution is an obvious one considering the age wise activities of both groups, young males mostly riding the vehicle to assist the older people in the family for their outdoor activities and societal roles. **Sukumar S (2018)[8]**studied a total of 34 cases of pillion rider fatalities they said almost all were involved the injuries. In our study 78 % were riders it may be due to that Sukumar S[8]conducted the study only on pillion riders. people from extremes of ages and less active age groups are generally dependent on younger family members and friends for their daily chores as regards to transportation which is also true for females, very few drivers proportionate to men of same age group, especially for motorcycles which also reflects in the present study where no female rider was observed. All the riders in the present study were males whereas equal numbers of pillion riders were affected from both genders although they contributed towards 100% female population and 12.3% male population of the study.

**Suggestion**

1. Adoption of the appropriate road safety policy is the main driving force essentially needed for the major reduction in road traffic fatalities.

2. Education of traffic rules and road safety should be implemented in school curriculum to inculcate road safety practices since childhood.

3. RTA must be considered like other notifiable diseases.

4. Fine on those persons not wearing helmet and not following rules.

**Ethical Clearance-** Taken from The Ethics Committee, S.M.S. Medical college and attached hospital, Jaipur.

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# The Effectiveness of Zinc Micronutrients From Pumpkin *(Cucurbita moschata D)* Extract on the Testosterone Levels of Mice *(Mus musculus L)*

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## Abstract

Various factors cause disharmony in the household. One of them is erectile dysfunction. This can be treated by providing aphrodisiacs and some minerals such as zinc to increase libido. One of the plants that contain zinc is pumpkin (Cucurbita morchata D ). This study was conducted to determine the differences in giving pumpkin skin, meat, and seed extracts to testosterone levels. This study uses a laboratory experimental research type. This study, using extract variables from the skin, meat and seeds of pumpkin (Cucurbita moschata D) and levels of the hormone testosterone. The sample used was male mice (Mus musculus) divided into control groups and three treatment groups. Data were analyzed using the One-way ANOVA statistical test.There were differences in testosterone levels in mice between the control group and the group of mice given extracts of meat, skin and seeds (p-value <0.05).The highest testosterone level in mice was in mice given pumpkin seed extract.

***Key words:*** *Zinc Micronutrients, Cucurbita moschata D, Extraction, Testosterone Levels, Mus musculus L*

## Introduction

Multiple factors cause disharmony in the household. One of them is erectile dysfunction and premature ejaculation. The incidence of erectile dysfunction increases with age, namely 12 cases per 1,000 people aged 40-49 years, 30 cases per 1,000 people aged 50-59 years, and 46 cases per 1,000 people aged 60-69 years [1]. In a study in the United States, there was a 5% incidence of erectile dysfunction [2]. There is no factual data on the incidence of sexual dysfunction in Indonesia, but it is suspected that less than 10% of married men experience erectile dysfunction [3]. according to the data in the Congress of Urology Asia IV in Singapore revealed that 14% of men aged over 18 years who underwent outpatient in Asia turned out to suffer from erectile capacity, and of that amount, the most experienced impotence is male urban China 25%, followed by Indonesian men 21% (second place).

From studies in the Journal of the American Health Association, three out of 10 men experience sexual problems. Generally, among others, complaints in the form of 21% premature ejaculation, 5% erectile dysfunction, and 5% low sexual desire. The proportion of cases of erectile dysfunction of all cases of sexual dysfunction is 50%. The prevalence of erectile dysfunction in men aged 40-50 years is 40-50% and increases with age. Erectile dysfunction is a

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complex and multifactorial problem [4].

Domestic harmony is closely related to sexual activity or quality conjugal relations with adequate quantity. However, from the above explanation, it turns out that data on cases of erectile dysfunction (premature ejaculation) occurs not only in Indonesia but also become a global problem. The ability of an erection has a linear correlation with libido and the hormone testosterone. Both are related to various things, especially food consumption such as protein, vitamins and minerals.

There are various forms of the term aphrodisiac stimulant or stimulants that bus a libido or sexual desire in biology. Aphrodisiacs themselves can be grouped into two. The first one affects physically and psychologically, for example, through sight, taste, smell and impressions such as perfume. Second, that affects from within the body, for example, food, drink, medicine or spices [5].

Aphrodisiacs (aphrodisiacs) are substances or drugs that arouse sexual desire or libido [6]. Aphrodisiac plants contain saponin derivative compounds, alkaloids, tannins, and other compounds from several studies, which physiologically can improve circulation in the central nervous system (cerebral) or peripheral blood circulation (peripheral). This increase in blood circulation will indirectly enhance body tissues' activity to improve organ function. Several types of plants with aphrodisiac content include earth pegs, clove flower buds, ginseng, stoic bamboo shoots, cinnamon, lavender, chocolate and pumpkin, etc. Apart from aphrodisiac ingredients, several minerals such as zinc also play a role in increasing libido. Yellow pumpkin is an example of plant foods with the highest zinc content. Meanwhile, from animal protein, oysters are foods that are high in zinc content.

Yellow pumpkin is a local food ingredient and is very easy to obtain. It contains a very suitable protein for consumption, has a digestibility of 99%, and beta carotene is relatively high, which is 1.569 µg / 100 g. Carotenoid levels, especially β carotene, β cryptoxanthin, lutein and zeaxanthin [7]. Carotene is known as a potential reducer of singlet oxygen species (ROS). Consumption of antioxidants such as carotenoids, polyphenols and tocopherols can prevent oxidative stress [8].

Seeds of Cucurbita moschata, also known as pumpkin seeds, watermelon seeds, and delicious snack, also have properties to prevent the prostate gland's benign enlargement. These seeds also contain mineral elements Zn (zinc) and Mg (Magnesium), which are very important for reproductive organs' health, including the prostate gland. 100 g of Cucurbita moschata seeds contain the mineral Zn of 6.5 mg [9].

Yellow pumpkin (*Cucurma morchata D* ) contains many vitamins and minerals, nutrients in every 100 g of 34 cal, pumpkin, 1.1 protein, 0.3 fat 08 minerals, 45 mg calcium and 0.3 mg zinc. Zinc deficiency can cause impaired growth of reproductive organs, especially testes in men and sexual maturity, impaired seminal and prostate vesicle secretion, seminal and prostate vesical secretion disorders. Impaired function of Leydig cells can cause a decrease in the production of androgens, mainly testosterone. Fertility disorders due to zinc deficiency can be in low semen volume due to decreased seminal vesical secretion, impaired sperm quality and reduced sperm quantity due to reduced testosterone secretion, which plays a role in the spermatogenesis process and sperm maturation in the epididymis [10].

The research from S. Aghaei et al. suggests the protective effect of pumpkin seed extract on the characteristics, biochemistry and histology of male rat sperm by giving cyclophosphamide. Stated that pumpkin seeds are rich in oxidants, reduce the toxicity of cyclophosphamide, and significantly increase rats' antioxidant levels. Yellow pumpkin contains alkaloid compounds useful for stimulating the nervous system, raising or lowering blood pressure and fighting microbial infections. Yellow pumpkin contains natural phenolic compounds that have potential as antioxidants and have medicinal bioactivity. This

compound can be found in stems, leaves, flowers and fruit. Flavonoids in the human body function as antioxidants, protect cell structures and increase anti-inflammatory and antibiotic vitamin C [11].

Decreased testosterone levels due to impaired Leydig cell function will cause a decrease in the frequency of sexual intercourse. Besides, it will affect sexual response because decreased libido causes the phase of sexual response is not optimal. That sexual dysfunction can arise, which can be erectile dysfunction, ejaculatory dysfunction and orgasm dysfunction [10].

This study will focus on the pumpkin as an aphrodisiac and zinc source because similar studies have not been widely conducted. This study is based on data on increasing reports of erectile dysfunction and premature ejaculation among men. Erectile dysfunction is caused by many things, one of which is inadequate food intake. Foods containing zinc and aphrodisiac ingredients can increase sexual desire and libido and reduce erectile dysfunction. One of the foods that are included in the aphrodisiac ingredients with the highest zinc content is pumpkin. Therefore, this study was conducted to determine the differences in the administration of extracts of skin, meat, and pumpkin seeds to the hormone testosterone levels in mice.

## Materials and Methods

### Plant preparation and extraction

The pumpkin (Curcubita moschata D) was identified in the plant taxonomy manual. The pumpkin fruit that has been identified is then separated based on the parts to be extracted, namely the skin of the fruit, the flesh, the seeds. The pulp, skin, and seeds extraction process, which already had constant weight, was macerated with methanol-water (4: 1) at room temperature (25-28 ° C) for 10 hours, respectively. The maceration results were filtered with Whatman 41 paper, and the filtrate was centrifuged for 10 minutes. Then the solvent was evaporated using a rotary evaporator at a temperature of 65 ° C to obtain a thick solution of the extract of the flesh of the fruit, skin and pumpkin seeds.

### Animal groupings and treatment

This study used 30 male mice obtained from PUSVETMA Surabaya. 30 male mice were grouped into 4 groups, namely control, seed treatment, skin treatment and meat treatment, each of which was 3 replications. Group, I was a control group given distilled water. Group II was a group given pumpkin skin extract. Group III was a group given pumpkin seed extract, and Group IV was a group given pumpkin seed extract. Oral administration of the extract solution using a needle with a dose of 2gr / kg BW and a volume of 0.2 ml is given for 36 days according to the time needed in the spermatogenesis cycle.

### Mice Blood Collection

Blood collection of mice was carried out on day 36, the mice were euthanized using chloroform, then the abdominal part of the mice was surgically removed, and 1 ml of blood was taken intracardiac. Then the blood is put into a vacutainer, left for 24 hours until the blood plasma is separated from the erythrocytes and coagulants.

### Testosterone Level Analysis

Blood plasma that has been separated from the coagulant is taken using a micropipette, then the blood plasma is inserted into the microtube, centrifuged for 10 minutes at a speed of 3500 rpm. Test of testosterone levels was carried out in the Laboratory of the Tropical Disease Center, Airlangga University using the ELISA method.

## Statistical Analysis

Values are presented in form of mean ± Standard Error of Mean and were analyzed in SPSS by One Way Analysis of Variance (ANOVA). A significant difference was recorded at $p < 0.05$.

## Results

The process of analyzing the testosterone hormone must be carried out as a whole. Simultaneously, the sample results in the form of blood serum must be stored in a freezer at a temperature of -50 ° C so that the hormones contained therein are not damaged. ELISA using Testosterone Mouse Kit from China. The results of the analysis of blood oxytocin levels from various treatment doses using ELISA can be seen in Figure 1

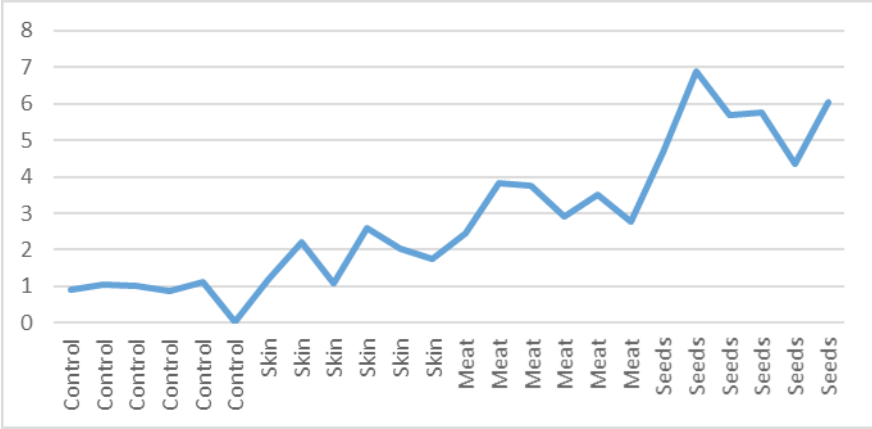


**Fig 1: Testosterone levels in the treatment group of mice ( Mus Musculus )**

The results of the study on the calculation of testosterone levels in male mice treated with extracts of pumpkin skin, fruit and seeds, showed a difference, the group that showed the highest increase in testosterone levels was in the group treated with pumpkin seed extract, which was an average of 5.573 ng. / ml, while the control group was the group with the lowest testosterone level at 0.837 ng / ml, then the pumpkin skin extract group was 1.814 ng/ml, and the pumpkin pulp extract was 3.202 ng/ml, respectively.

**Table 1: One-way ANOVA test results**

| Group | | Group | Mean Difference | Std. Error | Sig. |
|---|---|---|---|---|---|
| Lsd | Control | Skin | -.97650* | 0.37629 | 0.017 |
| | | Meat | -2.36483* | 0.37629 | 0 |
| | | Seeds | -4.73617* | 0.37629 | 0 |
| | Skin | Control | .97650* | 0.37629 | 0.017 |
| | | Meat | -1.38833* | 0.37629 | 0.001 |
| | | Seeds | -3.75967* | 0.37629 | 0 |
| | Meat | Control | 2.36483* | 0.37629 | 0 |
| | | Skin | 1.38833* | 0.37629 | 0.001 |
| | | Seeds | -2.37133* | 0.37629 | 0 |
| | Seeds | Control | 4.73617* | 0.37629 | 0 |
| | | Skin | 3.75967* | 0.37629 | 0 |
| | | Meat | 2.37133* | 0.37629 | 0 |

The highest testosterone levels in mice were in mice given pumpkin seed extract (mean = 5.5732). There was a difference in testosterone levels of mice between the control group and the group of mice given extracts of meat, skin and seeds. This can be seen from the significance value of the p-value (0.000), which is more than α (0.05), which means that there are differences from 4 groups (control, meat, skin and seeds).

From table 1, it can be concluded that there are at least two different groups. However, it turns out that all groups have significant differences with the group. The difference between the control group and the skin group has a p-value (0.017) less than α (0.05). The difference between the skin group and the meat group had a p-value (0.001) less than α (0.05). The difference between the control group and the seed group has a p-value (0.000) less than α (0.05).

## Discussion

In this study, the extract from the skin, fruit pulp, and pumpkin seeds was given orally so that it went through several stages of absorption to respond to increase testosterone levels. The process of zinc absorption is similar to iron in the body, where absorption requires transportation. This process occurs in the small intestine (duodenum); zinc is transported by albumin, and transferrin enters the bloodstream and is carried to the liver. Excess zinc is stored in the liver in the form of metallotheine; otherwise, it is brought to the pancreas and other body tissues. In the pancreas, zinc is used to make digestive enzymes released into the digestive tract at mealtime. Thus, the digestive tract receives zinc from two sources: food and digestive juices from the pancreas.

Zinc absorption is regulated by metallotheine, which is synthesized in the cell wall of the gastrointestinal tract. When the consumption of zinc is high in the gastrointestinal wall cells, some are converted into metallotheine as storage so that absorption is reduced. The amount of zinc that is absorbed ranges from 15-40%. Zinc absorption is affected by body zinc status. If the more zinc is needed, the more zinc is absorbed. Zinc is excreted mainly through faeces. Besides, zinc is excreted through urine, and body tissues are passed, such as skin tissue, small intestinal wall cells, menstrual fluids and sperm.

According to research conducted by Hayati, it was shown that the morphology of spermatozoa increased after giving the ethanol extract of pumpkin seeds [12]. The statistical analysis results showed that the treatment group experienced a significant increase in spermatozoa morphology ($p < 0.05$). Widowati stated that giving the mineral Zn caused a rise in spermatogenic cells due to increased testosterone [9]. Testosterone from the seminiferous tubules is bound by protein binding antigens and transported by seminiferous tubular fluid. Testosterone reaches the epididymis. While in the epididymis, the spermatozoa undergo a maturation process. In the epididymis, testosterone is converted by the five reductase enzyme into DHT, which functions to remove cytoplasmic remains that stick to the spermatozoa after leaving the seminiferous tubules. The increase in DHT causes the mechanism of eliminating cytoplasmic remains to be more effective, resulting in improved spermatozoa's typical morphology.

Widowati stated that giving the mineral Zn caused increased spermatogenic cells due to increased testosterone [9]. This mineral Zn is also contained in pumpkin seed extract, so providing the extract can increase testosterone. Testosterone in the epididymis is converted into DHT by the enzyme five reductases. This DHT hormone functions to remove cytoplasmic droplets in spermatozoa. The spermatozoa released from the epididymis have high motility. If the testosterone and DHT in the epididymis increase, the process of eliminating cytoplasmic droplets will be better, and the motility of spermatozoa that comes out of the epididymis will also increase.

The mechanism of spermatogenesis in the testes depends on the harmonious action of the

hypothalamic-pituitary-testis axis. Local factors and sometimes immunological factors are essential in this mechanism. Among them, nutrients and supplements such as zinc come from factors that can affect reproductive hormone secretion. Therefore, the presence of zinc in food can affect reproductive parameters. The hormone-releasing gonadotropins from the hypothalamus affect the anterior pituitary and cause increased secretion of LH, FSH, and then simulate testosterone's secretion. Simultaneous increase of FSH and LH in serum by the mineral zinc 100 ppm affects the hypothalamus-pituitary-testis axis. This can occur due to a rise in LH and FSH secretion from the front pituitary and following an increase in serum testosterone levels [13]. Overall, it can be seen that adding organic or mineral zinc can affect hormone levels in both dependent and independent reproductive systems.

Zinc is involved in more than 90 enzymes related to carbohydrate and energy metabolism, protein degradation/synthesis, nucleic acid synthesis, heme biosynthesis, CO2 transport (carbonic anhydrase) and other reactions. The most obvious effect is on metabolism, function and maintenance of the skin, pancreas and male reproductive organs, especially in converting testosterone to active dihydrotestosterone. In the pancreas, zinc has something to do with the amount of protease secretion needed for digestion. It also has to do with insulin, although it does not play a direct role in insulin activity.

Zinc has a direct effect on the maturity of sperm cells and maintains the germinal epithelium of the male sex glands. It also plays a role in the growth and development of male sexual organs and their biological activities. Zinc deficiency causes delays in testicular growth and development and inhibits the spermatogenesis process. Zinc supplements are useful in the reproductive cycles of many species and are essential for the synthesis of spermatogenesis. The effect of zinc on the prostate gland is apparent. Zinc deficiency reduces testosterone discharge and prevents spermatogenesis.

According to research from Azadeh, Relatively testicular weight increases with zinc supplementation [14]. In general, the weight and size of the testicles are directly related to their performance. Therefore, the increase in weight has a positive effect on the spermatogenesis activity, and the production of androgen hormones in the testes and the increase in testosterone increases testicular weight. The increase in testicular weight in this study can be ascribed to a rise in stem cells and primary spermatocytes. Lydig cells make androgen hormones which are transferred to blood cells and Sertoli. Part of the testosterone that reaches the Sertoli cells is converted into estradiol by the aromatase enzyme. Zinc deficiency in male goats causes smaller testes and causes decreased libido [15]. Lack of nutrition indirectly affects fertility in men. This nutritional effect results from metabolic effects. So, according to this study, an increase in serum testosterone levels synergizes with an increase in metabolic activity and an increase in testicular weight, influenced by the intake of nutrients derived from zinc.

Previous research conducted by Taufiqqurrachman states that giving 50 mg of pumpkin extract can increase LH and testosterone hormones levels compared to the control group [16]. According to Taufiqqurrachman, the increase in LH levels is caused by the pumpkin's sitosterol content, which stimulates the anterior hypnosis gland without affecting FSH secretion. Another study conducted by Juniarto, by administering 25mg / 2ml of pumpkin and 25mg / 2ml of earth peg to Spraque Dawley rats for 53 days was able to increase the number of spermatozoa significantly differently from the control group. According to Juniarto, the increase is due to the content of eurikomolactone and amarolinda, which can increase LH and FSH's secretion by improving the affinity of the anterior pituitary cell receptor membrane. This component also enhances the cell receptor membrane's association and even to the 5-α reductase enzyme, which plays a role in converting testosterone to dehydrotesterone [17].

In this study, the difference in the results from the testosterone levels analysis indicated that pumpkin seed extract administration affected the increase in testosterone levels of male mice.

## Conclusion

There was a difference in testosterone levels of mice between the control group and the group of mice given extracts of meat, skin and seeds ( p-value 0.000). The group that showed the highest increase in testosterone levels was treated with pumpkin seed extract, with an average of 5,573 ng/ml. In contrast, the control group was the group with the lowest testosterone level, namely 0.837 ng/ml, then the pumpkin skin extract group. 1,814 ng / ml and extract of pumpkin pulp 3,202 ng / ml respectively.

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**Ethical Clearance**: Not Required

**Conflict of Interest:** Nil

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# The Overview of Health Protocols for Preventing and Controlling of COVID-19 ; A Qualitative Exploration from Rural Area in Indonesia

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## Abstract

Background: The COVID-19 has been spread to all over the world. Numerous people still not obey the health protocols that imposed by government. This study aisms to examine in depth the application of health protocols in preventing and controlling of COVID-19 in Muna District. Methods: This research employed an descriptve qualitative research design using semistructed interviews and a purposive sampling approach. The data collection methods was included indept interviews with some key-persons that responsible for COVID-19 prevention and control programs in Muna Regency of Souteast Sulawesi Province of Indonesia and community members. The informants of this study were the the head of COVID-19 task force as key informant; the head of law deparment, a member of the legislative commission for health, a military member and a police, as well as the public. The data was analysed thematically using Nvivo V.12 Plus Software. Results: 1) The role of militery and police as supervisors in implementing health protocol has been carried out well. 2) The implementation of individual health protocols has been carried out, however people with low level of awareness was still lacking about the importance of implementing health protocols such as washing hands, maintaining distance and wearing masks. 3) The application of health protocols for business actors has been carried out, however business owners may still open their businesses (finances matter) but they are required to apply health protocols. 4) The implementation of health protocols in community groups has been carried out and there are some members of the community who care about handling the acceleration of COVID-19, together with local governments to assist with the implementation of health protocols. 5) The regional government provides guidance to the community to participate in efforts to prevent and control Covid-19, conduct security/surveillance in every day, target mass crowds in some areas. Conclusion: Supervision and implementation among citizen in rural area must be regulated strictly and community empowerment is crucial.

***Keywords:*** *COVID-19; washing hand; physical distancing; mask utilization; Indonesia ; Rural Area*

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## Background

The novel coronavirus disease 2019 or COVID-19 has become a critical public health issue worldwide. An outbreak of COVID-19 was first occurred in Wuhan (Hubei, China) at the end of 2019, and subsequently

spread to more than 200 countries. Recently, in 17th of April 2021 there were over 138,688,383 confirmed cases and approximately 2,978,935 confirmed deaths worldwide[1]. As of 17th of April 2021, Indonesia has recorded 1,589,359 positive cases involving 43,073 deaths [2].

Efforts to deal with the Covid-19 pandemic as a disaster management approach are important to pay attention to active community participation. Public awareness of the virus is a real step in fighting the pandemic so that it does not spread faster. The high rate of transmission has made every country appeal to its people to reduce their outdoor activities [3, 4].

WHO advises the public to make efforts to prevent Covid-19 transmission by such as washing your hands regularly with soap and water, or cleaning them with an alcohol-based hand rub, keeping a distance of at least 1 meter between you and people who cough or sneeze, avoid touching your face, cover your mouth and your nose when coughing or sneezing. As well as, stay at home if you feel unwell, refrain from smoking and other activities that weaken the lungs, Practice physical distancing by avoiding unnecessary travel and away from large groups of people [4-6].

In Indonesia, in preventing and overcoming the spread of the COVID-19 virus, efforts from the Government and the role of the community are needed to overcome it. Based on an appeal from the Indonesian government, as an effort to prevent and handle the transmission of the COVID-19 virus, it requires people to maintain a minimum distance of 1 meter and always wash their hands with soap. In addition, the community must remind each other of these calls. The public should also recommend it to avoid traveling to high-risk areas, contact with people with symptoms, and consumption of meat from areas with the COVID-19 outbreak. Basic hand hygiene measures are also recommended, including frequent hand washing and use of PPE such as face masks [7, 8].

In Indonesia, following up on the Presidential Instruction Number 6 of 2020 concerning the improvement of the discipline of health protocol enforcement in the prevention and control of the 2019 corona virus disease and the instruction of the Minister of Home Affairs number 4 of 2020 regarding technical guidelines for regional head regulations in the context of implementing discipline and law enforcement of health protocols as a preventive effort and controlling the 2019 corona virus disease, the Regional Government of Muna Regency issued a Regent Regulation Number 35 of 2020 concerning the Implementation of Health Protocols in the Prevention and Control of Corona Virus Disease 2019.

The spread of Covid-19 is very fast and widespread in Indonesia, to tackle and break the chain of the spread of Covid-19, this determination is very precise because until now the spread of Covid-19 has reached 493 districts / cities spread across 34 provinces or more precisely throughout provinces in Indonesia have recorded Covid-19 cases, from Aceh to Papua [9]. Muna district has been included in the red zone category based on the number of infected people. The status of the red zone in Muna is certainly a sorrow for the Muna, considering that Covid-19 is a type of virus that does not have a vaccine or until now no effective medicine has been found. In such conditions, collaboration between the local government and the community is needed to prevent and break the chain of spreading covid-19 in Muna.

The Muna government has issued various policies, one of which is through a circular, but the circular issued by the Muna regent is considered to have no binding legal force because it is only an appeal where the implementation can or may not be done. So, the Muna regent issued another policy by issuing Regent Regulation Number 35 of 2020 concerning the Application of Health Protocols in the Prevention and Control of Corona Virus Disease 2019 in Muna Regency.

Based on the results of the research obtained, it is concluded that there are still many people who do not comply with the health protocols established by the government besides that the supervision of the implementation of the Regent Regulation on the prevention and control of Covid-19 is considered ineffective because it is not only for the community but the enforcers of local regulations.

Based on the description of the problems above, the author considers it necessary to conduct research on the Application of Health Protocols in the Prevention and Control of Covid-19 in Muna Regency. This research focuses on the application of health protocols in the prevention and control of Covid-19 in Muna district and how factors affect the implementation of health protocols in the prevention and control of Corona virus disease 2019 in Muna district.

## Methods

### *Study Design*

This study was used an exploratory qualitative reserach design using semi-structured interviews (IDIs) and a purposive sampling approach. The data collection methods was included indepth interviews with stakeholders and general publics. The IDIs aims to explore the implementation of health protocols in Muna Regency, Southeast Sulawesi Province of Indoensia during the COVID-19 outbreak.

### *Study setting and study participants*

This study conducted on two communities from two different districts in Muna Regency; Batalaiworu District and Katobu District. The population of this district is predominantly Muslim. People living here belong mostly to the middle class to lower middle class. The areas have been selected purposively because the few members of these communities are already known to the coinvestigator. The coinvestigator served as a gate keeper for providing enterance to the general public and the key person of the COVID-19 prevention and control program in Muna Regency. Interview participants will be selected following the eligibity criteria.

The details of study participants in this study ; as key informant was a The Head of COVID-19 Response Acceleration Task force (Satgas COVID-19). Supporting informant were the head of law deparment of Muna Regency, a legislative member for health commission, the head of disctrict level health office, member of military (TNI) and a police. For the general publics there were ten indivuals participated.

### *Eligibility criteria*

The following are the criteria for inclusion and exclusion of study participants. Inclusion criteria: Residents of Muna Regency (Batalaiworu District and Katobu District). Exclusion criteria: Those who refused to participate in this study, those who have experienced COVID-19 and were undergoing treatment during the data collection, those who were suspected for COVID-19 and have been isolated/ quarantined, and family members of COVID-19 positive cases during the data collection time.

### *Data Collection Procedures*

A semi-structured interview guide was developed for stakeholders and community members. The initial questions on the guide will help to explore participant's perception about the impletentation of COVID-19 health protocols in Muna regency. Additional questions were assesed the compaliance of general publics on the health protocols regulation. All semi-structred interviews conducted by face to face interview with strict health protocols. The interviews was scheduled based on the participant's convenient day and time. Interviewed were conduted in December 2020.

### *IDIs with Stakeholders and General Public*

The inteviews was conducted among the stakeholders that responsible for the pevention and control program in Muna Regency. As wellas, there were 10 persons participated in this IDIs to explore the perceptions about the implementation of health

protocols in Muna Regency. The investagtors did not continue the inteview due to the saturation of the data are alredady achived from 10 informants. Data saturation is the point when no new themes emerge from additional interviews. Face to face interview was conduted with strict health protocols. Further, inform consent was given before the interview began. It reveals that every informant were agree that the interview could be audio-recorded and that written notes could be taken. The inteview was conducted for around 30-40 minutes for each person in Bahasa Indonesia and Muna languange. All informants should be assured that their information will remain confidential and that no identifying features could be mentioned on the transcript. The major themes included a general discussion about participants knowledge and perception about the COVID-19 pandemi, and the issue about the implementation of the health protocols. The data recording was transcribed by a transcriptionist within 24 hours of the interviews. An interview guide for IDIs is shown in Tabel 2.

### *Data Analysis*

All the data were transcribed and translated into English language by listening to the audio recordings in order to conduct a thematic analysis. Nvivo version 12 software was used to import, organize and explore data for analysis. Two independent reserachers read the transcripts at various times to develop familiarity and clarification with the data. An iterative process was employed to help the researchers to label the data and generate new categories to identify new themes. The recordes text divided into shortened units and labelled as a 'code' without losing the ain essence of the reserach study. Subsequently, codes will be analysed and merged into comparable categories. Lastly, the same categories were grouped into subthemes and final themes. For ensuring inter-rater reability, two independent investigators performed the coding, category creation and thematic analyses. Discrepancies between the two investigators were resolved thorugh consensus meetings to reduce researcher bias.

### *Ethics and dissemination*

All informants were asked to sign an inform consent prior to participate in this study. The informed consent form was signed directly before the interview began. Ethical approval for this study has been obtained from the Ethical Office of Universitas Muslim Indonesia. The study results disseminated to the scientific community and to the research subjects participating in this study. The findings might be help us to explore the implementation health protocols of COVID-19 prevention and control programms.

## Results

**Table 1. Characteristic of The Informants**

| Code | Age | Education | Position | Interview Location | Informant's Status |
|---|---|---|---|---|---|
| Mr. A | 52 | Master | The Head of Health District Office | Health District Official | Key Informant |
| Mr. B | 45 | Undergraduate | The Head of Law Department | Regional Government Office | Supporting Informant |
| Mr. C | 32 | Undergraduate | Legislative Member of Health Comssion | Regional Legislative Official | Supporting Informant |

***Cont...*** **Table 1. Characteristic of The Informants**

| | | | | | |
|---|---|---|---|---|---|
| Mr. D | 44 | SHS | TNI / Military member | TNI's regional official | Supporting Informant |
| Mr. E | 30 | SHS | Police Member | Police's regional official | Supporting Informant |
| Mr. F | 32 | Undergraduate | General public | Gatot Subroto Street | General Informant |
| Mr. G | 37 | SHS | General public | Laino Traditional Marker | General Informant |
| Mr. H | 45 | SHS | General public | Kelapa Street | General Informant |
| Mrs. I | 44 | Undergraduate | General public | Mawar Street | General Informant |
| Mr. J | 39 | Undergraduate | General public | Laino Traditional Marker | General Informant |
| Mr. K | 27 | SHS | General public | Laino Traditional Marker | General Informant |
| Mrs. M | 29 | Undergraduate | General public | Gatot Subroto Street | General Informant |
| Mrs. N | 36 | SHS | General public | Gatot Subroto Street | General Informant |
| Mr. O | 42 | SHS | General public | Seroja Street | General Informant |

**Senior High School*

**Table 2. Interview Guideline**

| No | Theme | Questions |
|---|---|---|
| | The Implementation of Health Protocols | 1. How is public awareness about the importance of wearing masks, washing hands and physical distancing? |
| | | 2. What are the regulation in Muna Regency regarding health protocols in order to prevent and control of Covid-19? |
| | | 4. What are the government doing to make the rules related to the implementation of health protocols will be run well? |
| | | 5. What is the government's action in overcoming violations of the implementation of health protocols in Muna District? |
| | | 6. How effective is the application of health protocols in the prevention and control of Covid-19 in Muna District? |
| | | 7. How is the supervision of local governments regarding the implementation of health protocols in the prevention and control of Covid-19 in Muna District |
| | The Determinants | 1. What are the legal factors that influence the implementation of health protocols in the prevention and control of Covid-19 in Muna District? |
| | | 2. How do law enforcement factors influence the implementation of health protocols in the prevention and control of Covid-19 in Muna District? |
| | | 3. What are the community factors that influence the implementation of health protocols in the prevention and control of Covid-19 in Muna District? |
| | | 4. What are the facilities and infrastructure factors that influence the implementation of health protocols in the prevention and control of Covid-19 in Muna District? |

***The Role of Supervisors in Implementing Health Protocols***

The role of the TNI or Military

Based on the interview, information was obtained about the role of the TNI as policy supervisor in implementing health protocols as revealed in the following interview results:

*"In accordance with the president's direct instructions, namely the president and the TNI commander, we swiftly carry out directives in terms of supervision in order to comply with this health protocol, because the covid-19 outbreak is very dangerous and affects the security and stability of the country, so we, as the TNI, have the role as The last guard of national defense needs to swiftly carry out instructions from the president and the TNI high commander to carry out this order professionally because again this is related to the State "(Mr D, 44 years).*

From the explanation above, it is quite clear that the involvement of the TNI in monitoring policies on implementing health protocols and handling Covid-19 in Indonesia is very necessary. The involvement is carried out by observing the parameters of the situation, even the more widespread involvement in the law enforcement and economic sectors even though the PSBB policy has been stopped.

***COVID-19 Response Acceleration Task force (Satgas COVID-19)***

Based on the interview, information was obtained about the role of the Covid-19 Task Force as a policy supervisor in implementing health protocols as revealed in the following interview results:

"For supervision, we continue to do it based on how it is implemented in the community and kWe together with the security forces, in this case the TNI / POLRI, we always supervise the policies that have been carried out by the Government, namely we as technical officers together with our friends at the Puskesmas monitor or carry out activities in the field for investigations cases, so if there is information from the profession or notification so we know. So we have a group for prevention so we also have to follow up and supervise it, something like that "(Mr A, 52 years old).

Based on the statement of a community informant who is in the Batalaiworu District, according to Mrs. I, this is not in line with the following:

"Regarding the implementation of the Covid-19 task force service, the supervision service is considered ineffective because we, the community, are not given an initial understanding in terms of complying with existing health protocols, we are only advised, for example, we are required to use masks, keep our distance, and wash our hands, however. We are not educated on the procedures and reasons why we should use it or implement this health protocol, because there is a different understanding from people whose education level is undergraduate with us people whose education may be below average so it is people like this who need to be given an understanding so that the implementation of supervision on the implementation of this health protocol can run and be carried out well "(Mother I, 44 years).

The explanation above related to the implementation of the supervisory role of the Covid-19 task force has been done well. However, in the view of the community it is still considered ineffective because the community is not given an initial understanding in terms of complying with the implementation of health protocols. In addition, there needs to be regulations made based on facts in the field.

***General Public***

Based on the interview, it was obtained information on the role of the civilian government as policy supervisor in implementing health protocols as revealed in the following interview results:

*"Yes, the Satpol PP agency was given additional tasks in terms of monitoring and enforcing the enforcement of policies issued by the new regional government in the form of a regulation on the implementation of health protocols in the prevention and control of Covid-19 in Muna as well" (Mr. B, 45 years).*

The information above Mr. B, as the Head of Law in Muna Regency regarding the role of the civilian government as a policy supervisor in implementing health protocols, it is quite clear that the civilian government is in handling covid-19 in Muna district. given additional tasks in terms of supervising and enforcing policies issued by the Muna regional government in the form of a Regulation on the implementation of health protocols in the prevention and control of Covid-19 in Muna District.

Based on the statement of a community informant who is in the batalaiworu sub-district, Mr. O stated that it is in line with the role of the civilian government as a policy supervisor for implementing health protocols. However, on the one hand, there is still a tendency in the behavior of an officer in taking action or warning against people who violate health protocols.

*"The supervision carried out by the civilian government, in this case the Satpol PP, I think has been carried out well, but on the one hand there is still a tendency in the behavior of an officer to take action against or warn the public because in the field, this officer reprimands the public for violating health protocols that are not accompanied. by providing an understanding of the community who violates "(Mr. O, 42 years)*

***Individual application of health protocols***

Based on the interview, information was obtained about the application of individual health protocols as revealed in the following interview results:

"Regarding the application of individual health protocols, in this case public awareness of the importance of using masks is strongly recommended to prevent the spread of the virus. in addition, using a mask, wash hands, and keep our distance as we protect ourselves and those around us so as not to be exposed to this virus, considering that Covid-19 is a type of virus that spreads very quickly and cannot be predicted so it is necessary to implement health protocols in standard activities to prevent it. Just earlier because of the more specific transmission, we do not know what kind of transmission, because to prevent it, such as wearing a mask, washing hands and maintaining distance is the same as preventing the spread of Covid-19 from spreading "(Mr A, 52 years)

Based on the statement of a community informant who is in the Katobu sub-district, Mr. G stated that the implementation of individual health protocols had been implemented properly. However, there are still those who do not comply with the policy of implementing health protocols because the people affected by the Covid-19 virus are already in trouble or have minimal income and must no longer be burdened by the rules related to the implementation of health protocols, there are sanctions for not using masks, and bringing hanzsanitizer, who is to buy them.

***Application of health protocols to business actors***

Based on interviews, information was obtained about the application of health protocols to business actors as revealed in the following interview results:

*"Regarding the connection with the implementation of health protocols as a business actor, it has had a very significant impact or effect since the Covid-19 pandemic. so that we, the local government, apply health protocols to business actors to comply with local government recommendations in terms of holding places for washing hands, wearing masks, and maintaining distance. So from these recommendations the public can judge the business actor that their place of business applies the health protocol as recommended by the government or the covid task force "(Mr B, 45 years old)*

The explanation above is related from the application of health protocols to business actors that in implementing health protocols they always comply with directions and apply health protocols themselves for the convenience of the community / visitors.

***Prevention and control of Covid-19 from the aspect of reducing the transmission of Covid-19 in Muna District.***

Based on interviews, information was obtained about the prevention and control of covid-19 from the aspect of reducing the transmission of covid-19 in Muna district as revealed in the following interview results:

*"In prevention and control, the local government provides guidance to the community to participate in efforts to prevent and control the 2019 corona virus disease (covid-19), conduct security / surveillance which is carried out every day targeting the crowd in the Muna district area, effective law enforcement efforts against violations. health protocol, prohibits the implementation of community activities that collect large numbers of people including the implementation of religious activities and the like, provides information on the prevention and control and handling of the spread of the corona virus disease (covid-19), informs the sub-district task force and coordinates with local health officials in finding members of the public who are suspected of meeting the symptoms of the corona virus disease (covid-19). (Mr A, 52 years)*

Likewise, it is different from Mr. J's statement, stating the following:

*"Yes, if you look at the conditions that have occurred in the past, the decline in Covid-19 transmission right now is actually a bit difficult if you want to know because to be honest, we, especially people like us, are not well informed about the number of decreasing numbers of Covid-19 transmission, besides that there is no official information. from the covid-19 task force, such as the demand for data related to the ups and downs or the entry and exit of covid-19 patients "(Mr J, 39 years)*

The explanation above is related from the prevention and control of covid-19 from the aspect of reducing the transmission of covid-19 in the Muna district, prevention and control, the local government provides guidance to the community to participate in efforts to prevent and control Corona Virus Disease 2019 (COVID-19), to carry out security or supervision every time, especially the day targeting the crowd in the Muna Regency area.

## Discussion

***The Role of Supervisors in Implementing Health Protocols***

Based on the results of the interviews, this research will discuss the relationship between research findings and related theoretical theories which are described as follows: this research is in line with the theory Aulia Fitri (2020)The TNI conducts supervision and handling of the Covid-19 pandemic and is in line with the circular issued by the Regent of Muna regarding the application of health protocols in the prevention and control of Covid-19 in Muna[10]. This research is in line with Kurniawan (2020) Police put forward preventive and discretional measures for the sake of public security and order. This is also in line with the circular policy issued by the Muna Regent regarding the application of health protocols in the prevention and control of Covid-19 in Muna [11]. The explanation above is not in line withbased on a circular issued by the Muna Regent regarding the application of health protocols in the prevention and control of Covid-19 in Muna.

***Individual application of health protocols***

This research is in line with A circular issued by the regional government of Muna district, in this case the regent's regulation policy number 35 of 2020 concerning the application of health protocols in the prevention and control of corona virus disease 2019. in the regent's regulation it is explained as guidelines

for implementing health protocols as an effort to prevent and control covid- 19 as stipulated in Muna Regent Regulation Number 35 of 2020.

***Application of health protocols to business actors***

This research is in line with Nismawati (2020) micro business actors remain open and implement health protocols and comply with a circular issued by the regional government of Muna district, in this case the regent's regulation policy number 35 of 2020 concerning the application of health protocols in the prevention and control of corona virus disease 2019. in the regent's regulation it is explained as guidelines for implementing health protocols as an effort to prevent and control Covid 19 as stipulated in Muna Regent Regulation number 35 of 2020 [12].

***Application of health protocols to community groups***

This research is in line with a circular issued by the regional government of Muna district, in this case the regent's regulation policy number 35 of 2020 concerning the application of health protocols in the prevention and control of corona virus disease 2019. in the regent's regulation it is explained as in the guidelines for implementing health protocols as an effort to prevent and control Covid- 19 as stipulated in Muna Regent Regulation number 35 of 2020.

This research is in line with Indriyani and Virus (2020) efforts to prevent the spread of covid-19. and in line with the circular issued by the regional government of Muna district, in this case the regent's regulation policy number 35 of 2020 concerning the application of health protocols in the prevention and control of corona virus disease 2019. the regent's regulation explains as guidelines in implementing health protocols as an effort to prevent and control of Covid-19 as regulated in Muna Regent Regulation number 35 of 2020 [13].

## Conclusion

Based on the results of the research in this study, it can be concluded that: the role of policy supervisors in implementing health protocols, namely the TNI / Polri, has been carried out by carrying out their duties related to the role of policy supervisor in implementing health protocols in schools. Individual health protocols have been implemented, but there are still people who are less aware of the importance of implementing health protocols such as washing hands, maintaining distance and wearing masks. Further, the application of health protocols to business actors has been carried out, in this case business owners may open their businesses but are required to apply health protocols according to applicable regulations. because it is related to finances and shop owners feel at a loss if they do not open their business. As well as, the implementation of health protocols in community groups has been carried out and there are some who are members of the community circle who care about the handling of the acceleration of Covid-19 in Muna District, in this case with the local government to help with the implementation of health protocols. In addition, prevention and control of covid-19 from the aspect of reducing the transmission of covid-19 in the Muna district in prevention and control, the local government provides guidance to the community to participate in efforts to prevent and control covid-19, conduct security / surveillance which is carried out every day targeting the mass crowd in Muna regency area.

**Suggestions**

During the corona virus pandemic (COVID-19) the local government should urge and advise all members of the district to always obey government rules and recommendations, by implementing the health protocol "social distancing / physical distancing" to keep your distance, always wear a mask , always wash your hands diligently, be vigilant and be careful when the arrival of foreigners or immigrants from outside the area, and get used to a healthy lifestyle by keeping

the environment clean and exercising diligently.

Futhermore, it is better if the implementation of individual health protocols by the community must have a level of awareness of the importance of implementing health protocols such as washing hands, maintaining distance and wearing masks. It is better if business actors and the government provide transaction patterns between consumers and producers via online in order to avoid meetings that can result in the spread of the virus. besides that, the government also guarantees business actors against the losses caused by covid-19. The application of health protocols to community groups must be more concerned about the dangers of covid-19.

Regarding the prevention and control of Covid-19 from the aspect of reducing the transmission of Covid-19 in Muna District in prevention and control, the local government should provide guidance to the community to participate in efforts to prevent and control Covid-19. In addition, for further research, it is expected to conduct research by comparing first making observations and seeing how important the policy of implementing health protocols is in prevention and control.

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# Alteration of Iron, Zinc, Vitamin A Breast Milk Levels During Lactation Period Among Mothers of Low Birth Weight Infant Born at Preterm and Term

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## Abstract

**Background**: Duration of lactation and preterm delivery were noticed as dominant factors affecting breast milk composition, including its micronutrient particularly iron, zinc and vitamin A. This study was to analyze the alteration of iron, zinc, vitamin A levels within colostrum and mature breast milk among mothers of low birth weight (LBW) preterm and term infants.

**Methods:** This cross-sectional study was conducted between July 2019 and April 2020 among mothers of LBW infant delivered at preterm and term at Dr. Soetomo hospital. Seventeen samples of breast milk were enrolled on each group. Respectively, colostrum and mature milk were collected between day 2 and 4, between day 15 and 20 after delivery.

**Results:** The iron levels on both groups did not change significantly during lactation period (respectively p=0,266 and p=0,845). Zinc levels were found significantly higher in colostrum of both groups, as well as vitamin A levels within colostrum in LBW preterm group (p<0,05).

**Conclusions:** Higher levels were found in zinc within colostrum of both groups, similar to vitamin A in LBW preterm group. In contrast, iron did not differ significantly during lactation period.

***Keywords:*** *Colostrum, Mature breast milk, Low birth weight*

## Introduction

Breast milk is the primary nutrition priority recommended to be given to all infants [1] as it has been known as an optimal nutrition source for infant growth and development for its macronutrients, micronutrients and unique immunology.[2] Data in the preclinical studies showed the importance role of breast milk micronutrients in normal brain development during the late fetal and early neonatal periods, however studies related to the role of breast milk micronutrients on preterm brain development are still limited compared to macronutrients.[3] Lactation period and preterm birth have been identified as the main causes that can affect the composition of breast milk. Given that exclusive breastfeeding is

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recommended until 6 months of age, it is important to have accurate data regarding the composition of breast milk.[4]

Iron, zinc, and vitamin A are important micronutrients in breast milk.[5] About 12% mortality of children under 5 years of age are known associated with deficiencies of five common micronutrients and 3 of them are iron, zinc and vitamin A, either one of them or in combination.[6] Infants with low birth weight (LBW), both preterm and term are significantly more susceptible to zinc deficiency than term infants with normal birth weight as well as iron deficiency.[7,8] Preterm infants with small for gestational age are also the most vulnerable group for vitamin A deficiency.[9] Many conflicting statements are found in the literature regarding the impact of preterm birth on nutrient levels, one of which is breast milk micronutrients. In most previous studies have investigated the differences in the micronutrient levels of breast milk for preterm and term infants without adding birth weight. Research on nutrition in term infants which adding birth weight discuss only macronutrient levels and not specifically examine the difference of breast milk levels between LBW and normal birth weight term infants.[10,11] On the other hand, information regarding the micronutrient levels of breast milk is still lack and inconclusive.

A better understanding of the breastfeeding components will also help in various aspects, one of which is breastfeeding education and better breastfeeding habits, as well as nutritional therapy for high-risk infants, one of which is in preterm infants.[12] On the other hand, information on the composition of both colostrum and mature breast milk in Indonesia is still lack, especially in LBW infants, both preterm and term. Therefore research in colostrum and mature breast milk of LBW, in particular iron, zinc, and vitamin A, preterm and term mother infants is very important.

## Materials and Methods

A cross-sectional study was conducted from July 2019 to April 2020 at Department of Child Health Dr. Soetomo General Hospital Surabaya. The sample were breast milk of mothers who gave birth at preterm and term gestational age by spontaneously, c-section and instrumental vaginal delivery. Colostrum samples were collected between day 2 and 4 and mature breast milk between day 15 and 20 after delivery. Data were analysed by homogeneity test using Shapiro-Wilk test, then analyzed using paired T-test for homogenous data distribution and Wilcoxon for heterogenous data. Two groups then being tested for the difference using the Independent T-test for homogenous data distribution and Mann-Whitney for heterogeneous. IBM SPSS 21.0 was used for all statistical analyses.

## Results

During the research period (July – December 2019), there were a total of 215 LBW infants who were born preterm and 39 LBW infants who were born at term in the delivery room and operating room of Dr. Soetomo General Hospital. Based on the sample size calculation formula, the minimum sample size in each group is 17, so that a total of 34 patients were included in this study consisting of 17 mothers who gave birth to LBW preterm infants and 17 mothers who gave birth to LBW term infants who met the inclusion and exclusion criteria. The number of samples was taken from the minimum sample size due to the limited research funding. During the study period, 1 were excluded (the mother died) and 4 mothers were dropped out (the infant died) in the LBW preterm infants and 2 mothers were excluded (the mother was on a ventilator) and 3 mothers were dropped out (the mother resigned) in LBW term infants group. The average age of mothers who gave birth to LBW preterm infants was 22-35 years with an average infant weight of 1000-2000 grams, while the average age of mothers who gave birth to LBW term infants was 28-36 years with an average infant weight 1800 – 2300 grams. The results of the homogeneity test on the basic characteristics of subjects eplained in table 1 show a homogeneous distribution in the sex and mode of delivery of the mother, while the data distribution is not homogeneous in the two groups

found on the maternal age, gestational age, birth weight, and combination of gestational age and birth weight.

The mean iron levels in colostrum and mature breast milk in the two groups listed in table 2 show insignificant differences (P> 0.05) while in table 3 shows that zinc levels in colostrum are significant (P<0.05) higher than mature breast milk, meanwhile in Table 4 shows that mean levels of vitamin A in the colostrum in LBW preterm infants were significantly higher than in the mature breast milk, while in the group of LBW term infants was not significantly different.

**Tabel 1 Basic Characteristics of Subjects**

| Characteristics | LBW preterm babies (n=17) | LBW term babies (n=17) | P-Value |
|---|---|---|---|
| Mother's Age (mean±SD), years old | 28,18±6,66 | 32,06±3,75 | 0,047 |
| Gestational age (mean±SD), weeks | 33,35±2,52 | 37,76±0,75 | <0,001 |
| Birth weight (mean±SD), grams | 1555±480,85 | 2087,76±291,98 | <0,001 |
| Combination of gestational age and birth weight, n (%)<br>Small for Gestational Age<br>Appropiate for Gestational Age | <br>6 (35,3%)<br>11(64,7%) | <br>13 (76,5%)<br>4 (23,5%) | 0,016 |
| Sex, n (%)<br>Male<br>Female | <br>7 (41,2%)<br>10 (58,8%) | <br>7 (41,2%)<br>10 (58,8%) | 1,000* |
| Mode of delivery, n (%)<br>Spontaneous vaginal delivery<br>CS / assisted delivery | <br>3 (17,6%)<br>14 (82,4%) | <br>6 (35,3%)<br>11(64,7%) | 0,338* |

* homogenous P-Value >0.05

**Tabel 2 Comparison of Iron Levels in Colostrum and Mature Breast Milk**

| Gestational Age | Iron | | P-Value |
|---|---|---|---|
| | Colostrum (Mean ± SD), mcg/dL | Mature breast milk (Mean ± SD), mcg/dL | |
| LBW preterm babies | 95,41 ± 36,93 | 107,18 ± 42,39 | 0,266 |
| LBW term babies | 116,06 ± 64,17 | 119,29 ± 44,05 | 0,845 |

**Tabel 3 Comparison of Zinc Levels in Colostrum and Mature Breast Milk**

| Gestational Age | Zinc | | P-Value |
|---|---|---|---|
| | Colostrum (Mean ± SD), mcg/dL | Mature breast milk (Mean ± SD), mcg/dL | |
| LBW preterm babies | 789,12 ± 358,67 | 527,29 ± 122,07 | 0,027* |
| LBW term babies | 529,65 ± 171,80 | 367,76 ± 114,91 | 0,010* |

* significant P-Value <0.05

**Tabel 4 Comparison of Vitamin A Levels in Colostrum and Mature Breast Milk**

| Gestational Age | Vitamin A | | P-Value |
|---|---|---|---|
| | Colostrum (Mean ± SD), mcg/dL | Mature breast milk (Mean ± SD), mcg/dL | |
| LBW preterm babies | 100,24 ± 15,55 | 91,41 ± 11,88 | 0,015* |
| LBW term babies | 87,76 ± 15,95 | 92,47 ± 10,92 | 0,207 |

* significant P-Value <0.05

## Discussion

The mean comparison results of iron, zinc, and vitamin A levels in colostrum and mature breast milk in preterm and full-term infants in this study showed mixed results. Lönnerdal states that many differences in the breast milk content of preterm and term infants are probably due to decreased blood flow and differentiation of mammary epithelial cells and the absence of tight junctions between epithelial cells in the mammary glands of preterm infants.[16]

There was no significant difference in iron levels in both colostrum and mature breast milk on LBW preterm and LBW term infants. This finding is in line with the results of a study by Sabatier et al in Switzerland which examined 27 mothers with preterm infants and 34 mothers with term infants which the mean iron levels in mothers' breast milk with preterm infants was 36 ± 23 mcg/dL, while in mothers with term infants was 44 ± 15 mcg/dL, however this study did not divide the studied breast milk by lactation period.[17]

The mean levels of zinc in colostrum were significantly higher than in mature breast milk for both LBW preterm infants and LBW term infants. The results of this study are in line with the study by Ting et al. which showed that there was a significant dynamic in zinc composition based on the lactation period, namely colostrum, transitional breast milk, and mature breast milk, each of which was 660 ± 390 mcg/dL; 330 ± 140 mcg/dL; and 220 ± 130 mcg/dL as well as a study by Erick which found that zinc levels in breast milk decreased with increasing lactation

period, however these studies did not group based on gestational age or birth weight.[18,19] Meanwhile, the results of this study are not in line with the study by Sabatier et al. which reported no significant difference in zinc levels in breast milk for mothers with preterm infants and mothers with term infants.[17]

Low birth weight preterm infants showed that means level of vitamin A in colostrum was significantly higher than mature breast milk. This in line with a study conducted by Lima et al. on breast milk from mothers with preterm infants who concluded that vitamin A levels were significantly more based on the lactation period ($p < 0.001$) with mean concentrations of the colostrum period. And mature breast milk respectively $81.38 \pm 30.09$ mcg/dL and $58.17 \pm 17.48$ mcg/dL.[20] Meanwhile, insignificant results were obtained in the LBW term infants group. This might happen because of many factors that affect vitamin A levels in breast milk, namely vitamin A intake, anthropometric status, socioeconomic status, and maternal education according to research from Dror and Allen.[21]

The findings of this study showed significantly higher levels of zinc and vitamin A in the colostrum of mothers who gave birth to preterm babies, this could be used as an education regarding colostrum nutrient levels and the importance of breastfeeding at the beginning of the lactation period as early as possible.

## Conclusion

Iron levels in colostrum and mature breast milk changed insignificantly for both preterm and term infants with LBW, while zinc levels in colostrum are significantly higher than in mature breast milk both in mothers of LBW preterm and term infants. Levels of vitamin A in colostrum were significantly higher than in mature breast milk in mothers with LBW infants, however it was not applied in the group of mothers with LBW term infants.

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**Ethical Clearance**: The identity of the subject was guaranteed confidentiality. This study was conducted after obtaining ethical clearance from the Ethics Committee of Dr. Soetomo General Hospital in Surabaya, Indonesia with certificate number 1213/KEPK/V/2019. Before the subject recruitment, the researchers had explained to the parents about the general research information and the consent.

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# The Effectiveness of Family Support Program based on Clean and Healthy Behaviour (CLHB) Indicators

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## Abstract

A clean and healthy lifestyle behavior (CHLB) in the household structure is essential to maintain and improve the health of family members. However, in its implementation, consistency is needed so that clean and healthy lifestyle behavior can be carried out optimally based on ten indicators of a CHLB of household arrangements that the government has set. Therefore, this research was conducted using a cross-sectional design with a quantitative descriptive study design—population in the sub-district of Banguntapan, Bantul District, consisting of 826 households. The sample of this study was collected using a technique with a random sampling sample of 100 families and data collection using primary data and secondary data, and field observations. Based on the ten indicators of a clean and healthy lifestyle in the household structure, four indicators have not been appropriately implemented, namely giving exclusive breastfeeding (21%), weighing babies and toddlers every month (14%), doing physical activities every day (34%), and not smoking in the home (23%), with clean and healthy lifestyle coverage in the excellent category (66%). Therefore, it can be concluded that the application of a clean and healthy lifestyle in the Banguntapan, Bantul, household characteristics is good (66%) but has not met the government's target because four indicators of a clean and healthy lifestyle have not been implemented optimally.

***Keywords:*** *Characteristics, CLHB, CLHB indicator, Household*

## Introduction

Clean and Healthy Living Behavior (CHLB) is an applying learning process. So that a person, family, group, or community can help themselves independently related to healthy life behavior[1]. CHLB in a household structure is one of the efforts made to empower every member of the household to have the awareness to practice clean and healthy living habits and to be able to play an active role in driving health in the community[2].

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In general, implementing CHLB increases the family's member independence and empowerment in health problems. In addition, the specific objectives are to increase the knowledge, attitudes, and behavior of the community in particular and households towards the Maternal and Child Health, Nutrition, Environmental Health, Lifestyle, and Public Health Service Assurance programs[3].

In the household structure, CHLB has ten indicators, namely: a) childbirth assisted by health workers, b) giving exclusive breastfeeding, c) weighing babies and toddlers every month, d) using clean water, e) washing hands with clean water and soap, f) using clean and healthy latrines, g) eradicating

mosquito larvae, h) eating fruit and vegetables every day, i) doing physical activity every day, j) not smoking in the house[1].

Based on the strategic plan in 2015-2019, the Ministry of Health targets a clean and healthy lifestyle is set to 80%. However, since 2013 the achievement of a clean and healthy lifestyle was 55%. It decreased from 2012, which equal to 56.5%. It is far from the target set in 2014, which is 70%. So it can be said that around 45% of households have not done CHLB[4].

The results of the Basic Health Research in 2018 contained three CHLB indicators. First, the national prevalence of smoking at the age of more than ten years increased from 28.8% (2013) to 29.3% in 2018. Meanwhile, the prevalence of smoking in the population aged 10-18 years in 2013 increased from 7.2% to 9.1% in 2018. Second, data from the Indonesian Health Profile in 2018 shows that 100% of the five districts in Yogyakarta have implemented a clean and healthy lifestyle policy. Then, the evaluation results of applying the clean and healthy lifestyle indicator at the household level carried out by the Yogyakarta Province in 2018 were 45%[5,6,7].

Inadequate achievement of this target is because of the limited promotion of health education due to a lack of personnel. In addition, the implementation of health promotion and community empowerment related to a clean and healthy lifestyle is still needing[4]. Therefore, this study aims to determine the characteristics of a clean and healthy lifestyle in the household structure in one of the districts in Yogyakarta, Banguntapan Bantul, in 2020, based on ten CHLB indicators.

## Research Method

This research was conducted using a cross-sectional design with a quantitative descriptive study design. It took placed in the entire community in Banguntapan, Bantul, which consists of 826 families. The research sample was collected using a random sampling technique, with a sample of 100 families, and data collection using primary and secondary data, which has been done in 2020. Moreover, the results of the analysis are presented in tables and graphs.

## Findings

**Table 1 presents the results from the respondents about a Clean and Healthy Lifestyle Behavior (CHLB) based on the ten indicators.**

| No. | Indicator | Yes | | No | | Not Given | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | N | % | N | % |
| 1 | Childbirth assisted by health workers | 88 | 88 | 2 | 2 | 10 | 10 | 100 | 100 |
| 2 | Giving exclusive breastfeeding | 69 | 69 | 21 | 21 | 10 | 10 | 100 | 100 |
| 3 | Weighing babies and toddlers every month | 76 | 76 | 14 | 14 | 10 | 10 | 100 | 100 |
| 4 | Using clean water | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 |
| 5 | Washing hands with clean water and soap | 91 | 91 | 9 | 9 | 0 | 0 | 100 | 100 |
| 6 | Using clean and healthy latrines | 100 | 100 | 0 | 0 | 0 | 0 | 100 | 100 |
| 7 | Eradicating mosquito larvae | 98 | 98 | 2 | 2 | 0 | 0 | 100 | 100 |
| 8 | Doing physical activity every day | 66 | 66 | 34 | 34 | 0 | 0 | 100 | 100 |
| 9 | Eating fruit and vegetables every day | 90 | 90 | 10 | 10 | 0 | 0 | 100 | 100 |
| 10 | Not smoking in the house | 71 | 71 | 23 | 23 | 0 | 0 | 100 | 100 |

Table 1 Frequency distribution of CHLB Indicators

The results of the first indicator show that 88 out of 100 respondents had implemented one of the indicators in PHBS, whose used health personnel when carried out babies. However, two people did not use professional health personnel, and ten others did not answer.

In the second indicator, the results showed that 69 breastfeeding mothers gave exclusive breastfeeding from a total sample of 100 breastfeeding mothers, so there is still 21% who have not given exclusive breastfeeding. Therefore, intensive health promotion is needed to achieve exclusive breastfeeding for babies can be 100%.

From the frequency distribution in Table 1, it shows that 76 respondents who have babies and toddlers weigh their babies every month, while 14 other respondents do not.

100% of the respondents have used clean water and healthy latrines, seen in indicators four and six. So, all the respondents are aware of both indicators, used clean water and healthy latrines, are necessary.

As shown in table 1, indicator five regarding washing hands with clean running water and using soap, most of them are aware. However, only 9% of respondents have not washed their hands using soap and clean water.

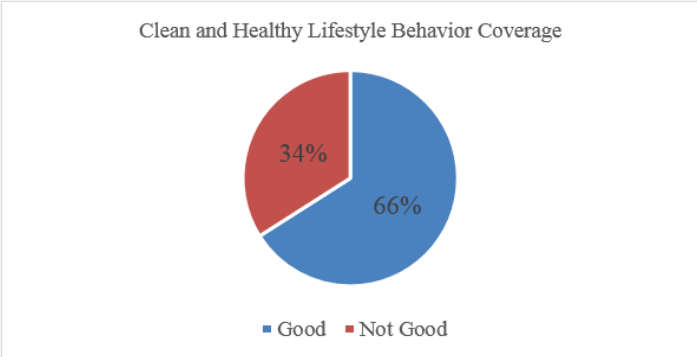
In the indicator of eradicating mosquito larvae at home, it appears that 2% have not carried out the eradication of mosquito larvae, but 98% of respondents have eradicated mosquito larvae. So it can be said that the community already understands the importance of cleanliness and health at home so that mosquitoes do not breed.

Indicator of doing physical activity shows that 34% of respondents do not do a physical activity because they are not used to doing physical activity alone. However, 66 respondents stated that they had been doing physical activity even though they only walked around their house.

The ninth indicator shows that the results show that 90% of respondents have eaten vegetables and fruit every day. In comparison, the remaining 10% of respondents have not made it a habit to consume vegetables and fruit. Therefore, it is crucial to change the community's behavior so that they get used to eating vegetables and fruit.

According to the tenth indicator regarding no smoking in the house, there are still 23% of the 100 respondents, so 71% still smoke. Based on these results, a good health promotion program will change the respondent's behavior not to smoke in the house.

Only four indicators that do not meet CLHB requirements, which are "giving exclusive breastfeeding" by 21%, "weighing babies and toddlers every month" (14%), "doing physical activity every day" (34%), and "no smoking inside the house" (23%).




**Graph 1. Coverage of CLHB conducted by Banguntapan Residents in 2020**

Based on Graph 1, it can be said that 66% of the PHBS coverage carried out by the residents of Banguntapan, Bantul, in 2020 is considered good.

## Discussion

Based on the research results that have been carried out, the results show that five indicators are not optimal in implementing the ten clean and healthy lifestyle indicators in the household structure. These five indicators are indicators of exclusive breastfeeding by 21%, weighing babies and toddlers every month 14%, doing physical activity every day 34%, and no smoking in the house 23%. Moreover, based on the clean and healthy lifestyle coverage results, most of them are in a suitable category. However, the achievement of a clean and healthy lifestyle in the household structure in Banguntapan District, Bantul, is still not optimal because several indicators have not been fulfilled.

A clean and healthy lifestyle in the household structure is intended to make more independent in maintaining health to avoid disease. A clean and healthy lifestyle is also a strategy in increasing community and family independence in the health sector. So there is a need for coordination and communication between health cadres with families and communities in conveying information and conducting health education[2].

The pre-test and post-test results show that health promotion to improve people's ability to apply a clean and healthy lifestyle has a significant effect[8]. Furthermore, the ten indicators affect community clean and healthy lifestyle behavior changes in household structures[8]. In addition to the health promotion being carried out, knowledge of the environment used as a parameter of personality conditions plays an essential role in having a clean and healthy lifestyle[9].

Other things that can affect the fulfillment of the clean and healthy lifestyle indicator in household structures are knowledge and attitudes. Therefore, the level of family education and family knowledge level is very influential. The higher the level of family education will affect the level of family knowledge so that it will form a habit of family members to have a clean and healthy lifestyle, but the lack of family education will lead to a lack of information about clean and healthy lifestyle[10,11,12,13].

One indicator that has less achievement value is not smoking in the house. Smoking habits are influenced by the social environment and the ease of getting cigarettes. In addition, not smoking in the house is influenced by habits that are difficult to change. So even though it is supported by good knowledge about the dangers of smoking, it will still be challenging to change its behavior[12,14,15]. The mother's attitude and behavior very much determine the role of the mother in "weighing the baby" as an indicator of a clean and healthy lifestyle. The more positive the mother's behavior to weigh the child's weight at the Integrated Healthcare Center, the better the mother's behavior to adopt a clean and healthy lifestyle[16].

Another indicator of a clean and healthy lifestyle is giving babies exclusively breastfed. In this study, only 69% of the respondents giving exclusive breastfeeding to their babies. There is study conducted that working mothers give less exclusive breastfeeding to their babies[17]. It is because less time to spent with them. In addition, many mothers say that their breastmilk production is trickly, so they cannot meet the baby's needs. The breast milk produced by the mother will decrease if it is not given to the baby on an ongoing basis because breast milk will be produced more depending on the stimulation of the suction from the baby[17].

However, other research shows that the determinant of knowledge about clean and healthy lifestyle is in line with having a clean and healthy lifestyle in household structure[16,18]. A clean and healthy lifestyle can be carried out by people with any level of education[18]. Besides, low-educated people carry out no difference in the implementation of a clean and healthy lifestyle. Moreover, many factors

can influence implementing a clean and healthy lifestyle[18]. On the other hand, most highly educated homemakers have not adequately carried out their clean and healthy lifestyle because of the minimum information received by them, especially about how to implement a clean and healthy lifestyle in the household structure[19].

The intervention needed to increase awareness of living with a clean and healthy lifestyle is increasing and maintaining clean and healthy living habits as well as the role of health workers who must always be active in providing information through health promotion so that people can consistently carry out a clean and healthy lifestyle and increase public knowledge[20].

Based on the above discussion, healthy living behavior in household arrangements can be caused by many factors, namely knowledge, attitudes, awareness, promotion, and dissemination of information about the importance of implementing a clean and healthy lifestyle in household structures. Meanwhile, the clean and healthy lifestyle in Banguntapan, Bantul, was in a suitable category but had not yet reached the target set by the government. Unfortunately, some clean and healthy lifestyle indicators have not been maximally achieved. It is because there are still CHLB indicators that have not been appropriately implemented.

## Conclusion

It can be concluded that the characteristics of the Banguntapan, Bantul, household in implementing a clean and healthy lifestyle in the household structure are already good, which scored 66%. However, that result has not met the target of the government. Suppose it is seen from those ten indicators, only four indicators that have not been maximally implemented. Future studies hope that other researchers can examine a clean and healthy lifestyle in Bantul more extensively.

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# Reduction Surgery of Giant Hemifacial Neurofibromas: A Case Report

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## Abstract

Neurofibromatosis type 1 (NF-1), first described by Von Recklinghausen (1882), is an autosomal dominant disease caused by a spectrum of mutations in NF-1 gene. We reported a case of giant facial neurofibromas in 26 years old male patient without family history of neurofibromatosis type 1. Features typical of neurofibroma, including an enlarged nerve fascicle composed of elongated nuclei and scant cytoplasmic cells, were identified. Excision was performed with preservation of parotid duct. The mass weight was about 5,5 kg. There were some challenges during the procedure, such as bleeding, hypovolemia, and preservation of vital structure.

***Key words:*** *Giant facial neurofibromas, benign tumour, neurofibromatosis type 1, surgical reduction*

## Introduction

Giant facial neurofibromas cause functional and neurological damage and deficits. Surgical resection is the mainstay of treatment and is a major challenge for surgeons with regard to restoration of defects after tumor resection. We presented a case of surgical reduction of facial neurofibroma of an enormous size and difficulties that posed a rare challenge for the surgeons. In this case report, the reduction was performed in an elliptical design, on the outer edge of the lateral canthus of the right orbital.

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## Case Presentation

We reported a case of 2016, a giant facial neurofibromas in 26 years old male patient without family history of neurofibromatosis type 1. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. Family history did not reveal any similar complaints in immediate or distant relatives. The patient was from a low social economic family, graduated from junior high school, unemployed.

He was found by health workers during a social service. Due to a lack of information, he had never sought medical opinion before. Even so, he had high

hopes for mass reduction surgery. From the results of the examination, the function of the right facial nerve was reduced. The ophthalmologist said the visual acuity in the right eye was within normal limits, except for the ptosis of the palpebra. There was café au lait all over his back and in the axillary region. General health conditions were within normal limits.



**Figure 1. Frontal view before operation**

Pre-operation

We had provided an explanation beforehand and the patient had understood the possible reduced function of the right facial nerve as a result of the surgery. We had designed a reduction incision near the lateral canthal area of the right eye. We had made the design by considering the possible bleeding that could occur.



**Figure 2. Designing the reduction**

During Operation

The anesthetist had prepared 3 bags of Whole Blood (WB) and 3 bags of Pack Red Cell (PRC). The blood pressure was 153/78 mmHg and pulse 92x m. Prolene 1.0 was inserted to the orifice of parotid duct, but we had trouble for further insertion. The reduction was performed in 4 hours by 3 operators, 2 assistants, 1 anesthetist, 2 anesthetist assistants, and 1 circulating nurse. During surgery, enlarged nerve fascicles, dilated blood vessels and brownish patches all over the tissue were found. The fluid was entered through 2 IV lines. Total blood loss was 3500 ml, post-operative blood pressure was 121/64, and the pulse was 88x /m. The administered fluid were 2500 ml Ringer Lactate + 1000 ml normal Saline, 1000 ml Voluven, 700 ml WB, and 230 ml PRC. Then, the hemovax drain was inserted.



**Figure 3. The reduction mass, weighing 5.5 kg.**



**Figure 4. After suturing**

**Post Operation**

On day 1, the hemoglobin level was about 8, the patient was administered with four bags of PRC. On day 4, th hemoglobin level was about 10, and the hemovax drain was taken out. The patient was allowed to discharge from hospital on day 5.



Figure 5. After 1 year

## Discussion

Neurofibromatosis type 1 (NF1), affecting approximately 1 in 3000 individuals, is one of the most common inherited genetic conditions [1]. NF1 is principally associated with cutaneous, neurologic, and orthopedic manifestations [2] that can cause damaging functional and cosmetic effects. The diagnosis of NF1 has been relied primarily on clinical criteria due to its high degree of accuracy and the absence of reliable molecular tests. Genetic testing can be particularly helpful for patients who present with an unusual phenotype or an incomplete clinical picture [3].

It is said that NF1 is caused by a mutation in NF1 gene that encodes tumor-suppressing neurofibromin protein, a Ras inactivator. It can be classified according to anatomical location: cutaneous, subcutaneous, intraneural, and plexiform. Plexiform neurofibromas (PN), histologically benign tumors of peripheral nerves which arise from Schwann cells, occur in 20%–50% of all patients with NF1 [4]. The nerves are transformed into a thick convoluted mass, which is likened to a bag of worms. Treatment of PN mainly consists of symptom management and / or surgical resection [5].

However, these tumors involve nerves, blood vessels, or other internal organs, thus complicating surgery with often incomplete resections followed by tumor regrowth, or morbidity. The challenges during the operation was how to keep hemodynamics stable and how to perform all actions as measured and as quickly as possible. Although there have been preclinical and clinical studies of several molecularly targeted compounds, no medical or therapeutic treatment has been approved to date for PN [6]. Continuous care of the patient is the main management of NF1 by monitoring clinical manifestations according to age, with the aim of early recognition and treatment of symptomatic complications [2].

Thus has been reported a case report of giant hemifacial neurofibromas, with an enormous size. The reduction has been done by considering the speed of surgery, also the possible amount of blood loss, so that the patient could recover early with optimal results. However it may still needed further reduction, but the amount of mass removed will be much smaller. In conclusion, this case report can be used as a reference in other cases of reduction of facial neurofibroma.

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work.

**Disclosure**: The authors declare that there is no conflict of interest in this work.

**Ethical Clearance**: Taken from Bina Sehat Hospital committee

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# Difference in DNA Methylation between Cleft Lip and Cleft Lip and Palate

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## Abstract

It is suspected that environmental exposure to non-syndromic oral clefts, which includes cleft lip (CL), cleft lip and palate (CLP) has an effect on epigenetic mechanisms, particularly deoxyribonucleic acid (DNA) methylation. DNA methylation will be expressed during facial morphogenesis and have an impact on facial development. This study aimed to observe differences in DNA methylation between CL and CLP, between CL mothers and CLP mothers, and correlation between CL and CL mothers, CLP and CLP mothers.

This observational study used a sample of 13 patients diagnosed with CL and 14 patients diagnosed with CLP and their respective mothers. The test was performed using ELISA MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric).

The median DNA methylation at CLP was 1.92 (0.23 - 14.07) and CL was 1.71 (0.08 - 8.47) (p 0.752 > 0.05)). Median DNA methylation in CLP mothers was 0.997 (0.03 - 6.14) and in CL mothers 0.72 (0.23 - 6.16) (p 0.798 (p> 0.05). Correlation test for DNA methylation of CLP with CLP mother r = -0.259 and (p = 0.394 >0.05). Correlation test for DNA methylation of CL patients with CL mothers revealed r = -0.492 and (p = 0.087 > 0.05).

The results of this study showed no difference in methylation between CL and CLP. This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers.

***Key words:*** *cleft lip, cleft lip and palate, DNA methylation*

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## Introduction

The non-syndromic oral cleft (NS), which includes cleft lip (CL), cleft lip and palate (CLP), and cleft palate (CP), consists of a variety of disorders affecting the lips and oral cavity.[1] One of the most common congenital disorders is NS cleft lip with/without cleft palate (CL/P), affecting 1 in 700 live births worldwide.

This deformity has a long-term effect on the patient's health and social integration. Residual defects due to scarring and abnormal facial development will cause functional and psychosocial problems for the sufferer.[1,2] Abnormalities of NS CL/P follow a multifactorial model supported by heritability studies with estimated genetic contributions varying from 45% to 85%, depending on the population.[3] There were several factors that influence the incidence of CL and CLP, ie. genetic factors, deficiency of vitamin B6, vitamin B12, folic acid and iron, as well as other factors, such as environmental exposure like organic solvents, air pollution, cigarettes and agricultural chemicals.[4,5]

Environmental exposure may have an effect on epigenetic mechanisms, particularly deoxyribonucleic acid (DNA) or histone methylation.[6] Methylation of DNA has an effect of changing gene activity without altering gene sequences and can be inherited.[7] Methylation of DNA will be expressed during facial morphogenesis and have an impact on facial development, so that DNA methylation can determine the type of cleft in CL and CLP.[8] Biologically, epigenetic changes may involve in the incidence of CL and CLP and their severity. However, there are very few studies on this in humans.

This study aimed to observe differences in DNA methylation between CL and CLP patients, between mothers of CL and CLP and correlation between CL patients and CL mothers, CLP patients and CLP mothers.

## Methods

This observational study has passed the ethical test with clearance letter no E.5.a/037/KEPK-UMM/III/2020. This study was an analytical study with a cross sectional design and was conducted at Cleft Lip and Palate (CLP) Center, Faculty of Medicine, University of Muhammadiyah Malang. This study used a sample of 13 patients diagnosed with CL and 14 patients diagnosed with CLP and their respective mothers. Written informed consent was obtained from the mothers. Examination was performed using ELISA MethylFlashTM Global DNA Methylation (5-mC) ELISA Easy Kit (Colorimetric) Base Catalog # P-1030.

## Results

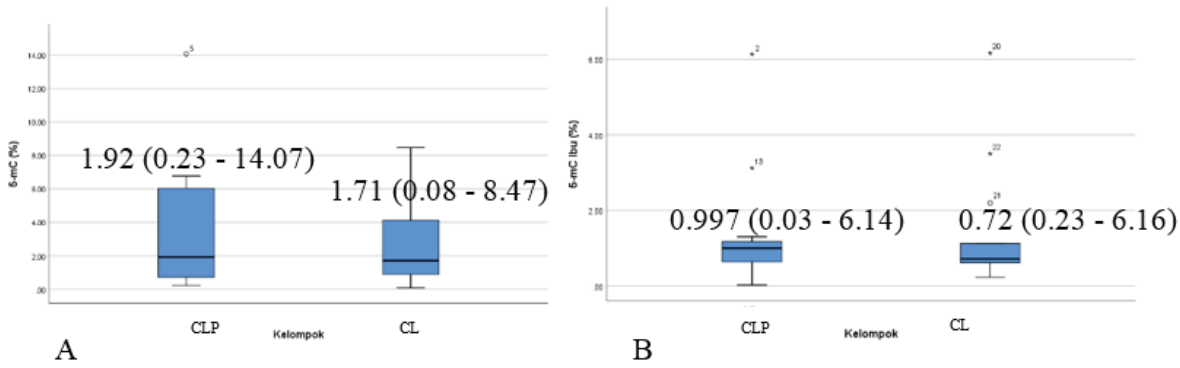


**Figure 1. (A) Median DNA methylation in CLP and CL groups; (B) Median DNA methylation in CLP mothers and CL groups**

The normality test results revealed normality of DNA methylation data in CL and CLP groups, while in CL mothers and CLP groups the normality test was not met, so the test was carried out with non-parametric statistical Mann-Whitney test. Likewise, the correlation test of DNA methylation in CL and CLP with DNA methylation of the mothers was performed non-parametrically using Spearman Rank correlation test.

Calculations yielded a median DNA methylation value in the CLP patient group of 1.92 (0.23 - 14.07) and in CL patient group of 1.71 (0.08 - 8.47). The Mann-Whitney statistical test obtained a p-value of 0.752 (p> 0.05), which indicated that there was no significant difference in DNA methylation. This test proved that DNA methylation in CL and CLP patient groups was relatively the same.

The calculation resulted in median value of DNA methylation in CLP mother groups of 0.997 (0.03 - 6.14) and in CL group of women of 0.72 (0.23 - 6.16). The Mann-Whitney statistical test obtained a p-value of 0.798 (p> 0.05), which indicates that there was no significant difference in DNA methylation. This test proved that the DNA methylation in the CL group and CLP mothers were relatively the same.

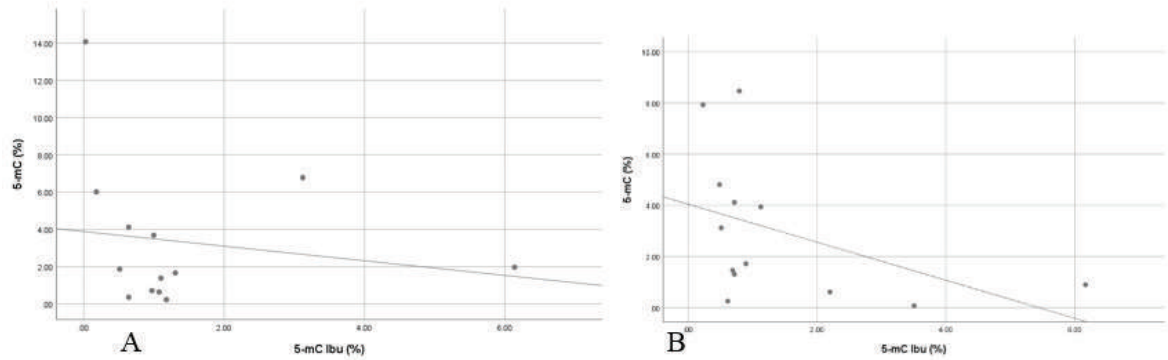


**Figure 2. (A) Scatter plot of the correlation between DNA methylation of CLP patients and CLP mothers. (B) Scatter plot of correlation between DNA methylation of CL patients and CL mothers.**

The Spearman Rank correlation test showed correlation between DNA methylation of CLP patients and CLP mothers with r = -0.259 and p = 0.394. This test proved that there was no significant correlation (p> 0.05) between DNA methylation of CLP patients and CLP mothers. Correlation test for DNA methylation of CL patients with CL mothers showed r = -0.492 and p = 0.087, which proved that there was no significant correlation (p> 0.05) between DNA methylation of CL patients and CL mothers.

## Discussion

Epigenetic influences on a person's traits involve changes in how DNA is packaged, expressed, and converted into proteins without involving variations in DNA sequence. There are chemical modifications that change the level of gene activity, even though the underlying DNA remains the same. These modifications can be attached to the DNA itself or to the histone proteins that are wrapped around the DNA. In DNA methylation, which is the most studied epigenetic mechanism, methyl group binding occurs in the DNA, which affects the expression of nearby genes, often rendering them inactive.[6] Methylation of DNA occurs by covalent addition of methyl group to 5-carbon of cytosine ring by DNA methyltransferases, yielding 5-methylcytosine (5-mC).

Research by Alvizi (2017) found positive correlation between DNA methylation from lip tissue and blood of patients with oral cleft, which suggests that blood is a suitable material for methylation testing in CL/P.[3] This fact underlies the method of collecting blood data in this study.

The results of this study found that there was no difference in methylation between CL and CLP. Similar results were obtained in a study by Khan et al., 2018 regarding the methylation of long interspersed nucleotide element-1 (LINE-1) in CL lip tissue which was examined by bisulfite conversion and pyrosequencing. The study found no significant difference between CL and CLP methylation levels, and no significant differences between methylation levels according to sex. The difference between the medial and lateral sides of the cleft was apparent in male infants but not female infants, and in infants whose mothers did not take folic acid supplements during the periconception period but not in the offspring of mothers who took the supplements.[9]

This study resulted in a median value of DNA methylation in CLP higher than in CL. A study by Sharp et al. (2017) found similar results that the DNA methylation profiles of CL and CLP are more similar to each other than DNA methylation profiles of cleft palate (CP). The methylated regions differed more between CP and CL, than between CP and CLP, and more differed methylated regions between CP and CPP than between CL and CLP. This suggests that the three subtypes have different DNA methylation profiles, but the DNA methylation profiles of CL and CLP are more similar to each other than the DNA methylation profiles of CP. The implication of this oral cleft study is to remind that CL, CLP and CP should be analysed separately and not combined into a single entity or CL/P for analysis.[10]

This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This is in accordance with a study conducted in Saudi Arabia by Al Faishal et al., (2018) who indicated the transmission of epimutation BRCA1 from mother to child. Verification of methylation in positive mother-infant pairs in the promoter region, analyzed by pyrosequencing in three pairs, found that maternal and neonatal leukocyte DNA showed similar methylation patterns and rates across CpG sites analyzed.[11] A study by Iacobazzi et al., (2014) obtained different results. Folic acid and hyperhomocysteine metabolism disorders are thought to play a role in the incidence of CL/P. A case-control study showed that mothers of children with CL/P NS had higher concentrations of total plasma homocysteine during fasting as well as after a methionine loading test, compared with control mothers. Research on the use of periconceptual folate has been shown to prevent CL/P.[12]

This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers. A slightly different finding was obtained by Jin et al. (2015) who suspected a possible correlation between hyperhomocysteine and CL/P by the involvement of abnormal DNA methylation, cell proliferation, apoptosis, and migration during embryogenesis. High plasma homocysteine levels have been observed in mothers after the birth of babies with CL/P.[13]

## Conclusion

This study found no difference in methylation between CL and CLP, with the median value of DNA methylation in CLP higher than that in CL. This study found that DNA methylation between CL mothers and CLP mothers was relatively the same. This study also found no correlation between DNA methylation of CL and CL mothers, and between CLP and CLP mothers. Further studies are needed to examine blood homocysteine levels in CL and CLP and in their respective mothers.

**Ethical Clearence**: This observational study has passed the ethical test with clearance letter no E.5.a/037/KEPK-UMM/III/2020.

**Conflict of Interest:** The authors declare that

there is no conflict of interest in this work.

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# Non-Communicable Diseases among the Elderly in Indonesia in 2018

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## Abstract

Non-Communicable Disease (NCD) is closely related to the aging process, social development, and increased risk factors. The study aims to analyze the prevalence of NCD among the elderly in Indonesia based on demographic characteristics. The study employed the 2018 Indonesia Basic Health Survey data. The survey used the multi-stage cluster random sampling method; it was a weighted sample of 85,358 elderly. In this analysis, the NCDs prevalence in the elderly includes hypertension, heart disease, bronchial asthma, chronic renal failure, diabetes mellitus (DM), stroke, and cancer determined based on a doctor's diagnosis. The study result shows the prevalence of NCD in the elderly population in Indonesia, most of which are hypertension, mental-emotional disorders, depression, DM, and heart disease. Based on age group, the prevalence of hypertension, mental-health disorders, and depression tends to increase with increasing age, stroke and bronchial asthma are higher in the 70-79. DM and cancer were higher in the 60-69. Heart disease and kidney failure did not differ relatively between age groups. The prevalence of hypertension, mental-emotional disorders, depression, DM, heart disease, and cancer was higher in women. Still, asthma, stroke, and chronic kidney failure tended to be more in men. The study concluded that the most prevalent NCDs among older people in Indonesia were hypertension, mental, emotional disorders, depression, DM, and heart disease. NCDs have demographic characteristics.

***Keywords:*** *non-communicable diseases, hypertension, heart disease, diabetes mellitus, stroke, cancer, elderly, community health, public health.*

## Introduction

The aging population is a global trend in the 21st century. WHO data estimates show the increase in people aged 60 and over from 11% in 2006 to 22% in 2050. In five decades, aging in developing countries is much faster than in developed countries; more than 80 % of the world's elderly live in developing countries compared to 2005 as much as 60%[1]. In Indonesia, an older person is someone who has reached the age of 60 (sixty) years and over[2]. In five decades, the elderly in Indonesia has doubled (1971-2020), namely to be 9.92% (±26 million), older women (10.43%) are more than the male elderly (9.42%). In 2020, six provinces in Indonesia had an old population structure, the number of seniors has reached 10%, namely Yogyakarta (14.71%), Central Java (13.81%), East Java (13.38%), Bali. (11.58%), North Sulawesi (11.51%), and West Sumatra (10.07%)[3].

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Increasing age in the elderly is accompanied by a decrease in the body's intrinsic capacity and functional capability, which affects the body's organ systems[4]. NCD is closely related to the aging process and social development. The increase in the burden of NCD is due to a rise in the number of older people and the prevalence of commonly preventable risk factors such as tobacco use, inadequate physical activity, unhealthy diet, and harmful alcohol use. Metabolic risk factors are increased blood pressure, overweight/obesity, hyperglycemia, and hyperlipidemia[5]. Non-communicable diseases, also known as chronic diseases, tend to be long-lasting and result from a combination of genetic, physiological, environmental, and behavioral factors. The main types of NCD are cardiovascular disease, cancer, chronic respiratory disease, and DM[6].

During the past month, about half of the elderly in Indonesia experienced health complaints both physically and psychologically (48.14%), and around 24.35% of the elderly were sick in the last month. However, the morbidity rate for the elderly in Indonesia in 2020 is the lowest point in the previous six years[7]. The majority of the elderly treated their health complaints, either by self-medicating or outpatient (96.12%)[3].

The elderly are at high risk for suffering from NCD, so the author compiles the article to analyze the prevalence of NCD in the elderly in Indonesia based on demographic characteristics. We expect to provide input for strategic policies for prevention and control of NCD in Indonesia.

## Materials and Methods

The study employed the 2018 Indonesia Basic Health Survey data. The survey was a national-scale survey conducted by the Indonesian Ministry of Health. The population in this study was the elderly (≥60 years old) in Indonesia. The survey used a multi-stage cluster random sampling method, and it was a weighted sample of 85,358 elderly.

The NCDs prevalence in the elderly includes hypertension, heart disease, bronchial asthma, chronic renal failure, diabetes mellitus, stroke, and cancer determined based on a doctor's diagnosis. Cancer in question was any cancer diagnosed by a doctor. Heart disease is any heart disease, including congenital heart defects that doctors diagnose. The prevalence of emotional-mental disorders is the elderly who are currently experiencing emotional-mental conditions. According to the SRQ-20 (Self Reporting Questionnaire), depression prevalence was the elderly who are currently experiencing depressive disorders according to MINI (Mini International Neuropsychiatric Interview).

The study carried out analysis by statistical descriptive by observing the distribution by demographic characteristics of the elderly. The demographic characteristics of the elderly who were involved were age, residence, gender, education, work type, and wealth status.

## Results and Discussion

Table 1 shows an overview of NCDs in the elderly population in Indonesia, most of which are hypertension, mental-emotional disorders, depression, DM, and heart disease. Based on the elderly age group, the higher the age, the higher the prevalence of hypertension, mental health disorders, and depression. The hypertension prevalence, mental-emotional disorders, and depression in women were higher than men's. The hypertension prevalence was higher in urban areas, while mental-emotional disorders and depression were higher in rural areas.

**Table 1. The prevalence of NCD among the elderly in Indonesia**

| Demographic Characteristics | Prevalence of Disease | | |
|---|---|---|---|
| | % or $^{0}/_{00}$ | 95% CI | N (Adjusted) |
| Hypertension (%) | 32.6 | 32.0 – 33.2 | 58.666 |
| Emotional mental disorders (%) | 12.7 | 12.3 – 13.1 | 82.304 |
| Depresi (%) | 7.6 | 7.4 -7.9 | |
| Heart Desease (%) | 4.4 | 4.2 – 4.6 | 85.358 |
| Asma Bronchaile % | 4.2 | 4.0 -4.5 | |
| Cronic Renal Failure (%) | 0.8 | 0.7 – 0.9 | |
| DM($^{0}/_{00}$) | 56.0 | 53.8 – 58.4 | |
| Stroke ($^{0}/_{00}$) | 43 | 40 - 45 | |
| Cancer ($^{0}/_{00}$) | 4.3 | 3.8 – 5.0 | |

Source: The 2018 Indonesia Basic Health Survey

The prevalence of hypertension and mental-emotional disorders had a higher tendency for uneducated elderly and depression at the low education level. The hypertension prevalence and mental-emotional disorders and depression were higher in the group who did not work than the group who worked. Based on wealth status, the hypertension prevalence was higher at the wealthies, whereas most depression and the mental-emotional disorder tended to be higher at the lowest.

The hypertension prevalence based on doctor's diagnosis in Indonesia increases with increasing age and wealth, tends to be higher for women who live in urban areas. Previous research mentions the possibility of a daily dietary diet, adipose activity, and psychosocial stress, which can cause blood pressure to increase with age and increase in wealth[8]. Hypertension in older women is due to the reduced protective effect of the hormone estradiol, which affects the structure and tone of blood vessels and vasodilation of the endothelium of blood vessels[8]. Previous studies have informed that the prevalence of hypertension is higher due to lifestyle changes such as lack of physical activity and dietary modifications[9].

The prevalence of mental-emotional disorders and depression is higher among women who live in rural areas, have low education, are higher in the unemployed, and have lower wealth. Globally, around 15% of adults aged 60 and over suffer from mental disorders, mental and neurological disorders that account for 6.6% of total disabilities. The most common mental and neurological disorders in the elderly in the world are dementia (5%) and depression (7%), anxiety disorders (3.8%)[10].

Kiely's research found that older women generally experience mental disorders such as depression and anxiety, whereas men experience more adverse mental health impacts related to death, including suicide. The gender pattern varies according to country and other social contexts, influenced by cultural and social norms, differentiation of gender roles, disadvantages,

and empowerment throughout life[11–13]. Meanwhile, older people with higher education and positive mental attitudes have better psychological adjustment and better perceptions of changes in aging, both physically, life, and professionally[14–16].

**Table 2. The prevalence of hypertension and mental health among the elderly by demographic characteristics in Indonesia**

| Demographic Characteristics | Hypertension (n = 58.666) | N (adjusted) | Mental Health (n=82.304) | | N (adjusted) |
|---|---|---|---|---|---|
| | | | Emotional Mental disorder | Depression | |
| Age | | | | | |
| · 60-69 | 30.7% | 37,773 | 11.6% | 7.0% | 53,719 |
| · 70-79 | 35.5% | 16,267 | 14.0% | 8.6% | 22,382 |
| · ≥80 | 37.4% | 4,626 | 17.1% | 9.8% | 6,202 |
| Residence | | | | | |
| · Urban | 35.2% | 31,608 | 12.1% | 7.5% | 41,756 |
| · Rural | 29.6% | 27,058 | 13.3% | 7.8% | 40,548 |
| Gender | | | | | |
| · Male | 27.1% | 25,739 | 10.0% | 6.1% | 39,279 |
| · Female | 36.9% | 32,927 | 15.2% | 9.0% | 43,025 |
| Education | | | | | |
| · No education | 33.7% | 11,091 | 14.4% | 8.3% | 16,729 |
| · Didn't graduate from elementary school | 32.0% | 15,770 | 14.4% | 8.5% | 22,962 |
| · Elementary school | 32.4% | 18,179 | 12.8% | 7.9% | 25,862 |
| · Junior high school | 33.8% | 4,854 | 10.3% | 6.5% | 6,268 |

***Cont...*** **Table 2. The prevalence of hypertension and mental health among the elderly by demographic characteristics in Indonesia**

| | | | | | |
|---|---|---|---|---|---|
| · Senior high school | 31.9% | 5,896 | 7.9% | 5.3% | 7,205 |
| · College | 32.4% | 2,877 | 5.7% | 3.4% | 3,277 |
| Occupation | | | | | |
| · Not works | 39.1% | 27,922 | 16.5% | 10.0% | 35,518 |
| · Public servant/army/ police | 30.6% | 792 | 5.8% | 3.5% | 972 |
| · Private sector | 21.5% | 808 | 7.8% | 5.0% | 1,114 |
| · Entrepreneur | 28.9% | 6,114 | 8.0% | 5.3% | 8,255 |
| · Farmer | 24.0% | 15,833 | 10.7% | 5.8% | 25,899 |
| · Fisherman | 21.4% | 249 | 12.1% | 5.4% | 433 |
| · Labor/Driver/Maid | 26.2% | 2,940 | 10.0% | 6.9% | 4,864 |
| · Others | 34.5% | 3,801 | 8.8% | 6.7% | 4,987 |
| Wealth | | | | | |
| · Poorest | 31.5% | 11,995 | 13.9% | 8.1% | 18,880 |
| · Poorer | 31.4% | 11,042 | 13.8% | 8.1% | 16,182 |
| · Middle | 32.4% | 10,788 | 12.7% | 8.2% | 15,055 |
| · Richer | 32.9% | 11,140 | 12.6% | 7.6% | 15,313 |
| · Richest | 34.4% | 13,702 | 10.3% | 6.3% | 16,872 |

Source: The 2018 Indonesia Basic Health Survey

Table 3 shows, based on age group, the prevalence of heart disease and kidney failure is relatively not different, while for asthma bronchiale, it tends to be higher in the 70-79 age group. The heart disease prevalence was higher in women, but asthma and chronic kidney failure tended to be more in men. The majority of heart disease and chronic renal failure was higher in urban areas, whereas asthma bronchiale was higher in rural areas.

Yazdanyar's (2009) study found that the prevalence of heart disease increases with age, almost

the same for men and women at the age of 60-79, but more in women at >80[17]. A previous study reported that the high prevalence of heart disease in urban areas is mainly due to the high prevalence of lipid profile disorders in urban communities in China. The situation is possible due to a more sedentary urban lifestyle and a diet high in fat[18]. The factor that affects the cardiovascular risk of the elderly in Malaysia is a lack of physical activity[19]. The asthma prevalence in males is higher likely to be influenced by smoking behavior in men[20]. The asthma prevalence is high among the elderly who live in rural areas, did not complete primary school, and work as fishermen[21].

**Table 3. The prevalence of heart disease, asthma bronchiale, and chronic renal failure among the elderly by demographic characteristics in Indonesia (n=85,358)**

| Demographic Characteristics | Heart Disease | Asthma Bronchiale | Chronic Renal Failure | N (adjusted) |
|---|---|---|---|---|
| Age | | | | |
| · 60-69 | 4.3% | 3.8% | 0.7% | 54,487 |
| · 70-79 | 4.5% | 5.2% | 0.8% | 23,588 |
| · ≥80 | 4.3% | 4.4% | 0.8% | 7,284 |
| Residence | | | | |
| · Urban | 5.5% | 4.1% | 0.9% | 43,373 |
| · Rural | 3.1% | 4.4% | 0.7% | 41,985 |
| Gender | | | | |
| · Male | 4.2% | 5.2% | 0.9% | 40,375 |
| · Female | 4.4% | 3.4% | 0.7% | 44,983 |
| Education | | | | |
| · No education | 2.9% | 4.4% | 0.6% | 17,943 |
| · Didn't graduate from elementary school | 3.4% | 4.6% | 0.7% | 23,763 |
| · Elementary school | 4.3% | 4.3% | 0.7% | 26,589 |
| · Junior high school | 5.9% | 3.6% | 1.1% | 6,381 |
| · Senior high school | 7.6% | 3.9% | 1.1% | 7,342 |
| · College | 8.9% | 3.2% | 1.5% | 3,340 |
| Occupation | | | | |
| · Not works | 5.4% | 4.8% | 0.9% | 38,090 |

***Cont...*** **Table 2. The prevalence of hypertension and mental health among the elderly by demographic characteristics in Indonesia**

| | | | | |
|---|---|---|---|---|
| · Public servant/army/ police | 7.7% | 2.4% | 2.3% | 981 |
| · Private sector | 5.6% | 3.6% | 0.2% | 1,121 |
| · Entrepreneur | 4.8% | 3.9% | 0.7% | 8,301 |
| · Farmer | 2.4% | 3.9% | 0.7% | 26,178 |
| · Fisherman | 1.8% | 5.8% | 0.2% | 438 |
| · Labor/Driver/Maid | 3.1% | 3.4% | 0.5% | 4,897 |
| · Others | 6.4% | 3.9% | 0.7% | 5,067 |
| Wealth | | | | |
| · Poorest | 2.8% | 4.2% | 0.6% | 19,869 |
| · Poorer | 3.3% | 4.3% | 0.8% | 16,814 |
| · Middle | 3.6% | 4.4% | 0.6% | 15,586 |
| · Richer | 5.0% | 4.3% | 0.8% | 15,733 |
| · Richest | 7.2% | 4.1% | 1.1% | 17,357 |

Source: The 2018 Indonesia Basic Health Survey

The prevalence of chronic kidney failure among the elderly tends to be almost the same in all groups of the elderly, higher in men, living in urban areas, increasing along with the higher education level and the wealthies. A previous study stated that the highest prevalence of chronic kidney failure was 70-79 years, then 80-89 years, and 60-69 years, respectively. The progression of chronic renal failure in men occurs more rapidly than in women because of the hormone testosterone[22]. The hormone testosterone, which is the primary steroid hormone in men, affects increasing renal tubular apoptosis and accelerating the severity of chronic kidney failure in men[23].

**Table 4. The prevalence of DM, stroke, and cancer among the elderly by demographic characteristics in Indonesia (n=85,358).**

| Demographic Characteristics | Diabetes Mellitus (0/00) | Stroke (0/00) | Cancer (0/00) | N (adjusted) |
|---|---|---|---|---|
| Age | | | | |
| · 60-69 | 64.1 | 39 | 4.6 | 54,487 |
| · 70-79 | 47.4 | 49 | 4.0 | 23,588 |
| · ≥80 | 23.2 | 47 | 3.7 | 7,284 |
| Place of residence | | | | |

***Cont...*** **Table 4. The prevalence of DM, stroke, and cancer among the elderly by demographic characteristics in Indonesia (n=85,358).**

| | | | | |
|---|---|---|---|---|
| · Urban | 78.8 | 52 | 5.5 | 43,373 |
| · Rural | 32.5 | 32 | 3.2 | 41,985 |
| Gender | | | | |
| · Male | 48.3 | 46 | 3.0 | 40,375 |
| · Female | 62.9 | 40 | 5.6 | 44,983 |
| Education | | | | |
| · No education | 30.4 | 32 | 3.0 | 17,943 |
| · Didn't graduate from elementary school | 43.4 | 38 | 3.2 | 23,763 |
| · Elementary school | 51.0 | 42 | 5.0 | 26,589 |
| · Junior high school | 81.7 | 58 | 5.8 | 6,381 |
| · Senior high school | 109.8 | 64 | 5.6 | 7,342 |
| · College | 156.3 | 61 | 9.1 | 3,340 |
| Occupation | | | | |
| · Not works | 74.8 | 68 | 5.9 | 38,090 |
| · Public servant/army/ police | 117.3 | 50 | 7.3 | 981 |
| · Private sector | 73.3 | 24 | 0.4 | 1,121 |
| · Entrepreneur | 69.8 | 31 | 4.8 | 8,301 |
| · Farmer | 20.6 | 15 | 2.2 | 26,178 |
| · Fisherman | 19.9 | 18 | 1.2 | 438 |
| · Labor/Driver/Maid | 28.8 | 15 | 3.4 | 4,897 |
| · Others | 88.7 | 46 | 4.1 | 5,067 |
| Wealth | | | | |
| · Poorest | 26.6 | 35 | 3.4 | 19,869 |
| · Poorer | 37.7 | 37 | 3.3 | 16,814 |
| · Middle | 46.7 | 41 | 3.2 | 15,586 |
| · Richer | 60.8 | 45 | 4.8 | 15,733 |
| · Richest | 111.5 | 56 | 7.0 | 17,357 |

Source: The 2018 Indonesia Basic Health Survey

Table 4 shows the highest prevalence of DM in the 60-69 age group, living in urban areas, females tend to be high in education and government employees' occupation, according to several studies. Kirkman reported that the incidence of DM increases with increasing age until 65 years and after 65 years, both the incidence and prevalence rates decrease[24]. A previous study says that the DM prevalence tends to be high in the elderly because there is a degeneration process that decreases the function of pancreatic

β cells in producing insulin. Older women have a higher chance of increasing the body mass index to become obese and post-menopause. It is easier for fat accumulation to occur so that the translocation of glucose transporter to the plasma membrane will decrease, resulting in insulin resistance in muscle and adipose tissue[25].

The stroke prevalence tends to be higher in the 70-79 age group, in urban areas, higher in men, increases with education levels and wealth status. The prevalence of cancer was higher in the 60-69 age group. The situation is different from the previous research that stated the higher the age, the greater the cancer risk because increasing age will decrease resistance to cancer[26]. The prevalence of cancer was higher among older women and living in urban areas. The 2013 Indonesia Basic Health Survey reports that the highest majority of primary malignancies are cervical cancer and breast cancer. Previous research has found that lifestyle factors in urban areas, including smoking, diet, alcohol consumption, reproduction (pregnancy, breastfeeding, age at first menstruation, menopause), obesity, and lack of physical activity, are thought to be the main contributors to cancer growth[27]. The cancer prevalence in the elderly is increasing with the higher education level and wealth status.

## Conclusions

The most NCDs prevalence among the elderly in Indonesia was hypertension, mental-emotional disorders, depression, DM, and heart disease. Based on demographic characteristics, the higher the elderly, the higher the prevalence of hypertension, mental health disorders, and depression. Meanwhile, stroke and bronchial asthma tend to be higher in the 70-79 elderly age group, DM and cancer were higher in the 60-69 age group, however, the prevalence of heart disease and kidney failure did not differ relatively between age groups.

The prevalence of hypertension, mental-emotional disorders, depression, DM, heart disease, and cancer was higher in older women. Still, asthma, stroke, and chronic kidney failure tend to be more in men. The prevalence of hypertension, DM, heart disease, chronic kidney failure, stroke, and cancer in the elderly was higher in urban areas. It tends to increase with increasing levels of education and expenditure levels. At the same time, mental-emotional disorders, depression, and bronchial asthma were higher in rural areas and tended to be higher at low education and low wealth.

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# The Motivation of Preclinical Students to be A Doctor: A Turkish Perspective in the Private Educational Sector

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## Abstract

Global estimates confirm the future need for more health cadres, including doctors. The study aims to investigate reasons to choose medicine as a career among students at a Turkish private medical school. A web-based cross-sectional study was conducted among the first-, second-and third-class medical students receiving education in the second semester of the 2017-2018 academic year at the BezmialemVakif University, Istanbul, Turkey. The universal sampling technique using a semi-structured questionnaire was recruited to collect the data. The SPSS 16.0 package used to analyze the data, and the statistically significant was considered at less than 0.05. The mean age of students was 20.3 + 4.3 years (range 18-23 years). More than half (57.3%) were females, and most of them (76.5%) lived with their parents. The majority (87.5%) of them inquired about medical college, and 85.1% of them won the medical school in the first trial. The main reasons to study medicine were personal preference(75.7%), studying medicine is appealing(52.4%),humanitarian profession(52.2%), the ready availability of work for physicians(42.2%), challenging carrier providing an opportunity to solve mysteries(40.8%), childhood dream(39.2%), profiting profession(35.7%), my parents' vision and choice(31.8%), respectively. Almost the motives behind choosing to study medicine are similar across societies but different in the priorities.

***Keywords:*** *Motivation; Perspective; Preclinical, Future career, Medicine, Students; Turkey*

## Introduction

In Turkey, thousands of high school graduates are applying to join medical schools annually[1]. However, very little is known about their interest to practice medicine1. In fact, choosing the type of undergraduate study may be the most critical challenge facing the high school students after passing the national pre-university or "the national Students Selection and Placement Center (OSYM) exam"[2,3]. Literature shows that choosing medicine as a career among students from different worldwide regions may share similar motivations such as demographic characteristics, prestige, income, faculty role model, substantial early interest, lifestyle, and curiosity in the specialty[1]. However, significant differences have been reported from culture to culture. Family pressure is among the most common factors influencing the student's decision to study medicine in developing countries, but it is not the case in developed

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countries[2,4,5].In the last few years, the career goals for Turkish students have slightly been changed toward remuneration, advancement, training, efficient leadership, and secured employment[6].The quality aspects related to the professional skills of physicians were on the top priority of the Turkish population compared to communication or accessibility and the organizational skills of health care providers[7].The Turkish government has developed the health system over the past two decades to become more responsive to patients' needs and expectation[7,8]. An exceptional effort has been made to improvethe work environment in health institutions through the implementation of the universal health insurance (UHI) system, the application of patient's classification system (PCS) such as diagnosis-related groups (DRGs), and the payment for performance (P4P)[8-10]. The consequences were significant improvement in most health indicators, increased accessibility, availability of health resources and enhancement of quality, and increased studying medicine among Turkish youth of both sexes[7,11]. This study aims to investigate the motivations of choosing medicine as a career among students from the private educational sector.

## Material and Methods

### Study Design

A cross-sectional (web-based) descriptive study designed to explore the motivation of choosing medicine as a future career among the preclinical students enrolled in the faculty of medicine during the second semester of the 2017-2018 academic year at the BezmialemVakif University (Private sector), Istanbul, Turkey. The universal sampling techniques were recruited to collect the data through semi-structured and self-administered questionnaires. The study protocol was approved by the Ethics committee of the Faculty of Medicine, BezmialemVakif University (Reference number: 8/74 on 27th March 2018).

### Inclusion and exclusion criteria

All medical students in the 1st, 2nd, and third-year classes, both gender, Turkish nationality, and willingness to participate, are included in the study. Students from other classes or other colleges, post-graduates, other nationalities, and those who were unwilling to participate are excluded from the study.

### Data Collection

A semi-structured questionnaire was developed to collect the data. The questionnaire was divided into three parts:

**Parts one:**The socio-demographic data including age, sex, accommodation places, and whetherone or two of their parents are either health-related employer or academician.

**Part two:**Questions related to admission to medical schools, such as the current stage they in, the type of high school that they graduated from, the number of trials to pass the OSYM exam,and the source of the tuition fees.

**Part three:**Sources of pre-enrolment information about the medical school.

**Part four:**Reported reasonsbehind choosing medical college as a future career.

## Data Analysis

The data presented in the form of percentages, mean, and standard deviation. The SPSS 16.0 package used to analyze the data, and the statistically significant was considered at less than 0.05. A P-value of $\leq 0.05$ was considered statistically significant.

## Results

### Socio-Demographic characteristics

Two hundred and fifty (response rate=86.8%) completed questionnaires were undergone to the final analysis. Table 1 shows the socio-demographic characteristics of respondents. The mean age of them was 20.3 + 4.3 years (range 18-23 years).More than half of them were female (146, 57.3%), and three-quarter195(76.5%) accommodated with their parents

(Homestay) during medical school education. Among one-quarter of surveyed students, one or two of their parents are either health-related employer 69(27.1%) such as medical, dentist, pharmacist, nurse, etc., or and academician62 (24.3%) in university, institution, school, etc., respectively (Table 1).

**Table 1 Sociodemographic factors (n=255)**

| Questions | Categories | N (%) |
|---|---|---|
| Age | Mean (SD): 20.3 + 4.3, range 18-23 years | |
| Gender | Male | 109(42.7) |
| | Female | 146 (57.3) |
| Accommodation places for students during medical school education: | Homestay (with parents) | 195(76.5) |
| | Government dorm | 6(2.4) |
| | Private dormitory | 12(4.7) |
| | Stand-alone private house | 32(12.5) |
| | Private house with friends | 10(3.9) |
| One or two of your parents isHealth-related job (medical, dentist, pharmacist, nurse, etc.: | Yes | 69(27.1) |
| | No | 186(72.9) |
| One or two of your parents isAcademician (university, institution, school, etc.: | Yes | 62 (24.3) |
| | No | 193(75.7) |

**Admission to medical school**

More than one-third (38.4%) of students were from the third stage, compared to 32.5% and 29.1% from the first and second stages, respectively. Most of the students graduated from either public (53.3%) or private (43.1%) high schools. The highest percent (85.1%) of students win medical school from the first trial in the national pre-university test (OSYM). The tuition fees were mixed (government and self-sponsored) in about half 50.2% of students; however, still one-third (32.2%) of students are self-sponsored (Table 2).

**Table 2Factors related to admission to medicine (n=255)**

| Question | Categories | N (%) |
|---|---|---|
| Currently, which medical stage are you in? | 1st Class | 83(32.5) |
| | 2nd Class | 74(29.1) |
| | 3rd Class | 98(38.4) |
| Which type of high school that you graduated from? | Government | 136(53.3) |
| | Private | 110(43.1) |
| | International | 9(3.5) |
| Acceptance for medical school was after:- | First trial | 217(85.1) |
| | Second trial | 36(14.1) |
| | More than two | 1(0.8) |
| Tuition fees are: | Self-sponsored | 82(32.2) |
| | Government | 45(17.6) |
| | Mixed Scholarship | 128(50.2) |

**Pre-enrolment knowledge about the medical school**

Most of the students (87.5%) inquired about medical college before applying for study. The primary sources providing information to students about the medical school were students (58.8%) from the medical group (medical, dentist, pharmacist, nurse, etc.), parents and family (48.6), and physicians (51.4%), respectively. The information has a positive value among 56.1% of students, and 40.8% considered the information as enough (Table 3).

**Table 3 Pre-enrolment knowledge about the medical school(n=255)**

| Question | Categories | N (%) |
|---|---|---|
| Have you been inquired about medical college before applying for study? | Yes | 223(87.5) |
| | No | 32(12.5) |
| Whom of the following persons provided you with information about medical college? | Student (medical, dentist, pharmacist, nurse, etc.) | 150(58.8) |
| | Parents and family | 124(48.6) |

***Cont...*** **Table 3 Pre-enrolment knowledge about the medical school(n =255)**

| | | |
|---|---|---|
| | A physician | 131(51.4) |
| | Friends | 109(42.7) |
| | A worker in the health profession (dentist, pharmacist, nurse, etc.) | 88(34.5) |
| | Others | 57(22.4) |
| How to describe the information you received? | The information was enough | 104(40.8) |
| | The information had positive value | 143(56.1) |
| | The information affected the personal decision | 82(32.2) |
| | Others | 23(9.0) |

**The reported reason for choosing a medical college**

As shown in Table 5, the most frequently reported reason for choosing a medical college (75.7%) is a personal preference. Humanitarian reasons (52.2%) and considering studying medicine is appealing (52.4%) were reported by more than half of students, respectively. About two-fifth of students reported three reasons related to "Ready availability of work for physicians (42.2)", "Challenging carrier providing an opportunity to solve mysteries (40.8%)," and 'Childhood dream(39.2)".About one-third of students considered medicine is "Profiting profession (35.7)" and also respond to the "Parents' vision and choice (31.8%)".Less than one-third of the students believe that "the country is in dire need of doctors' services (29.0%)" in addition to "the positive evaluation that doctors receive from the community(28.6%)". In comparison, "24.7% believe that the reason has a very high secondary school graduation mark". About 22.0% of students reported the other three different reasons behind the desire to join medical school are "Religious causes (exploring the glories of God's creation)," "Preserving my health and that of my family" and then choosing "Happened without an advanced plan". The remaining four reasons were reported by less than 20% of the students: "Personal experience with the disease or another person's disease," "Having physicians as relatives in the family," "Preserving my health and that of my family," "Trying to change the perceived negative image of physicians in the community," and "Positive physicians image reflected by media", respectively.

**Table 5 Reported reason for choosing a medical college (n=255)**

| No. | Reported reasons | N(%) |
|---|---|---|
| 1 | It was my personal preference | 193(75.7) |
| 2 | Studying medicine is appealing | 134(52.4) |
| 3 | Humanitarian profession | 133(52.2) |
| 4 | The ready availability of work for physicians | 108(42.2) |
| 5 | A challenging carrier providing an opportunity to solve mysteries | 104(40.8) |
| 6 | Childhood dream | 100(39.2) |
| 7 | Profiting profession | 91(35.7) |
| 8 | My parents' vision and choice | 81(31.8) |
| 9 | My country badly needs the services of physicians | 74(29.0) |
| 10 | Positive community appraisal for physicians | 73(28.6) |
| 11 | Having a very high secondary school graduation mark | 63(24.7) |
| 12 | Religious causes (exploring the glories of God's Creation) | 58(22.7) |
| 13 | Preserving my health and that of my family | 57(22.4) |
| 14 | Happened without an advanced plan | 50(19.6) |
| 15 | Personal experience with the disease or another person's disease | 43(16.9) |
| 16 | Having physicians as relatives in the family | 40(15.7) |
| 17 | Trying to change the perceived negative image of physicians in the community | 35(13.7) |
| 18 | Positive physicians image reflected by media | 18(7.1) |

## Discussion

In this study, three-quarters (75.7%) of the surveyed students had made their choice independently without interference from their parents or the family. Similar findings had been reported in studies from Iraq[2], India[12], Saudi Arabia[13], Polish[14]. However, 31.8% of our sample influenced by their families. Our results are similar to those reported in India (76.0%)[12] but higher than those recorded in Saudi Arabia (11.6%)[13], and lower than those observed in Iraq[2]. Considering that more than half (57.3%) of the students were female, it is consistent with the phenomenon of feminization of medicine[11]. However, previous studies reported that female doctors are more burned out from work and frustrated by job than male[15] and about 81.5% of medical students who were exposed to violence during the clinical training were females[16]. Therefore, we found that 76.5% of students reside with their families, reflecting the influence of lifestyle associated with the family entity and its impact on maintaining individuals and dominating professional decision-making.About a quarter of the participants in the study are either from professional medical (27.1%) or academic families (24.3%), which may help to understand the family pressure where the most successful parents often play a fundamental role in the decision-making

process inside their family. Moreover, the correlation of parents 'medical background with students' career preference aligns with our expectations and previous research findings[17]. Like previous studies conducted in India[12], Saudi Arabia[13], Poland [14], more than fifty-two percent of the students chose the profession of medicine career for humanitarian consideration.The medical profession is attractive to many individuals, including our respondents, because it is based on saving lives and the positive community appraisal. Hence, it is a matter of happiness, gaining community respect, and making family members proud [2,13,18]. Moreover, the graduated doctors do not have difficulty finding a job in Turkey because of the high employment rate of doctors compared to neighboring countries such as Iraq[2,19]. Furthermore,the World Health Organization report estimated the needs-based shortage of health care workers in 2013 at about 17.4 million globally, including nearly 2.6 million physicians[20].Likewise to earlier studies[2,14], a childhood dream of becoming a doctor was at the top of the reasons for 39.2% of students. This finding is interesting because we found that most students were well-prepared to join the medical school. About 43.1% of students cost private secondary school fees to achieve the highest graduation grades that qualify them to compete for medical school. Therefore most of them (85.1%) passed the OSYM exam from the first trial. In fact, there is a clear interest on the part of the Turkish family to obtain a medical school. Therefore, we found that one-third (32.2%) of the students completely relied on family financial support, while half of them (50.2%) relied on family support with multi-source scholarships.More than a third (35.7) of students considered medicine to be a profitable profession, which is a natural behavior consistent with the previous studies[2,13,14,17,21]; however, about one-third of the students stated they wanted a positive community evaluation of physicians and the concern for personal and family health.Furthermore, about thirty percent of the students realized that their country desperately needed the services of doctors.Turkey and most developing countries suffer from brain drain, including medical professionals. Frequent views on social media of violence cases against health service providers may lead to the reluctance of young people to apply to medical colleges and health professions. Several reasons lie behind the continuous emigration of doctors, foremost of which are job burnout, job dissatisfaction, low income, few financial incentives, fewer opportunities to specialization, liable to more virulent microorganisms, insecurity, and high prevalence of violence at workplace [16, 22,23, 24].Most of the surveyed students (87.5%) worked hard to inquire about medical school before making the final decision. Similar to findings from Iraqi study[2], colleagues' students from the medical group were the main source of information (58.8%), parent (48.6), physician (51.4), friend (42.7), and health worker (34.5). The information received by the students was satisfactory in about forty percent of the students and affected their decisions positively by about 56.1%. These data show that Turkish students had a wide space for counseling, unlike students in Iraq[2].Most public and private Turkish universities adopt a media program that explains in detail the advantages of admission to the medical school, the study method, and the curriculum, with the opportunity to meet with some professors to help answer their questions about how to apply to medical colleges and what to expect from the medical profession.This study complaint some limitations. Although the response rate was seventy percent, the web-based survey and optional response might lead to selection bias. The cross-sectional design and descriptive analysis may prevent the determination of the causative reasons behind the choice of profession.The study sample was limited to one private university, and therefore its results cannot be generalized at the national level.

## Conclusion

The reasons for choosing medicine as a future career do not differ among Turkish students from their counterparts in other countries except in the priorities. Unlikemany previous studies, students' decision was not affected by their family members, and it was their

personal choice in about 75.7%, indicating a wide space of freedom to make a decision among Turkish youth. The priorities of studying medicine were because it is an attractive and humane profession with high employment opportunities. Moreover, medicine was the childhood dream and also a profitable profession. The choice of students was positively affected by the sufficient information they received from different sources.Most of the students showed early readiness to gain medical school accompanied by psychological and financial support.

**Source of Funding**: Self

**Conflict of Interest**: Non

**Ethical Clearance**: The study was performed under the guide lines supervision of Ethical Committee for human at a Turkish private medical school**.**

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# Fluvoxamine Provide a Gastro-Protection Against Vitiated Insult

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## Abstract

The etiology of peptic ulcer disease is multifactorial and remains an enigma over the last decades. The central parameter is the acid secretion; whose control is under the coordination of gastrin, acetylcholine, histamine, and prostaglandin. The treatment of peptic ulcers is a bi-armed tool, directed toward fighting microbial growth alongside acid suppression. However recent studies reported failure of the therapy due to recurrence of symptoms. Therefore, additional parameters should be considered including patient mood and psychological status. The present study aimed to introduce a new approach to the therapeutic regimen of ulcer disease using commonly used antidepressant drugs (fluvoxamine and fluoxetine) in a laboratory animal model of peptic ulcer induced by stress insult to act as a mood upset model in an attempt to mimic mood changes in human. The study was conducted on 4 groups of laboratory animals using control negative and control positive (misoprostol) against the tested drugs group (fluvoxamine and fluoxetine group). The result confirmed that fluvoxamine confers gastroprotective effects against ulcer insult compared to both fluoxetine or misoprostol groups. These results might significantly mean that antidepressant drugs could be utilized in peptic ulcer diseases or added at low doses to prevent ulcer insults due to whatever precipitating factors, such as, infection, alcohol, smoking, NSAIDs, and stress ulcer.

***Keywords:*** *fluoxetine, fluvoxamine, ulcer score, misoprostol, indomethacin.*

## Introduction

Gastric ulcers have been linked to steroidal and non-steroidal medicines, smoking, alcohol consumption, trauma, sepsis, shock, Helicobacter pylori, and stress; with the exception of ulcers, stress is among the most invasive causes that underpin many other disorders, such as depression. Amongst the most prevalent approaches for creating ulcer models is stress[1]. Many individuals with gastrointestinal system ulcers have depression, which is often associated with mental and somatic symptoms[2]. Evidence that some antidepressants can also have anti ulcerative properties[3] is of attention to the present study.

Through the use of tricyclic antidepressants (TCAs) for the management of gastrointestinal ulcer disease was the first known use of antidepressants for Intestinal disease[1]. Fluoxetine, bupropion, dothiepin, maprotiline, mianserin, trimipramine, monoamine oxidase-B (MAO-B) inhibitors, imipramine, amitriptyline, and mirtazapine, have been shown to

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have antiulcer properties[4,56]. In animal models, higher susceptibility to depression and anxiety is linked to ulceration, the same will be valid in human beings[7,8]. Furthermore, typical antidepressants and anxiolytics can greatly minimize the onset of stress ulcers, possibly to a significant degree than typical therapy like cimetidine and antacids[9,10].

Fluvoxamine, an SSRI medication, suppresses the CYP 1A2 enzyme, which has been linked to oxidative damage[11]. Nonetheless, in around 60%–80% of ulcer disorders, the causative factors are unknown, and the pathophysiologic circumstances in the course of sickness are comparable[1]. Higher levels of free radicals (ROS), for example, have been linked to the mechanisms of stress and indomethacin-induced stomach injury[12]. Experimental evidence[1,12] suggest the significant role of oxygen-derived ROS and lipid peroxides (LPO) in immediate gastric injuries generated by nonsteroidal anti-inflammatory medications (NSAIDs) such as indomethacin. Although some SSRIs have been shown to increase upper GIT hemorrhage when coupled with NSAIDs[13], fluvoxamine's protective effects on the CYP 1A2 enzyme and subsequent reduction in cell stress may be advantageous to the digestive system[11]. It's also been observed that boosting redox variables and reducing oxidant parameters are important in the antiulcer action mechanism of mirtazapine, an antidepressant medicine[1]. Unfortunately, no evidence about fluvoxamine's antiulcer activity is presently available. The focus of this research was to look at fluvoxamine's impacts in a rat ulcer model focusing on the histopathological appearance of intestinaltissues.

## Materials and Methods

A total of 40 Wistar rats (male, 130-160g, 10-weeks-of-age) were utilized for this study. Under standard laboratory animal conditions suitable for rat breading including temperature 22°C and good air conditioning, the studied groups were housed in animal house facilities provided by the university of Mosul. During the period of study, the rats were fed by commercially available pellet food and with free access to food and water. The researcher has followed the standard rules of laboratory animal breeding and treatment approved by Mosul University's local animal protection committee.

The animals were sub-classified randomly into four groups of 3 rats each; as follows, Group I (treatment-free control group using sterile water as a reference), Group II (fluvoxaminegroup), Group III (fluoxetine group), and Group IV (misoprostol group). Doses and suppliers are listed in table 1.The administration of the drug solution was manually done daily by orogastric tube (for 3 weeks duration) to overnight fasted rats. The daily doses of drug solution (50 mg/kg ) were freshly prepared by dissolving the drugs in sterile water. On the last day, a single dose of indomethacin insult was initiated to create the ulcer model and confirm protection provided by tested drugs, if any.A day before ulcer induction, rats were starved of food but had access to water.

**Table 1. The doses, origin, and suppliers of tested drugs.**

| Tested Drugs | Dose (mg/kg) | Supplier | Country |
|---|---|---|---|
| Fluvoxamine | 50 | Abbott, Illinois | United States |
| Fluoxetine | 20 | Bristol, Berkhamsted | United Kingdom |
| Misoprostol | 20 | Pfizer | United State |
| Indomethacin | 30 | Medochemie | Cyprus |

After 4 hours elapsed following ulcer induction, the laboratory animals were killed for their histopathological studying. The stomachs were then dissected and treated in a 10% buffered formalin solution for 24 hours before being treated in different amounts of alcohol and ultimately in xylene. After that, the tissues were fixed in paraffin, repeatedly sliced to a thickness of 4µm, placed on slides, and stained with hematoxylin and eosin (H&E; Sigma). The samples were then subjected to a blinded histological analysis by a team of experts. A competent pathologist used a Model BM-2101 light microscope to perform a blinded histopathological evaluation on the specimens (Olympus, Yuyao, China). The outcome of tissue remark for injury or lesion were listed in table 2 below.

**Table 2. Scores and remark of the all pathological changes**

| Remark | Score |
|---|---|
| Almost normal mucosa | 0 |
| Vascular congestions | 1 |
| One or two lesions | 2 |
| Severe lesions | 3 |
| Very severe lesions | 4 |
| Mucosa full of lesions | 5 |

## Results

The sectioning of the stomach of rats following indomethacin exposure revealed histopathological changes at mucosal and submucosal levels compared to the indomethacin-free control group. The mucosa of rats was examined using a light microscope and the result showed the highest score (approximately 5) of structural damage including ulcer formation, erosion formation, necrosis of epithelium of gastric mucosa, hemorrhage, infiltration of inflammatory cells, and dystrophic calcification (score and relevant degree of injury listed in table below). The control group has shown a completely negative outcome for the aforementioned parameters regarding mucosal and submucosal tissues (see Figure 1 and Table 3).

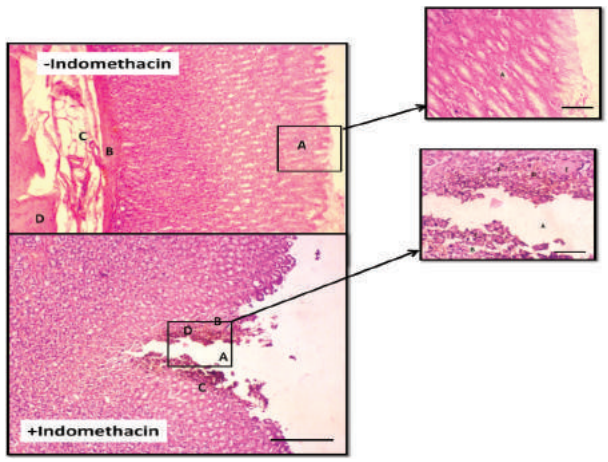


**Figure 1. Normal versus an ulcerated histological architecture of the rat stomach. No evidence was noticed of erotic and ulcerative changes of gastric layers mucosa (A), muscularis mucosa (B), submucosa (C) and muscularis (D) in normal compared to the ulcerated stomach by indomethacin; following staining by H&E stain, scalebar100µm.**

The sectioning of the stomach of rats of the studied groups following indomethacin exposure plus tested agents (fluvoxamine, fluoxetine, and misoprostol) revealed histopathological changes at mucosal and submucosal levels at various levels of severity (see Figure 2 and Table 3) in fluoxetine and misoprostol compared to fluvoxamine group. The mucosa of rats was examined using a light microscope and the result showed the highest scores of structural damage including ulcer formation, erosion formation, necrosis of epithelium of gastric mucosa, hemorrhage, infiltration of inflammatory cells, and dystrophic calcification in fluoxetine and misoprostol group. The fluvoxamine group has shown mild damage (scores close to 0) for the aforementioned parameters regarding mucosal and submucosal tissues (see Figure 2 and Table 3). Fluoxetine and fluvoxamine are SSRI (selective serotonin reuptake inhibitor) which is frequently have been used to manage pain, panic attacks, and alcoholism. Serotonin (5-hydroxytryptamine; 5HT) transporter are thought to be inhibited. Because ulcers are linked to a decrease in serotonin levels in stomach tissues[14,15], researchers

wanted to see if fluoxetine or fluvoxamine could have comparable properties on ulcer onset and development.

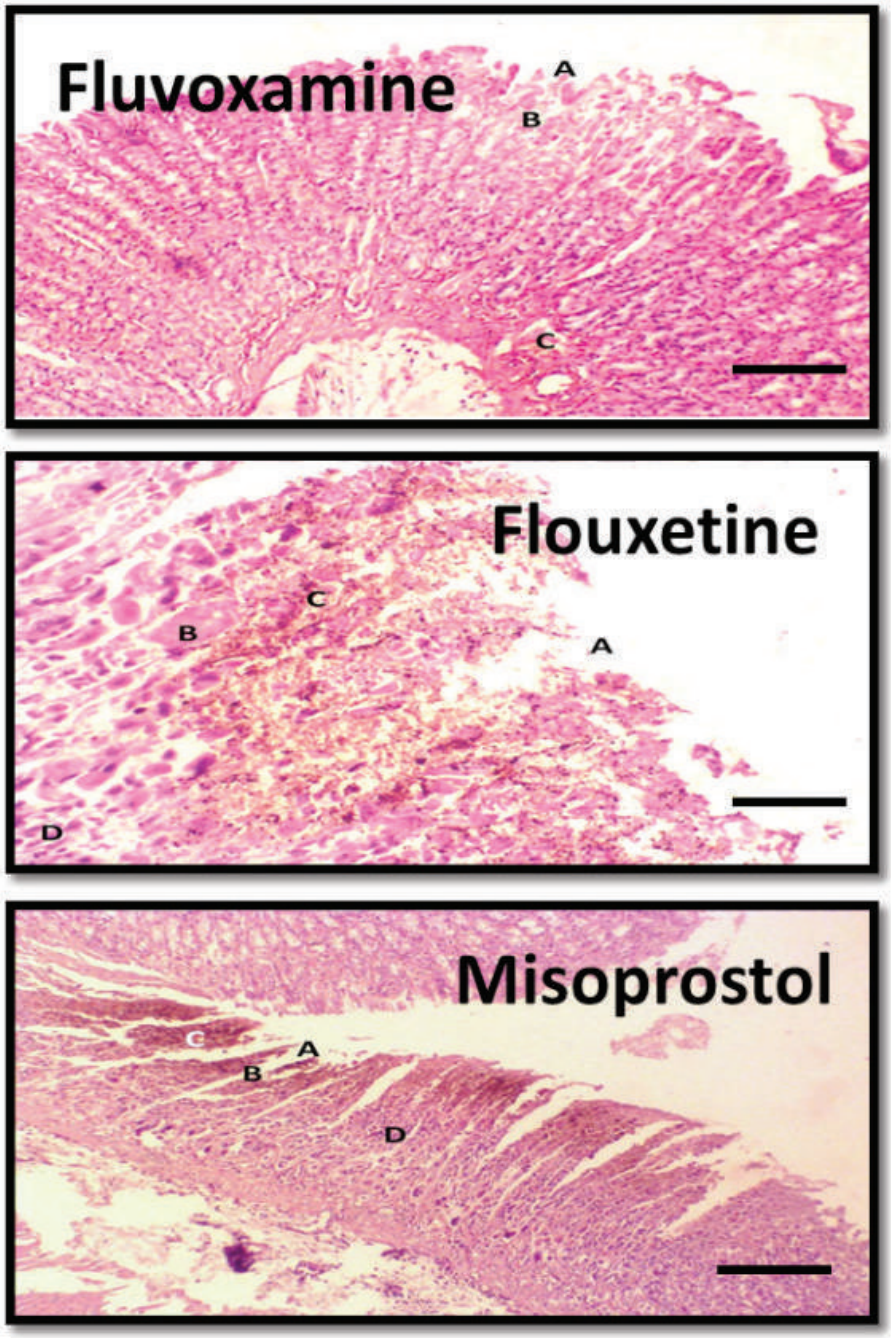


**Figure 2. A representative image for the histology of rat stomach in different studied groups. Fluvoxamine showed mild evidence of erosion of gastric mucosa (A), necrosis of epithelium of gastric glands (B), hemorrhage (C), and congestion of blood vessels (D), compared to severe score associated with both fluoxetine and misoprostol group, following staining by hematoxylin and eosin stain, scalebar100µm.**

**Table 3.Scores of the lesions of the groups of the study.**

| The lesions | -Drugs | +Indomethacin | +Fluvoxamine | +Fluoxetine | +Misoprostol |
|---|---|---|---|---|---|
| Ulcer formation (reaching muscularis mucosa) | 0 | 5 | 0 | 2 | 4 |
| Erosion formation | 0 | 5 | 1 | 3 | 5 |
| Necrosis of epithelium of gastric mucosa | 0 | 5 | 1 | 3 | 4 |
| Hemorrhage | 0 | 5 | 2 | 3 | 5 |
| infiltration of inflammatory cells | 0 | 4 | 1 | 2 | 3 |
| Dystrophic calcification | 0 | 5 | 0 | 0 | 1 |

## Discussion

Fluvoxamine's antiulcer impact in animals was studied in the present research utilizing an indomethacin-induced ulcer model. Fluvoxamine's beneficial effects were compared to fluoxetine and misoprostol to confirm that the action is specifically related to fluvoxamine. Fluvoxamine was demonstrated to reduce indomethacin-induced ulcers selectively versus the same member of its group-fluoxetine. Fluvoxamine's antiulcer activity was found to prevented indomethacin-induced ulcers more effectively than misoprostol.

As compared to several other NSAIDs, indomethacin has been demonstrated to cause more gastrointestinal injury in rats[16]. As a result, it seems to be the medication of choice for producing ulcer lesions. Antidepressant medicines have indeed been demonstrated in numerous trials to reduce histamine release from mast cells, limit stomach acid secretion, and block leukotriene (LTC4, D4, E4) receptors, resulting in antiulcer actions[17]. Beyond those mechanisms, redox lipid peroxidation is the most important dominant driver in indomethacin-induced stomach injury.Fluvoxamine, an SSRI, inhibits the CYP 1A2 enzyme, which is known to create reactive oxygen species (ROS)[9].

Intrinsic 5-HT plays a role in gut function modulation, inhibiting stomach acid production while boosting mucus secretion[7]. The current findings revealed that indomethacin and alcohol decreased stomach serotonin concentration, which is consistent with prior research that established that lesions developed in parallel with serotonin deficiency in stomach tissues[14]. Comparable to the CNS, the enteric nervous system has a serotonin reuptake mechanism that is suppressed by serotonin reuptake inhibitors. This might reflect why fluvoxamine was able to maintain the rats' gastric lesionsminimumcompared to misoprostol or fluoxetine, whereas fluoxetine from the same class of fluvoxamine was unable to do so, the reason behind that is unclear[13,18].

In rats, indomethacin (INDO) induced severe stomach mucosa lesions. Animal primed with fluoxetine, fluvoxamine, or misoprostol lowered ulcer scores to various extents. However, fluvoxamine seems to be the most effective one (see Figure 2 and Table3).

## Conclusion

We discovered that fluvoxamine possesses antiulcer properties. Indomethacin induces stomach injury through its potent irritation and systemic damages. Fluvoxamine but not fluoxetine seems to

activate its antiulcer actions in gut tissues via activating serotonin pathways and further investigation was required to confirm the differences between the mechanism of fluoxetine and fluvoxamine.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Taken from College of Pharmacy Research Ethics Committee

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# The Effect of TROP2 Expression on Papillary Thyroid Carcinoma Development in Iraqi Patients

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## Abstract

**Background** : Papillary Thyroid Carcinoma (PTC) is common type of the thyroid cancers, it's onset in female at 30-50 years more than male and account for about 80% of all thyroid cancer cases . PTC diagnosis depend on histo-pathological finds of abnormal papillary architectures in the tissue as golden standerd of PTC diagnosis . Trophoblast cell surface antigen 2 (TROP2) is trans-membrane glycoprotein receptor ,encoded by Tacstd2 gene , observe in various cancer types specially epithelial cancers . TROP2 has the up-regulation ( overexpression) in cancer cells such as pancreatic , thyroid and ovarian cancers. **Objective of the Study**:It's shown up-regulation of TROP 2 in PTC Iraqi patients by Immunohistochemical method.

**Materials and Methods**: The current study done in Al-Yarmook Teaching Hospital from March 2020 to January 2021 , was included 30 cases of PTC patients and 30 healthy subjects , all of subject's age were more than 30 years . This study depend on collect of paraffin embedded PTC biopsies then used Immunohistochemical method to examined the present of TROP2 in all cases.

**Results**: This qualitative study shows significant expression of TROP2 in PTC patients compare with control groups .

**Conclusion**: The current study demonstrate effect of TROP2 expression on PTC development

***Keywords***:*Papillary Thyroid Carcinoma(PTC), TROP2 and Immunohistochemical.*

## Introduction

Papillary Thyroid Carcinoma (PTC) is the common thyroid cancer disturbance about 80% of all thyroid cancers, and it's more frequent in females than males at age more than 30 years [(1)] . The histological analysis consider golden standard for diagnosis ,PTC's diagnosis by histology depend on present of papillary architecture association with nuclear features that involve elongation and irregular contour with grooves [(2)] .

Trophoblast cell surface antigen 2 (TROP2) is trans-membrane glycoprotein receptor ,encoded by tumor associated Ca signal transducer 2 (Tacstd2) gene located on chromosome 1p32 .It's found in cell lines of human trophoblasts and choriocarcinoma ,also the TROP2 expression observed to be correlated with the development and progression of tumors in several epithelial malignancies [(3)] . TROP2 has the up-regulation ( overexpression) in cancer cells such

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as pancreatic , thyroid and ovarian cancers [4].

The study aim is shown up-regulation of TROP 2 in PTC Iraqi patients by Immunohistochemical method.

## Materials and Methods

The current study done in Al-Yarmook Teaching Hospital from March 2020 to January 2021 was included 30 cases with PTC patients (pre-thyroidectomy) and 30 cases of health persons (control) ,the subjects studied ages were more than 30 years of both sexes. The all of individuals in this study were examined by ultrasound and computerized tomography scan to excluded any other tumor conditions .The sampling depend on collect of paraffin embedded PTC biopsies from PTC group and control group ,then used Immunohistochemical method to examined the present of TROP2(as qualitative measurement ) in all cases .

After examined TROP2, used chi-square method for statistic analysis to show different of TROP2 presenting by comparison between PTC and control groups ,and p-value (p-value > 0.05 mean significant value .

## Results

The present study explain a different of TROP2 expression results between PTC and control groups . This result used Chi-square assay to find different between groups . See table 1.

**Table 1: Comparison of PTC Patients and healthy persons groups according to TROP2 expression results via Chi-square assay method**

| Parameters | PTC group (30 cases) | Control group (30 cases) | P-value |
|---|---|---|---|
| TROP2 expression | 28 Positive | 0 Positive | 0.02* |
| | 2 Negative | 30 Negative | |

*Significant value

## Discussion

In this study the immunohistochemical method was used to assess the TROP2 expression in PTC patients compares with healthy persons . The study results demonstrate that a significant value in TROP2 expression in PTC patients between PTC and control groups , that agree with SAFFAR ,et al 2021 [5] . The present study found that increase TROP2 expression in PTC group.

TROP2's expression detected in high sensitivity and specificity PTC based on these finding , TROP2 immunohistochemistry staining has been shown to be a strong diagnostic marker for PTC ,but there are various studies show finding demonstrate the utility of TROP2 expression in the PTC diagnosis in clinical settings ,no mechanism was explored as to why TROP2expression was particularly elevated in PTC and not in other thyroid cancers [6] . The TROP2 expression also facilities tumor genesis by activating the MAPK / ERK pathway , which has significant implications for different cellular pathways lead to cancer cell proliferation ,migration ,invasion and survival [7].

This study results agree with Trerotola et al and others studies that shown that TROP2 is up-regulated in all PTC cells and support cell proliferation , and also regulate the TROP2 transcription [8].

## Conclusion

The current study demonstrate effect of TROP2 expression on PTC cancer cell proliferation ,migration ,invasion and survival immunohistochemical method.

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# Study of Asphervon Gum Effect on Diuresis, Spermatogenesis and Its Effect on Testosterone Level in Rat Male Blood

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## Abstract

The effect of Asphervon resin gum on spermatogenesis, on the level of testosterone in the blood of rats was studied, and the effect of two dosages of the drug on diuresis of rats with an aqueous load was also considered. Data have been obtained on the quantitative change of sperm in the rat ejaculate, on the ability of Asfervon to reduce testosterone levels in the rat's blood, and also, a pronounced dose has been revealed - the dependent diuretic effect of the drug Asfervon.

***Keywords:*** *diuresis, testicle, spermatogenesis, pH, urine, testosterone.*

## Introduction

Today, in connection with the need to find effective and safe drugs in medicine, medicines of plant origin are increasingly used. Medicinal plants with diuretic effect accelerate the removal of stagnant fluid from the body during edema, urinary problems, diseases of the heart and urogenital system.

Due to the fact that preparations of plant origin have practically no side effects, and their effect is milder than drugs of chemical origin, they are often prescribed to pregnant women, children and people with chronic diseases.

Basically, swelling, opacity are diverse in nature. It should be emphasized that they occur both in severe pathologies and in healthy people.

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Patient conditions such as pregnancy, premenstrual syndrome, obesity, and the use of certain groups of drugs can be accompanied by swelling.

Swelling in most of its cases does not threaten the patient's health, due to the fact that it is associated with congestion, which can be aggravated.

Intercellular fluid in tissues is able to collect almost anywhere, swelling in the face is especially noticeable. A greater manifestation of edema is observed in patients who do not observe a healthy lifestyle and have an unhealthy eating habit. Regardless of the reason for admission from the hospital, patients need help. In most cases, patients can adjust their condition themselves by using mild diuretic drugs.

Diuretics are mainly represented by the following groups:

1) diuretics "loop" and acting on the cortical segment of the Genle loop;

2) potassium-saving diuretics;

3) osmodiuretics.

Diuretics have a different duration of effect

on diuretics, and the force of exposure can also be different. This primarily depends on their physicochemical properties, mechanism of action.

To date, the effectiveness of medicinal plants with diuretic effects has also been proved[1].

The therapeutic properties of Asafetida are diverse. In treatises on Tibetan medicine, they claim that the plant "eliminates anxiety in the heart and rejuvenates organs that collapse from old age."

According to some authors, the composition of the plant is unique and the main therapeutic component is dried in the air milky juice (gum) obtained from the roots of the plant. Gum resins in the pharmaceutical industry are made by water infusions, emulsions, pills used in nerve diseases, hysteria; asthma, cough, anticonvulsant pills, digestive pills.

Ferula assa foetida L. grows wildly in Central Asia, especially in Iran and Afghanistan. Asafetide, oleo gum resin, is obtained from F. assa-foetida by cutting roots or removing stems[2].

In Iranian folk medicine, asafetide is used as a diuretic, antispasmodic, windmill and painkiller[3].

Asafetide normalizes metabolic processes in the body, removes mucus, improves the peristalsis of the gastrointestinal tract, has, calming, antimicrobial and bactericidal, anesthetic and spasmolytic effects, improves potency.

According to literature, asafetide treats many female diseases. Smelly ferula is used for infertility, the threat of miscarriage, painful menstruation and pain[4].

Our studies also showed antioxidant and anti-inflammatory activity of the given drug plant[5].

Asafetida is also a popular spice in Asian cuisine. It is used in Ayurvedic, Tibetan and many other folk medicine - with its help they successfully treat epilepsy, jaundice, hysteria, seizures, stomach diseases, etc. Speaking of Tibetan medicine, it should be noted that asafetide is considered the best aphrodisiac (effective dose - from 300 to 1000 mg). Asafetide contains essential oil, resin and gum. Oleum Asafoetida has been shown to enhance libido[6].

In Ayurveda and traditional medicine of various countries, such as Iran, America and Brazil, asafetide was used as an excitatory agent, experiments were carried out proving the effect of the plant on sprermatogenic activity in rats[7].

A number of researchers also emphasized the effect of Ferula's aphrodisiac assa-foetida, an increase in libido was noted[8].

AYOUBI A.R. and other co-authors in their studies note the ability of the plant "Ferula assa-foetida" to reduce testosterone levels, which is an important factor in the therapy of patients with benign prostatic hyperplasia[9,10].

This paper presents the results of a study of the effect of Asafetida resin gum on the urinary system, namely the diuretic effect, as well as the ability to affect spermatogenesis and testosterone levels in the blood of male rats.

We obtained gum resin together with employees of the ABDU-S emergency, in the form of a powder obtained during the separation from the upper layer - the fat part of the resin. The resin is obtained from the incision of the plant Ferula assa-foetida growing in the Jizzak region of Uzbekistan. Asferwon powder is obtained as a result of simple technological procedures in accordance with patent No. IAP 06453[11].

In the first part of the experiment, diuretic properties and the ability to affect changes in the level of pH in the urine of rats after the use of Asfervon were studied, since in folk medicine in Iran this plant is used as a diuretic. Also, in the second part of this study, we set ourselves the goal to study the effect of Asferwon, which contains the plant Ferula assa-foetida on spermatogenesis and testosterone levels in the blood of male rats.

Material, research methods: all studies were carried out on healthy animals quarantined for at least 10-14 days. The object of our study was the drug "Asfervon," which includes: the plant Ferula assa-foetida.

Study of diuretic action. Experiments were carried out on an aqueous load model. During the experiment, all animals were kept under standard vivarium conditions. At the same time, the temperature of the room was in the range of 18-25 ° C, the relative humidity in the range of 40-70%.

The experiment was conducted on 24 white, non-fertile female rats weighing 233-246 grams. The animals were divided into 4 groups of 6 rats in each group. A day before the experiment, the animals were placed in separate cells adapted to collect urine with a standard diet. Water was freely available to animals. The animals were kept stationary in vivarium.

Rats 2 hours before the experiment were orally (via a special probe) administered once according to the following regimen:

test group No. 1 - 1 ml of a 1% aqueous Asphervon solution was administered to animals at a dose of 50 mg/kg;

test group No. 2 - animals were injected with 1 ml of a 2% aqueous solution of the preparation "Asfervon" at a dose of 100 mg/kg;

control group (control) - distilled water was introduced in the corresponding volume;

intact group - no water load was produced in intact group.

The experiment was conducted for 5 hours. As a result, the collected volume of urine was studied according to the following criteria: amount of diuresis, odor, transparency, urine response (determined in parallel using a potentiometer and standard universal indicator paper). Data pH collected for 5 hours of urine.

**Study of spermatogenic action**. We also studied the effect of the Asferwon preparation containing the plant Ferula assa-foetida on spermatogenesis. The study was conducted at the Med Standard Test Laboratory.

To study the effects of the drug on spermatogenesis, we used data from Seyyed Majid Bagheri and others. The following procedure was used in the work. Extracting rat sperm was performed through a notch and extracting a small portion of the testicular caudal appendage[12].

Referring to this technique, we also after 2 weeks a small part of the testicular caudal appendage of each rat excised and evaluated the change in sperm count and parameters. The observation was carried out using a light microscope, with an increase of 10Kh times.

To conduct an experiment to study the spermatogenic effect, 12 sexually mature male rats weighing 245-247 gr were selected. During the experiment, all animals were kept under standard vivarium conditions.

At the same time, the temperature of the room was in the range of 18-25 ° C, the relative humidity in the range of 40-70%. Rats were divided into 2 groups, 6 rats in each group.

The test group - animals for 10 days before testicular separation, orally (by means of a special probe) was administered once 1 ml of 1% aqueous solution of the preparation "Asfervon" at a dose of 50 mg/kg.

Control group (control) - distilled water was introduced in the corresponding volume.

**Study of effects on testosterone levels in rat blood.** The effects of Asferwon on blood testosterone levels were studied using a procedure based on a change in testosterone levels in intact rats. During the experiment, all animals were kept under standard vivarium conditions. At the same time, the temperature of the room was in the range of 18-25 ° C, the relative

humidity in the range of 40-70%. The experiment involved 18 male rats weighing 190-240 g. Rats were divided into 2 groups, 9 rats in each group.

The test group - animals for 10 days before decapitation, orally (by means of a special probe) was administered once 1 ml of 1% aqueous solution of the preparation "Asfervon" at a dose of 50 mg/kg.

Control group (control) - distilled water was introduced in the corresponding volume.

To determine the level of testosterone in the blood of male rats, blood was removed by decapitation for analysis. The seized blood samples were delivered to the laboratory of the joint venture SWISS LAB LLC, where on the equipment Immulite 2000 xpi, Siemens Healthcare Diagnostics Limited, UK using special enzymatic-enhanced chemiluminescence technology, a study of testosterone levels in blood samples of intact and experimental groups was carried out.

All the results obtained were processed by the method of variation statistics according to the Student's criterion at $p = 0.05$ [13,14]. The tables show the mean arithmetic values (M), their corresponding standard errors of the mean value (m), Student criterion (t), number of samples (n), confidence boundaries (lower confidence boundary upper confidence boundary).

## Results

Diurez. From our experiments, we managed to find out that the drug "Asfervon" at a dose of 50 mg/kg in comparison with the control had a 16.24% more pronounced diuretic effect. At a dosage of 100 mg/kg, Asferwon compared to the control had 33.58% more pronounced diuretic effect (Table 1). The obtained data are similar to those of S. M. Bagheri, H. Mohammadsadeghi, M. H. Dashti-R, S. M. M. Mousavian, and Z. A. Aghaei, where a comparative study was conducted on the plant "Ferula assa-foetida[15].

**Table 1.: Urine volume per 5 hours (M ± tm; p = 0.05; n=6)**

| № | Intact group | Control + H2O (5 ml/100 g) | Group No. 1. Asphervon 50 mg/kg + H2O (5 ml/100 g) | Group No. 2. Asphervon 100 mg/kg + H2O (5 ml/100 g) |
|---|---|---|---|---|
| Result | 3,00 (2,7863÷3,2303) | 4,56 (4,2395÷4,8737) | 5,05 (4,9342÷5,1590) | 5,57 (5,1330÷6,0002) |
| Increase in diuresis in% | - | +51,46% | +67,7% | +85,04 % |

**Urine color**. In the experiment, the collected urine volume of the test and control groups ranged from thatched yellow to saturated yellow. The experimental group receiving the drug "Asfervon" was more often straw - yellow.

**The urine odor** in the experimental and control groups was sharp and specific.

**Urine reaction**. The obtained data on pH (hydrogen ion concentration) indicators showed that the experimental groups receiving the preparation "Asfervon" in two dosages showed practically no statistically significant changes in the urine pH of rats, compared with the control and intact groups.

In group 1. A 50 mg/kg + H2O (5 mL/100 g) Asphervon preparation, compared with the control group, showed a change in the pH index by 0.43, towards the alkaline medium. As a percentage, this

figure is + 7%.

Group 2. The preparation "Asfervon" at a dose of 100 mg/kg + H2O (5 ml/100 g), compared with the control, showed a change in the urine pH index by 0.36, towards the alkaline medium. The percentage is + 5.93 per cent (Table 2).

**Table 2 : Determination of urine pH level (M ± tm; p = 0.05; n=6)**

| № | Intact group | Control + H2O (5 ml/100 g) | Group No. 1. Asphervon 50 mg/kg + H2O (5 ml/100 g) | Group No. 2. Asphervon 100 mg/kg + H2O (5 ml/100 g) |
|---|---|---|---|---|
| Result | 6,20(6,0033÷6,3866) | 6,07(5,9641÷6,1825) | 6,50(6,2471÷6,7595) | 6,43(6,1505÷6,6994) |
| Increase in diuresis in% | | | +7% | +5,93 |

In the literature, there are also no statistically significant changes in the level of rat urine pH when administering the preparation from the plant "Ferula assa-foetida."

Spermatogenesis. Examination of the 50 mg/kg Asferwon effect on rat spermatogenesis showed that the drug improved the quantitative index of sperm presence in the test incision of the caudal appendage of the rat testicle. Compared to the control group by 60.5%. The study of the qualitative index of sperm, occurred under the measurement of the relative length of the sperm. In comparison to the control, the Asferwon group at 50 mg/kg increased the sperm length by 37.03% (Figure 1).

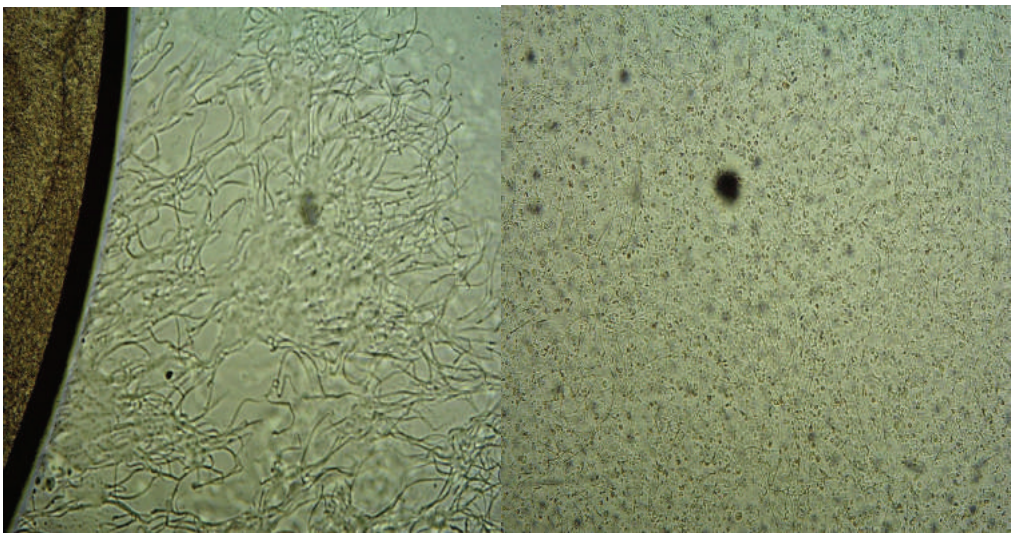

**Figure 1.: The amount of sperm in the recovered material in the test and control groups**.

**Experimental "Asfervon" 50 mg/kg Control**

In order to obtain comparative data and analyze them, we took a drawing in the control and experimental group, under the same magnification we took an area of 2 cm2. Where we were able to record that the number of sperm in the field of view is an average of 10.16 (8.6216 sound11.7116) pcs in the experimental group and 6.33 (5.2493A 7.4173) pcs in the control group. The ratio of average sperm length to control was 2.22: 1.62. (Table 3).

**Table 3. Characteristics of rat sperm (M ± tm; Ó =; n=6)**

| | Control (H2O) | Experimental "Asfervon" 50 mg/kg |
|---|---|---|
| length | 1,62 (1,5187÷1,7378) | 2,22 (2,1379÷2,3187) |
| Number in view | 6,33 (5,2493÷7,4173) | 10,16 (8,6216÷11,7116) |
| Change in% is long | | +37,03 |
| Change to% Quantity | | +60,50 |

In the experimental group, the sperm have a more shaped and mature shape compared to the control.

When studying the results of statistical processing, it was revealed that in comparison with the intact group, the animals to which the preparation from the plant "Ferula Assafoetida" under the trade name "Asfervon" was introduced reduced the level of testosterone in the blood by 4.7 times (Table 4).

**Table 4. A study of the effect on total testosterone levels in the blood of rats with Asferwon at a dose of 50 mg/kg (M ± tm; p = 0.05; n=9)**

| № п/п | Name-group | Blood testosterone level, nmol/l |
|---|---|---|
| 1 | Intact | 43,96 (41,0612÷46,8672) |
| 2 | Experiment | 9,29 (8,5817÷9,9912) |
| | | 4.73 times decrease in testosterone level compared to intact group |

## Result

The study showed that when the drug Asferwon was administered in experimental animals, testosterone levels were reduced. The results obtained are similar to those obtained by Seyyed Majid Bagheri[16] et al. These results allow us to approach a larger study of the therapy of benign prostatic hyperplasia, without additional prescribing drugs that reduce testosterone levels. Perhaps, in this case, the patient will be able to dispense with two directed pharmacological effects of one drug.

## Discussion of the Results

The results of the studies we obtained showed identity with the given data of the literature. In this regard, there is a need for further deeper study of the various dosages of the drug "Asferwon" on the urogenital system of the rat.

This study clearly showed that the drug has a moderate diuretic effect, as well as improves the quantitative and qualitative indicators of rat sperm. The findings are in line with theoretical expectations and will serve as material for our further research.

The discovered ability to reduce testosterone levels in the blood of rats is necessary in the therapy of benign prostatic hyperplasia and will allow patients to dispense with additional drug therapy.

**Conclusions** in the presence of diuretic activity, Asfervon does not change the level of urine pH of rats, the drug showed spermatogenic activity, and the ability to reduce testosterone levels in the blood of rats.

**Ethical Clearance -** Taken from pharmacological committee

**Source of Funding -** Self

**Conflict of Interest -** Nil.

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# Long-Term Consequences of Intraoperative Spillage of Bile and Gallstones During Laparoscopic Cholecystectomy

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## Abastract

Compared to open cholecystectomy, laparoscopic cholecystectomy is linked to a greater rate of iatrogenic gallbladder perforation. The long-term effects of gallstones and spilled bile are unknown. Over a three-year period, data were gathered prospectively from 1059 patients who had laparoscopic cholecystectomy. The operating techniques and postoperative outcomes of individuals who had gallbladder perforation were examined in detail. Long-term follow-up (varying from 24 to 59 months) was available for 92 % of patients. The gallbladder was perforated in 306 patients (29%); it was more prevalent in men and was linked to increasing age, body weight, and the presence of omental adhesions (all P less than 0.001). Patients with acute cholecystitis had no higher risk (P = 0.13). Pyrexia was more likely postoperatively in individuals who had gallbladder contents spilled (18% vs. 9%; P less than 0.001). Intra-abdominal abscesses formed in 1 (0.6%) of 177 patients with just bile leakage and 3 (2.9 %) of 102 patients with both gallstones and bile spilling, but no intra-abdominal abscesses formed in the 696 patients in whom the gallbladder was removed intact (P less than 0.001). During laparoscopic cholecystectomy, intraperitoneal leakage of gallbladder contents is linked to an increased risk of intra-abdominal abscess. Attempts should be undertaken to irrigate the operating field in order to drain spilt bile and retrieve all gallstones that may have been spilled during the process.

***Keywords*** *: Gallbladder, laparoscopic, gallstones, abscesses, cholecystectomy.*

## Introduction

Laparoscopic cholecystectomy has supplanted open cholecystectomy as the "gold standard" for the surgical treatment of symptomatic cholelithiasis. Although laparoscopic cholecystectomy has a slightly greater incidence of iatrogenic biliary tract damage than open approaches, overall complication rates appear to be comparable. Iatrogenic gallbladder perforation occurs more commonly after laparoscopic cholecystectomy, resulting in intraperitoneal leakage of bile and gallstones, as we and others have shown [1,2]. Although some writers suggested that gallbladder perforation during surgery should precipitate conversion to an open procedure [3], most institutions now remove as many stones as feasible while irrigating the peritoneal cavity to clear the spilled bile.

Although gallbladder contents spilling is regarded to be generally harmless, the long-term repercussions of intraperitoneal bile and gallstone spilling remain unknown. Experiments on animals have shown inconsistent results. Several investigations have shown that intraperitoneal stones cause a minor fibrotic response [2, 4, 5], whereas others have shown that abscess development occurs.[6] There have also been several accounts of difficulties resulting from spilling bile and gallstones[7 – 15]. The goal of this study was to establish the variables that predispose to gallbladder perforation during surgery, as well as the incidence and range of unfavorable consequences associated to bile leakage and gallstones.

## Material and Methods

For symptomatic cholelithiasis, 1139 consecutive patients underwent attempted laparoscopic cholecystectomy between July 2017 and August 2020. Prospectively gathered clinical, diagnostic, treatment, and follow-up data. 80 patients (7.0 %) who were converted to open cholecystectomy due to dense adhesions (n = 26), severe inflammatory alterations (n = 22), substantial spilling of bile or gallstones (n = 10) or other causes (n = 22) were excluded from the study. In 753 patients (71%) the gallbladder was removed intact, but in 306 patients (29%) the gallbladder was perforated during the procedure. The particular specifics of the operating technique were evaluated in these individuals.

Depending on a clinic visit two to four weeks postoperatively, the follow up of Short term is done. While the follow up of long term was achieved by telephone conversation or questionnaire in 977 patients (92%) at a mean of 3.3 years (range

2.1 to 5 years). 26 of the 82 patients who had not received satisfactory follow-up had died, nine had been incarcerated, eight had moved out of Iraq, and 39 had refused to complete questionnaires. To rule out selection bias, the hospital records of these patient subgroups were thoroughly examined. Gallbladder perforation was equally common in patients with and without good follow-up data (29% vs. 32

%). Patients with intact gallbladders experienced no notable early problems, whereas two patients with intraoperative gallbladder rupture developed perihepatic abscesses and two had superficial wound infections. The incidences of postoperative complications in the following results are based exclusively on patients who have completed long-term follow-up.

### Operative Technique

An attending surgeon or a resident performed the laparoscopy under the supervision of the medical personnel. The study took into account both elective and emergency situations. The procedure was performed using a four-trocar method and a 30-degree angled laparoscopic video camera [16]. The cystic artery and cystic duct were ligated with titanium clips, and gallbladder dissection was done with a mixture of electrocautery and blunt dissection with fine graspers. Either the umbilical or epigastric port was used to reposition the gallbladder. When the gallbladder perforated, efforts were undertaken to recover all lost stones, and the peritoneal cavity was irrigated with saline solution to remove the spilt bile. Antibiotics, most often a cephalosporin, were given to patients once before surgery and once thereafter. Broad-spectrum antibiotics were prescribed for a longer duration in patients with acute cholecystitis, especially when the bile culture was positive, depending on the clinical circumstances.

### Statistical Analysis

The chi-square test or Fisher's exact test were used to do statistical comparisons of proportions, as applicable. The Wilcoxon rank-sum test was used to compare continuous variables. P values of less than 0.05 were deemed statistically significant. The mean and/or standard deviation are used to convey summary parameters in the text.

## Results

Between July 2017 and August 2020, 1059 patients received successful laparoscopic cholecystectomy. Iatrogenic gallbladder perforation was found in 306 individuals (29 %, with a 95 % confidence interval ranging from 26 % to 32 %), with 191 (62 %) having just bile spilling and 115 (38 %) having both bile and gallstone spilling (Table I). The group of gallbladder perforation had a larger proportion of male patients than the intact group (43 % vs. 28 %; P less than 0.001). Also, they had a higher mean age than the intact group (56 ± 15 years vs. 52 ± 16 years; P less than 0.001), and the perforated gallbladder group had a higher mean weight (80 ± 18 kg vs. 76 ± 17 kg; P less than 0.001). Regarding the history of abdominal surgery, an increased risk of intraoperative gallbladder

perforation was not linked to it. Gallbladder perforation was more likely when there were adhesions between the gallbladder and the omentum (42 % vs. 30 % ; P less than 0.001). Although the perforated group had a slightly greater incidence of acute cholecystitis than the intact group (11% vs. 8.5%), the difference was not statistically significant.

**Table I. Patient and operative characteristics**

| | Gallbladder status | | P value |
|---|---|---|---|
| | Intact | Perforated | |
| Patients | 753 (71%) | 306 (29%) | |
| Bile only | | 191 (62%) | |
| Gallstones and bile | | 115 (38%) | |
| Sex | | | |
| Male | 214 (28%) | 132 (43%) | <0.001 |
| Female | 539 (72%) | 174 (57%) | |
| Mean age (yr) | 52 -± 16 | 56 -± 15 | <0.001 |
| Mean weight (kg) | 77 ± 17 | 81 ± 18 | <0.001 |
| Acute cholecystitis | 64 (8.5%) | 35 (11%) | NS |
| () mental adhesions | 226 (30%) | 127 (42%) | <0.001 |
| Mean surgical time (min) | 100 ± 38 | 106 ± 38 | 0.008 |
| Operation performed by surgical trainee | 182 (24%) | 79 (26%) | NS |

The rate of iatrogenic gallbladder perforation was greater in the first year of our experience with laparoscopic cholecystectomy (40%) in 2017, but it gradually decreased each year following, reaching 24 % in 2020. Perforation of the gallbladder occurred in 47 % of patients during dissection of the gallbladder from the liver, 21% during extraction through the abdominal wall, and 14% as a result of intraoperative retraction. Patients in the perforated group had a little longer operation time (100 ± 38 minutes vs. 106 ±38 minutes; P less than 0.01), although this was not clinically significant. Surgical residents conducted the same number of laparoscopic cholecystectomies in both patient groups (26% vs.24%; P = 0.573).

**Postoperative Complications**

There were no bile duct damage or perioperative fatalities. Ten patients (1%), including two in the

intact group (0.3%) for persistent cystic duct stump leaks closure and eight in the group of gallbladder perforation (3%) for intra-abdominal abscesses drainage, two for empyema decortication, and two for an iatrogenic cautery injury repairing that happened to the duodenum. Wound infection, pulmonary problems, bile leakage, or ileus across groups have no differences in the incidence postoperatively (Table II). Pyrexia developed after surgery in 53 patients (18%) in the group of gallbladder perforation and 66 (9%) in the intact group (P less than 0.001). Although the group of gallbladder perforation had a higher postoperative white blood cell count, (9800 ± 3200 vs. 9800 ± 3400; P equal to 0.02, a difference of minimal clinical significance), there were no clinically significant changes in the preoperative white blood cell count. Regarding the use of oral analgesics or the use of parenteral analgesics or the need for an antiemetic following surgery, there were no difference between the two patient groups. The perforated gallbladder group had a longer hospital stay (2.1 ± 3.2 days vs. 1.6 ± 1.3 days; P less than 0.01); however, there was no significant difference in the meantime to return to work (13.6 ±10.7 days vs. 17.0 ± 31.8 days; P = 0.3). In both groups, the majority of patients were happy with their surgical operations (92 % vs. 96 %; P = 0.29).

**Table II. Complications: Intact vs. perforated gallbladder (long-term follow-up)**

| Complication | Intact (%) | Perforated (%) | P value |
|---|---|---|---|
| Intra-abdominal infection | 0 (0) | 4 (1.4) | 0.001 |
| Ileus | 9 (1.3) | 4 (1.4) | NS |
| Pulmonary infection | 1 (0.1) | 2 (0.7) | NS |
| Bile leakage | 2 (0.3) | 1 (0.4) | NS |
| Hemorrhage | 2 (0.3) | 2 (0.7) | NS |
| Wound infection | 17 (2.4) | 3 (1.1) | NS |
| Residual gallstone symptoms | 72 (10.9) | 30 (11.1) | NS |

Four (0.4 %) of the 977 individuals for whom long-term follow-up data was available had intra-abdominal infections. All of them had a perforated gallbladder (P = 0.001). Two more patients in the perforated gallbladder group were found to have developed intra-abdominal abscesses after no long-term follow-up. One of the patients died of prostate cancer before the follow-up survey, while the other refused to answer the questions. Four of the six patients experienced bile and gallstone spilling, whereas the other two just had bile leakage. Three of the six patients developed a perihepatic abscess, two of whom also had right-sided empyema. In the other three individuals, a subhepatic abscess developed.

Only one patient with an intra-abdominal abscess was reported to have gallstones left after the treatment. These were not removed laparoscopically due to their inaccessibility. Four individuals had signs

of intra-abdominal infection within 10 days following laparoscopic cholecystectomy; however, one patient developed infection 28 days after the procedure and another patient after 34 months.

Four patients had their intra-abdominal abscesses percutaneously drained under CT guidance, although three of them needed surgery afterwards (Table III). Symptoms in one patient improved following CT drainage, but the patient suffered recurrent right upper quadrant discomfort six months later, necessitating laparotomy. The symptoms disappeared after a tiny chronic subhepatic abscess was discovered, which included three big, mixed stones (**Fig. 1**).

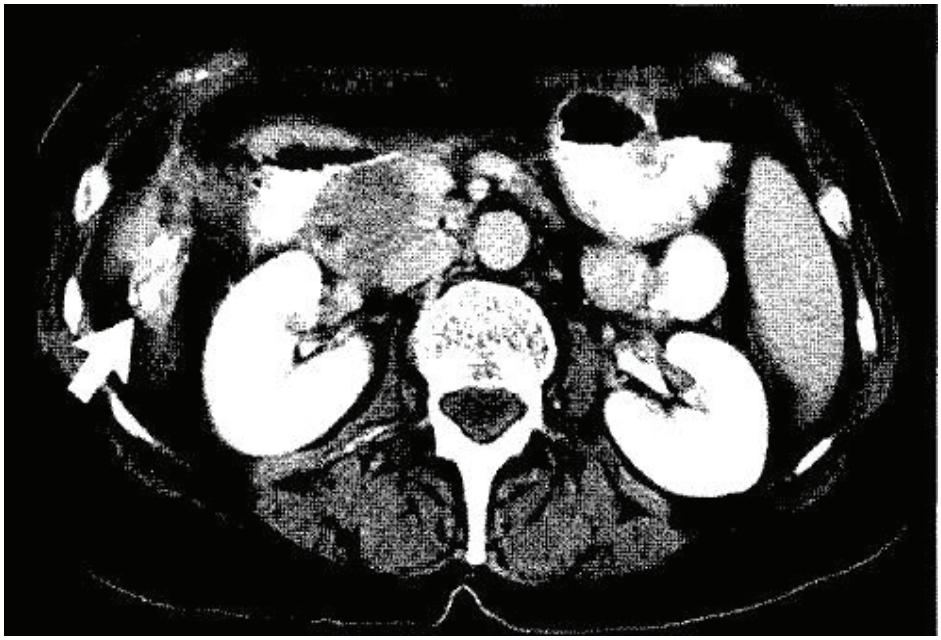

**Fig 1. CT scan demonstrating intraperitoneal gallstones (arrow) with surrounding inflammatory reaction and fluid collection.**

**Table III. Major infective complications secondary to spilled bile and 'gallstones**

| Patient | Spillage | Site of infection | Percutaneous CT drainage | Operative intervention |
|---|---|---|---|---|
| 1 | Bile | Perihepatic | Successful | None |
| 2 | Bile | Perihepatic, right chest | Unsuccessful | Right thoracotomy and decortication of empyema, drainage of perihepatic abscess |
| 3 | Bile + gallstones | Subhepatic | Not attempted | Laparotomy, removal of intraperitoneal gallstones; postoperative pulmonary embolus |
| 4 | Bile + gallstones | Subhepatic | Unsuccessful | Laparotomy, drainage of abscess |
| S | Bile + gallstones | Subhepatic | Unsuccessful | Laparotomy, drainage of abscess |
| 6 | Bile + gallstones | Perihepatic, right chest | Not attempted | Right thoracotomy and decortication of empyema, removal of gallstones and drainage of perihepatic abscess |

In two other patients, a laparotomy was performed to drain an intra- abdominal abscess. Empyema caused by perihepatic abscess necessitated transthoracic decortication in two patients (**Fig. 2**).

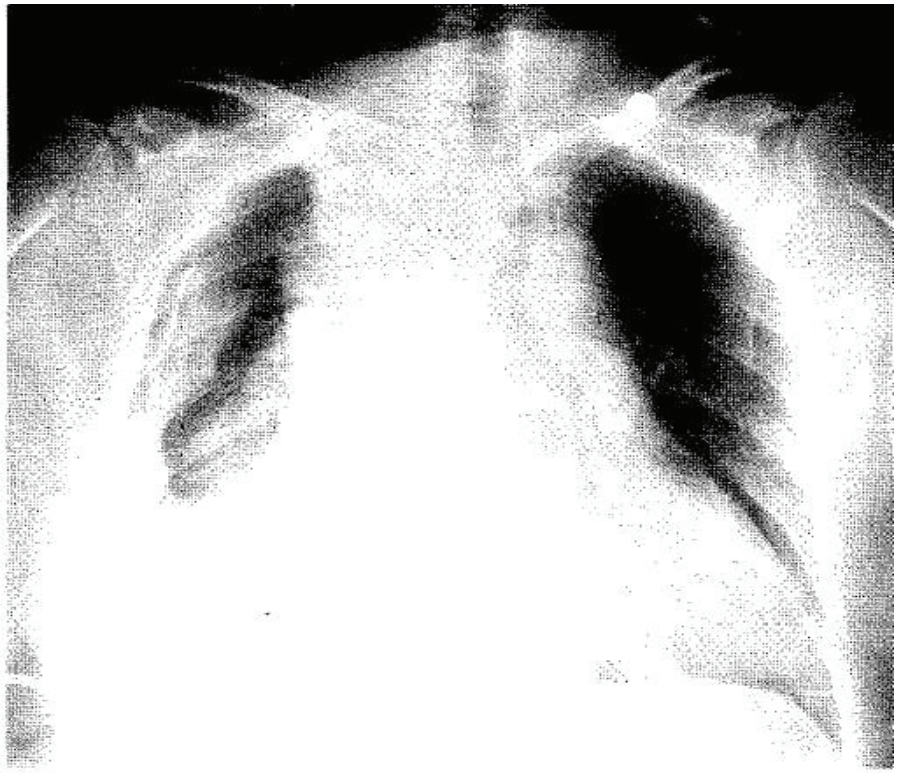


**Fig. 2. A, Right-sided empyema due to a perihepatic abscess caused by gallstones that have not been removed. Thoracocentesis, right thoracotomy, and decortication were all done.**

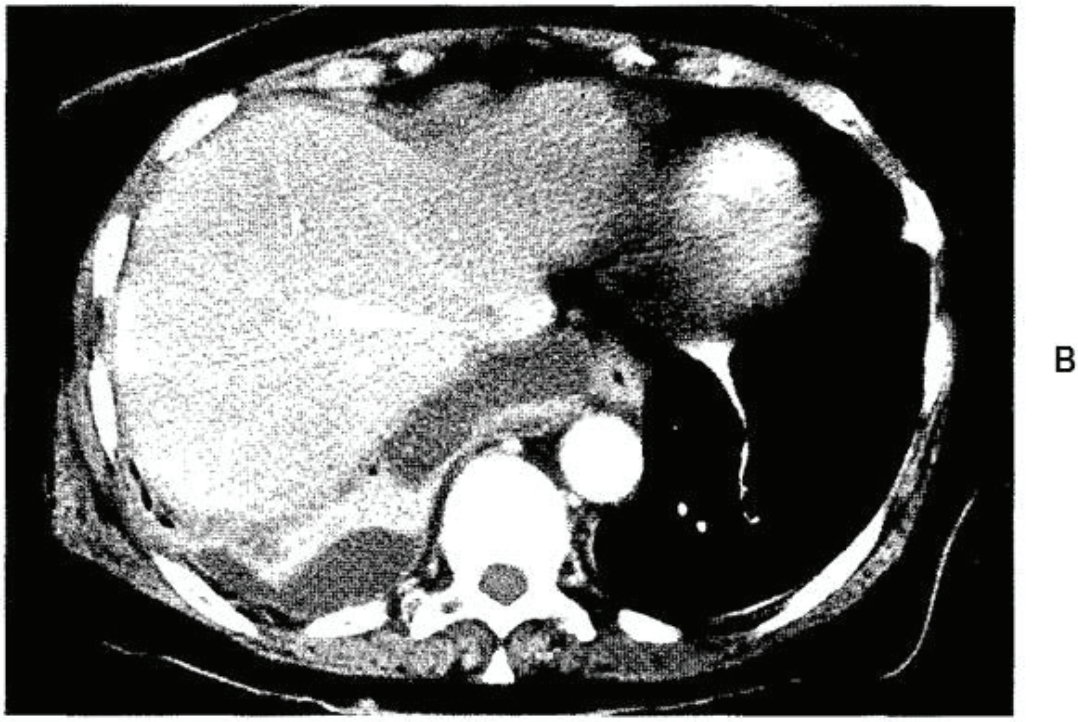


**Fig. 2. B, Patient A's CT scan revealed a subhepatic abscess that required surgical drainage.**

## Discussion

Laparoscopic cholecystectomy has quickly become the standard treatment for symptomatic cholelithiasis since it was first reported in 1989 [17]. The procedure, however, is not without risks, the most serious of which is a higher risk of biliary tract injuries than open cholecystectomy [18–21]. Despite this, 5 years of clinical experience and numerous prospective [22-25] and retrospective [26-28] trials have shown that laparoscopic cholecystectomy is a safe procedure with a low risk of major complications. Despite the fact that many studies have looked at the clinical outcomes of laparoscopic cholecystectomy, few have specifically addressed the consequences of bile and gallstone spillage into the peritoneal cavity, which occurs more frequently with laparoscopic than with open cholecystectomy [1,2]. Gallstones that were lost during surgery have been reported to cause intra-abdominal abscesses, empyema,[9] abdominal wall abscesses, [1,11,12] cutaneous sinus tracts, [13-14] and bladder fistulas.[15] Although these complications appear to be uncommon, there is no way of knowing how common they are.

306 (29%) of 1059 patients who underwent laparoscopic cholecystectomy had bile spillage alone or bile and gallstones spillage into the peritoneal cavity. This rate is similar to Jones et al. [29]. 32 % incidence of gallbladder perforation, but it is significantly higher than the perforation rate described in multicenter study in Canada (9% ). Male sex, increasing age, and weight were all associated with a higher risk of intraoperative gallbladder perforation. Jones et al. discovered similar connections. Larger abdominal wall adipose tissue, increased liver mass and friability (frequently fatty infiltration), which puts higher force on the gallbladder during cephalad retraction, and a bigger quantity of fat around the cystic duct are all likely to make the surgery more technically hard in larger male patients. The most common time of iatrogenic gallbladder perforation in our study was during gallbladder dissection from the liver. Electrocautery was used in 11 of our 1059 cholecystectomies. We can't draw any conclusions regarding the relative risk of perforation using alternative methods of dissection because just a few patients had the procedure done using laser dissection. The removal of the gallbladder through the abdominal wall was the second most common cause of iatrogenic gallbladder perforation. When a large gallstone burden prevents the gallbladder from being extracted through one of the ports, the gallbladder can be placed in a specimen bag before crushing or extracting stones with a stone forceps, or the fascia! The impact at the port site can be increased. Gallbladder perforation and its subsequent infective complications should be reduced as a result of these steps.

The frequency of acute cholecystitis was comparable in the intact and nonintact patient groups, a result that was also reported by others [2]. Although a severely inflamed gallbladder may appear to be more friable on the surface, the edematous and thicker gallbladder wall may guard against unintended perforation during several phases of the operating procedure. In our early experience, when the gallbladder was extremely inflamed, there was a low threshold for converting to open cholecystectomy, which likely contributed to the low frequency of gallbladder perforation in these patients. The incidence of gallbladder perforation was higher during the first year of laparoscopic cholecystectomy at our institution, as one might expect; however, after that, the iatrogenic perforation rate stabilized at around 25%.

Despite the high rate of gallbladder perforation during surgery, spillage of bile or gallstones did not cause serious complications in the majority of patients. Surprisingly, both patients with an intact gallbladder and those with a perforated gallbladder had the same rate of wound infection. Even when spillage into the port site was looked at separately, there was no evidence of a link to subsequent wound problems. In the group of patients of the gallbladder perforation, only six had intra- abdominal abscesses; in two of them, decortication required due to

empyema developed. Empyema is thought to have developed as a result of spilled gallstones causing a perihepatic abscess and subsequent diaphragm erosion into the right pleural cavity. This complication has been previously reported [9]. Despite the fact that percutaneous CT guided drainage was attempted in four patients, three of them required surgical intervention due to insufficient drainage, most likely due to the inability to remove the inciting gallstones.

In animal studies, gallstones plus bile have been shown to increase the risk of abscess formation,[6] whereas sterile gallstones only cause a mild inflammatory response [5]. In our study, 4 of 6 patients who developed intra-abdominal abscesses had known bile and gallstone spillage. Because of their frequent association with bacterobilia, brown pigmented stones may be more problematic when left in the abdomen [31]. Because bile culture or stone analysis were not routinely performed, no conclusions about the effects of infected bile spillage or the types of gallstones spilled can be drawn.

## Conclusion

After intraoperative leakage of gallbladder contents after laparoscopic cholecystectomy, the risk of significant sequelae is minimal. Only patients who had bile and/or gall stones spilled after laparoscopic cholecystectomy developed an intra-abdominal abscess (1.4 %). There were no intra-abdominal abscesses in the 753 patients who had their gallbladders removed intact. It is appropriate to irrigate the peritoneal cavity with a large (>1 liter) quantity of saline solution If an iatrogenic gallbladder perforation with bile leakage or gallstones occurs. It's unclear whether topical antibiotics are necessary. If gallstones are intentionally spilled within the abdominal cavity, all gallstones should be removed. Conversion to laparotomy is not always recommended after gallbladder perforation because infective complications are uncommon. However, if the majority of gallstones cannot be removed laparoscopically, conversion to an open procedure should be considered, especially if bacterobilia is suspected or confirmed by a Gram stain of the bile. Furthermore, unless the inciting gallstones can be removed, percutaneous drainage is unlikely to be effective if a trans-abdominal abscess develops.

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**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee

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*Research article*

# Medical Negligence Pertaining to Medical Records: A Retrospective Study

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## Abstract

**Background:** Medical record is a vital document, and the doctor/hospital has to maintain these records properly. Most medical negligence cases rely heavily on medical records to establish a case of negligence against the doctor. Poorly filled or incomplete medical records will usually work against the favour of the treating doctor. **Methods:** A total of 242 cases of medical negligence cases decided by the National Consumer Disputes Redressal Commission (NCDRC) from 2015 to 2019 (5 years) were analysed, and negligence/deficiency in service was proven in 126 cases of these lacunae in the medical records were found in 37 cases. **Results:** Some of the common mistakes were missed entry in the medical records (37%), deficient consent form (20%) and missing medical records (17%). **Conclusion:** Medical records play a vital role in medical negligence cases. Not maintaining proper medical records can leave the doctors/hospital vulnerable in liability cases.

***Keywords:*** *Medical negligence, Medical records, Consumer court cases, Liability, National Commission.*

## Introduction

The medical record is a very important document and plays a vital role in liability disputes concerning medical negligence. It includes documentation of the patient's history, clinical findings, diagnostic test results, preoperative and postoperative care, operation notes, a daily record of the patient's progress, a valid consent form etc[1].The Hon'ble Supreme Court and the National Consumer Commission in various judgments, have held hospitals/doctors liable for medical negligence for nonproduction of medical record and non-maintenance of medical records[2]. Poorly maintained medical records, e.g. missing pre-anaesthetic checkup, incorrectly filled consent forms, improper discharge summary etc., can play a huge role in deciding on the doctor's sentencing or acquittal. Almost every case of medical negligence will involve careful analysis of the patient's medical records, which is critical in establishing a case of negligence. These records will be scrutinised by experts in that field and opine on whether the treatment was given as per standards of medical practice. It is the primary responsibility of the treating doctor to make sure that the medical records are in order, and any lacunae found in not maintaining medical records properly, the liability will fall on the treating doctor/hospital. This study was done to identify the type of lacunae in the medical records in consumer dispute cases.

## Material and Methods

A total of 242 cases of medical negligence cases decided by the NCDRC from 2015 to 2019 (5 years) were analysed (data was obtained from the monthly periodical "Consumer Protection Judgements", DLT publishers). Of the 242 cases, negligence/deficiency in service was proved, and compensation was awarded

in 126 cases (Fig. 1). And in the 126 cases, lacunae in the medical records were found in 37 cases (Fig. 2).

## Results

Of the 37 cases with lacunae in the medical records (Fig. 3), the commonest mistake was missed entry in the medical records found in 15 (37%) cases which includes the following: the doctors signature was missing, name of the doctor conducting the procedure not furnished, pre-anaesthetic checkup details not included, preoperative treatment history not recorded, operative notes of the procedure not entered, no entry of the treatment given to the patient, preoperative evaluation of the patient was not recorded, postoperative progress notes were not entered, postoperative treatment not recorded and pre-evaluation of the patient prior to the operation was not entered. Some of the interesting cases in this category are as follows:

A pregnant lady died due to postpartum haemorrhage. The allegation was that the nurses conducted the delivery, and no doctor was present at that time. The medical records, i.e. the case sheet, bedhead ticket and prescriptions were devoid of the doctor's signature. There was no evidence to prove that a doctor was present during the delivery, amounting to deficiency of service[3].

A 70-year-old lady underwent corrective surgery for a left leg fracture. She developed a loss of sensation and blueish discolouration of the left leg a day after the operation but was assured that she would be fine. The leg became gangrenous and was amputated. Detailed progress notes were absent with respect to the condition of the patient after the surgery amounting to a breach of the standard of care. The doctor was found guilty of not rendering proper postoperative care[4].

An adult male underwent corrective surgery for a fracture of his left femur. Postoperatively he had undergone prolonged treatment in the hospital for pain and swelling at the fracture site to no avail. A second opinion revealed non-union of the bones. The medical records were devoid of the treatment given postoperatively and no explanation of how the infection had set in and what steps were taken to treat the complication[5] .

A female developed vesicovaginal fistula (VVF) post caesarean section and alleged that the operation was conducted carelessly, leading to VVF. The medical records did not reveal who performed the procedure. As the letterhead had the treating doctor's name (an MBBS graduate), it was presumed that she had conducted the operation and was found guilty of negligence for not being qualified to perform the procedure as she was only an MBBS graduate[6].

Deficient consent form was found in 8(20%) cases which includes the following: columns in the forms were not filled up or struck out, procedure not mentioned, blanket consent was taken, risks of the procedure was not mentioned, informed consent for procedure not taken, consent form singed by next of kin instead of the patient when concious. The following are some examples.

A female developed quadriparesis following surgery for atlantoaxial dislocation. No deficiency in treatment could be proved against the doctor. However, it was found that the consent form was not filled properly, many blanks were not filled up, and unwanted columns were not struck out, amounting to deficiency in service[7].

An adult female diagnosed with gall bladder sludge underwent endoscopic papillotomy. She developed postoperative pancreatitis and expired due to acute pancreatitis with septicemia and respiratory failure. The patient underwent a risky procedure with a lot of pros and cons. This should have been explained in detail to the patient and the relatives well ahead of the procedure and given adequate time to decide. In this case, the consent obtained was a "Blanket Consent," which was not acceptable[8].

A pregnant woman underwent a caesarian section and was transfused two units of blood. A few months later, she started falling ill frequently, and a blood test revealed HIV positive status. The consent form did not reveal the nature of the operation, the risks involved in the procedure, the necessity of blood transfusion, and the perils involved in transfusion. A valid consent was not taken, amounting to a deficiency in service[9].

An adult female was diagnosed with acute cholecystitis with cholelithiasis and underwent endoscopic retrograde cholangiopancreatography (ERCP) and laparoscopic cholecystectomy (LC). During the procedure, she suffered a cardiac arrest and died a month later. ERCP was a high-risk procedure, and the medical records showed that the doctor had failed to take informed consent from the patient to conduct the operation[10].

Missing medical records were found in 7(17%) cases which includes the following: medical records were not available, purchase records not available, absence of records of the anaesthetist, physician nor anaesthetist certifying the patient fit for surgery was absent, consent form was lost. Some examples are as follows:

A baby developed foetal distress after delivery and was diagnosed with birth asphyxia. The suit was filed five years after the incident. The case was taken into consideration as the consequence of the birth asphyxia was noticed in the defects which occurred during the developmental milestones establishing a continuous course of action. The defence claimed that the medical records were not available, and as per MCI guidelines, the medical records need to be maintained only for a period of 3 years. The Commission was of the opinion that this was a complicated case of birth asphyxia and had a potential for litigation in the future; hence the doctor/hospital should have been more vigilant and preserved the records for a longer period[11].

An adult male sustained a fracture, and plaster was applied. He subsequently developed compartment syndrome, and his leg had to be amputated. No medical records were present, and all evidence was based on the affidavits filed by the doctors. The doctors in their defence claimed that the necrotic area of the skin was small, but the extent of the necrosis could not be confirmed due to the lack of medical records. It was quoted by the National Commission, "Poor medical records mean poor defence; no records mean no defence"[12].

An elderly patient underwent an operation, and a biliary stent was placed; she, unfortunately, died ten days later due to complications. It was alleged that the stent was defective. The doctors were found not negligent in conducting the operation; however, the stent's purchase records were missing. It could not be proved whether the stent used was defective or expired, as alleged, without the purchase record. Hence compensation of Rs. 1 lakh was paid for an administrative lacuna and not due to negligence[13].

Deficient discharge summary was found in 6(15%) cases which includes no precautions/ follow up instructions and treatment, details of the procedure not mentioned, injuries caused during operative procedure not mentioned.The following are examples that fall in this category.

An elderly lady underwent partial cholecystectomy instead of complete cholecystectomy as planned, and she did not get relief. The doctor was found not negligent however the compromised cholecystectomy was not informed to the patient, nor was this information recorded in the discharge summary, which amounted to deficiency in service[14].

An adult male with a complaint of irritation in his eye underwent a cataract operation at the hospital. Postoperatively he continued to have pain and swelling in his eye and was referred to another centre. There he was diagnosed with endophthalmitis, and his eye was removed. The allegation was negligence in conducting the first operation. On perusal of the medical records, it was found that the patient received no follow-up instructions and precautions to be taken following the first operation, amounting to failure in

duty of care[15].

Error in entry was found in 3(7%) cases which includes wrong date of death entry, wrong cause of death recorded in the discharge summary, wrong blood group entered in the records. The following are examples that fall in this category.

The patient was provisionally diagnosed with empyema (the investigations were not conclusive of empyema) but later was diagnosed with septicaemia, leading to acute renal failure. The doctors were found not negligent in the treatment given to the patient. However, the provisional diagnosis of empyema entered in the hospital record was subsequently entered in the discharge summary due to clerical error. The Commission held that the medical record is a vital document, and in this case, the documentation was done casually. The hospital was liable for the wrong act of its employees, and punitive damages of Rs. 5 lakhs was awarded to the complaint[16].

A 70-year-old male underwent a CABG operation and expired ten days later. The doctors were found not guilty of negligence; however, it found the hospital deficient in not maintaining proper medical records. A part of the treatment history (over a week) before the operation was missing, and the date the patient died was wrongly recorded as on the day of surgery when in fact, he died about ten days later[17].

Other lacunae were wrong reporting 1(2%) case and tampering with records 1(2%) case with the following examples.

A 60-year-old female underwent endoscopic polypectomy and did not recover consciousness after the operation. The allegations were that proper consent was not taken before conducting the surgery, and preoperative tests were not done. The consent form did not mention the procedure performed on the patient, and details of the pre-anaesthetic checkup were not recorded. There was an apparent disparity in the CT scan reports issued by the same radiologist on the same day, one stating acute injury to the brain and another stating normal findings. All amounting to deficiency in service[18].

The delay in caesarean section CS and heavy doses of syntocinon resulted in foetal distress and brain damage. Medical records were tampered with, i.e. the dosage of syntocinon was altered by erasing, amounting to professional misconduct for which ten lakhs in punitive damages was levied[19].

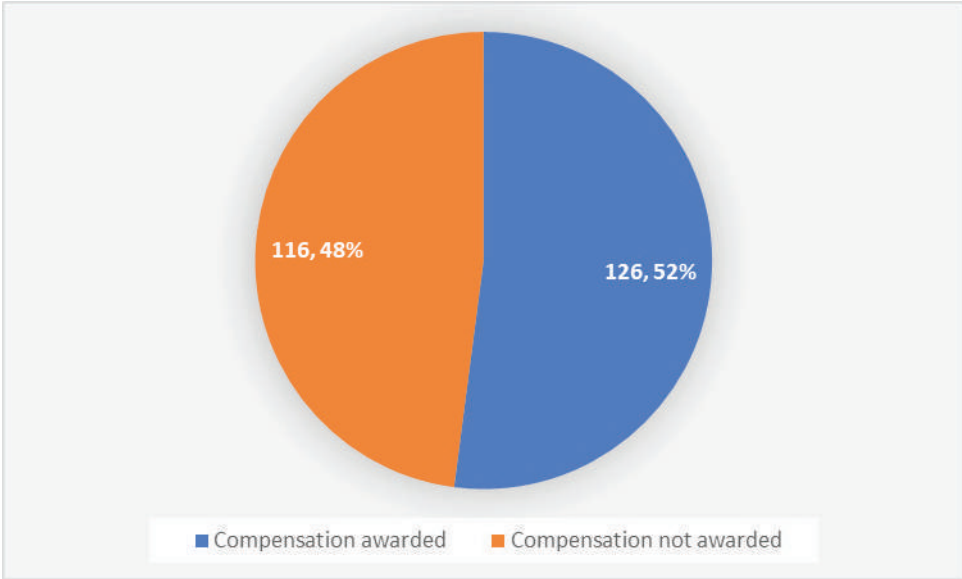


**Figure 1: Distribution of Medical negligence cases.**

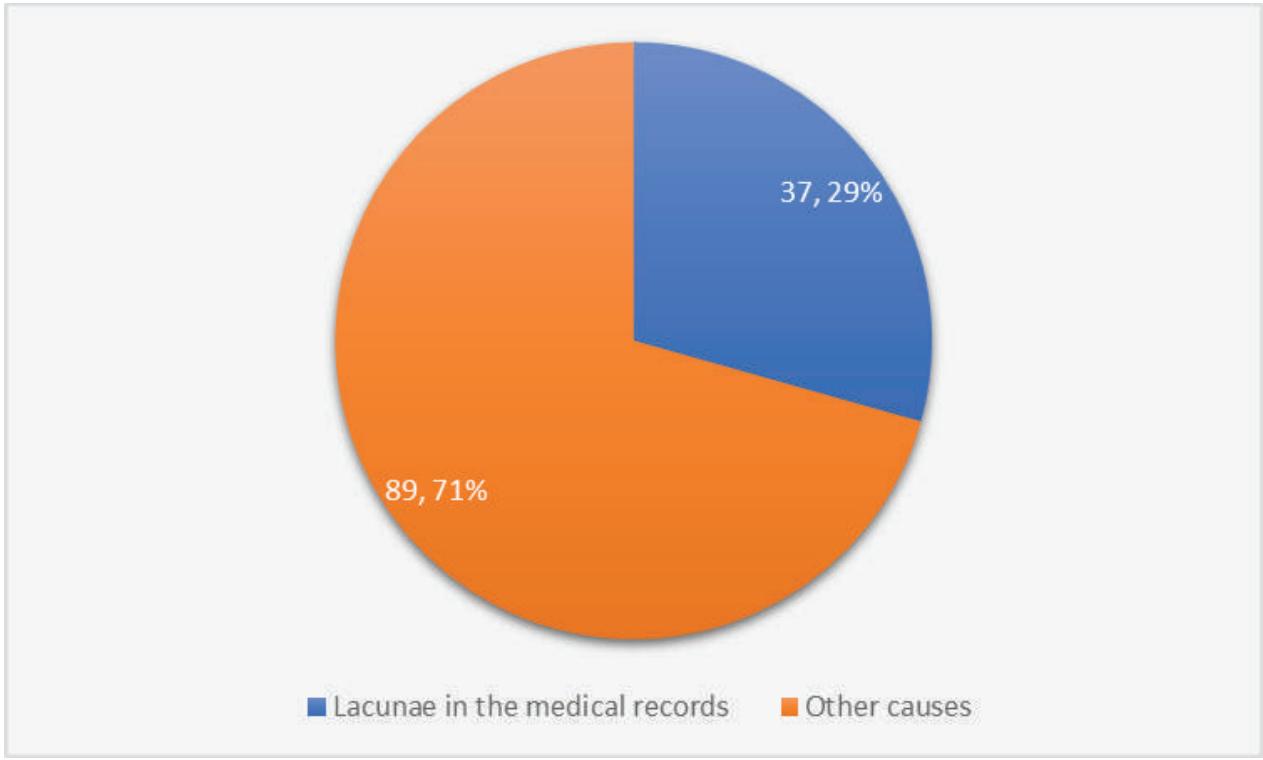


**Figure 2: Cases with lacunae in the medical records**

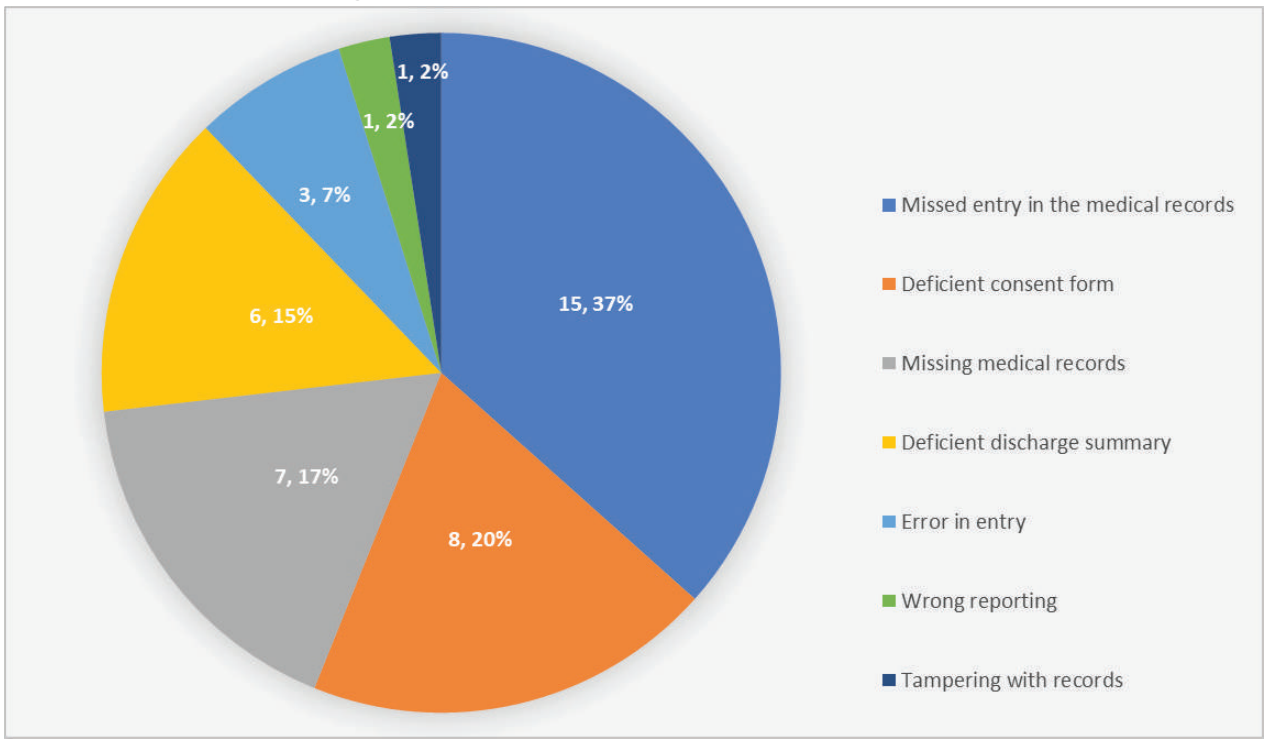


**Figure 3: Type of lacunae in the medical records**

## Conclusion

From the above cases, it is evident that medical records play a vital role in medical negligence cases. Not maintaining proper medical records can leave the doctors/hospital vulnerable in liability cases. Incomplete or poorly filled medical records can attract punitive damages even when the doctor is found not guilty of negligence. Doctors should be prudent in preserving medical records, especially in cases that have a potential for litigation, and should be maintained for a more extended period. Careless entry into the medical records leading to errors will reflect poorly on the treating doctors/hospital. The discharge summary should have all details of the treatment / operation /procedures and follow-up instructions. Medical records should never be tampered with. The doctor/hospital, if found guilty of tampering, can face severe penalty.

**Ethical Clearence:** The data was obtained from monthly periodicals. The research was done following ethical standards

**Source of Funding:** This research did not receive any grant from funding agencies.

**Conflict of Interest** No conflict of interest reported.

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# Antibacterial effects of Ceftriaxone/Zinc Oxide Nanoparticles Combination Against Ceftriaxone resistant *Escherichia coli* isolated from Urinary Tract Infections

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## Abstract

This study was designed to shed light on the issue of pathogenic bacteria resistance to antibiotic, and to overcome it by taking the advantages of nanotechnology. A hundred and twenty urine samples were collected from patients of different ages and of both genders who showed symptoms of urinary tract infections, from three hospitals in Baghdad. Ceftriaxone *E. coli* was detected and isolated from these samples. More recently, nanotechnology application has grown in importance in this problem. In this study, we used Zinc oxide nanoparticle to enhance the activity of ceftriaxone. Zinc oxide nanoparticle (ZnO NP) was prepared by biological method of processing of a fresh leaf aloe vera plants. Further characterization methods, which are Fourier, transform infra-red Spectroscopy (FTIR), and Atomic Force Microscopy (AFM) (which showed that the average diameter of the newly created ZnO NP is 45.55 nm) were applied for checking the created nanoparticle. Antimicrobial ability of ZnO NP showed an inhibition zone with a 13 mm in diameter. The isolates of *E. coli* showed a resistance to ceftriaxone at a concentration of 100 µg/ml. The results of inhibition activity of ceftriaxone antibiotic against *Escherichia coli* isolates of the current study showed a remarkable change when mixed with ZnO NP, in which ceftriaxone became affective in inhibiting *Escherichia coli* growth.

***Keywords:*** *Urinary tract infections, Zinc oxide nanoparticles, b-Lactamase*

## Introduction

Antibiotics modification is a frequently used strategy for rendering an antibiotic ineffectiveness and a large number of modified antibiotics such as aminoglycoside antibiotics (e.g. kanamycin, streptomycin, and gentamycin), β-lactams, chloramphenicol and others are known to exist in producer bacteria[1,2]. Nanoparticles are new strategy in drug modification. Nanoparticles (NPs) in general have many properties that are different from those of traditionally used materials[3,4,5]. They have dimensions typically below 100 nm, which allows them to reach specific sites inside the body and even to be permeable to tissues and cells. Therefore, they can deliver the drugs in active forms at sites that conventional drugs may not reach by themselves and thus minimize the undesirable side effects[6].

In the past two decades, ZnO NPs have become one of the most common metal nanoparticles in biological applications because of their excellent biocompatibility, with high economic and low toxicity[7]. ZnO NPs have showed promising candidates in biomedicine, especially in anticancer and antibacterial fields, which are involved with their ability to trigger an excess reactive oxygen species (ROS) production, release zinc ions, then induce

cell apoptosis[8]. Zinc oxide (ZnO) nanoparticles (NPs) have been implicated in the studies of next-generation nanoantibiotics development against pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and others[9,10,11,12], since they have a unique physicochemical properties[10]. ZnO NPs can be combined with antibiotic and anti-inflammatory drugs to enhance antimicrobial activity against pathogenic microorganisms[13,14]. Their application has been extended to antibiotic drugs or medical devices, theragnostic, implants, and other devices used in clinics[15]. Their particle size and particle size distribution levels are the key parameters that determine NP uptake into biomembranes and thereby influence antimicrobial activity against pathogenic microorganisms[16]. Furthermore, large specific surface area levels of ZnO NPs/MPs facilitate membrane adsorption for antimicrobial actions[17].

Up till now few reports are available on synergistic bactericidal activity of inorganic nanomaterials in combination with b-lactam antibiotics. Combinations of ZnO NPs/MPs with other antibiotic drugs, metal oxide NPs/MPs, and devices have been used to enhance antimicrobial activity against pathogenic microorganisms[18,19]. They have synergistic or improved antimicrobial activity against *E. coli, S. aureus, Aeromonas veronii, P. aeruginosa, B. subtilis*, and *Klebsiella pneumoniae*. This work aimed to explore the antibacterial activity of Ceftriaxone/Zinc Oxide Nanoparticles hybrid against Ceftriaxone resist *E. coli* isolated from urinary tract infections.

## Material and Methods

### Synthesis of Zinc Oxide Nanoparticle (ZnO)

Fresh leaf aloe vera plants were collected, weighed (25 g) and washed with tap water and then with distilled water to remove the impurities[20]. The washed leaves were crushed and boiled for about 15 min in 100 ml distilled water. The boiled extract was cooled and filtered through Whatman filter paper. The filtered plant extract solution was used for the synthesis of ZnO NPs. The plant extract was stored in a refrigerator for further use.

### Preparation of ZnO Nanoparticle

Aqueous solutions of zinc acetate di-hydrate (5, 10 and 50 mmol kg−1) were prepared using distilled water. The above-prepared aloe vera plant extract was added drop wise to these solutions (~5ml min−1) in a round bottom flask under constant stirring and heating at 70◦C. About 15 min after the addition, freshly prepared 0.2 mol kg−1 KOH solution was added dropwise to the reaction mixture to maintain the pH of 11.5. After constant stirring and heating for 4 hrs., the ZnO NPs were collected and washed 3– 4 times with distilled water and then with ethanol. The NPs were dried in a hot air oven at 55−60˚C before further use. The formation of ZnO NPs was confirmed by the appearance of yellowish white color precipitates in the solution mixture

### Antimicrobial Activity of Zinc Oxide Nanoparticles

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods by using sterilized loop. After that, 1 well was made in the agar. 50 µl of each dilution of ZnO nanoparticle was added to the well. The dish was sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

### Ceftriaxone Dilution

By using micropipette, 100 µl of the stock solution (10 ml of distilled water was add to 1 gm of the antibiotic) was added to 900 µl of DW in an eppendorf to make dilution #1. Serial dilution method was done to prepare 4 dilutions.

### Ceftriaxone Resistance

*E coli* were cultured in the Muller – Hinton media and a ceftriaxone disk (35 µg) was put on the agar. Bacteria were allowed to grow overnight.

**Detecting the MIC**

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods. Two wells per each dish were made. Fifty µl of each dilution of ceftriaxone was added to the wells. Dishes were sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

Ceftriaxone – Nanoparticles Combination

Fifty µl of each dilution was put in 5 eppendorfs separately. By end of this step, 5 eppendorfs; each having 50 µl of a specific dilution of ZnO nanoparticles, was prepared. Fifty microliters of the targeted dilution/concentration of ceftriaxone were added to each eppendorf to have 100 µl of the solution in each.

Ceftriaxone/Zinc Oxide Nanoparticles Sonication

All eppendorfs were then mixed by using ultrasonic bath. The eppendorfs were sonicated in the device for 15 minutes and at 37 ºC.

Ceftriaxone/Zinc Oxide Nanoparticles Antimicrobial Activity

*E. coli* was cultured in the Muller – Hinton dishes from the broth by streaking methods. After finishing the culturing, 2 wells per each dish were made. 100 µl of each solution was added to the wells. Dishes were sealed and left in an incubator at 37 ºC overnight, to be read in the next day.

Atomic Force Microscopy (AFM)

The surface morphology of the ZnO nanoparticles was visualized by AFM under normal atmospheric conditions. ZnO NPs powder sample is fitted atop the scanner tube with less than 8mm thickness. The sample is placed on a 15 mm diameter steel disk.

Fourier Transform Infra-Red Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy analysis was carried out using FTIR spectrometer (8400S, Shimadzu, Japan) in attenuated total reflection mode and spectral range of 4000–400 cm -1 with a resolution of 4 cm. The antibiotic suspension was dropped onto the glass slides with the help of a pipette and allowed to dry at 30◦C in incubator.

## Results and Discussion

Production of ZnO Nanoparticles

The FTIR result of ZnO NPs exhibited many characteristic bands, which includes the bands at 3425.34 and 3434.98 $cm^{-1}$, which were due to the stretching vibration of O-H bond; and the bands at 420.45 and 567.03 $cm^{-1}$, which was due to due to the vibration of Zn-O.

The AFM appeared the surface roughness of the ZnO NPs[21]**.** The result showed that the 2D and 3D images of the sample have uniform height distribution around 45.55 nm as an average, as shown in figure-1 and figure-2.

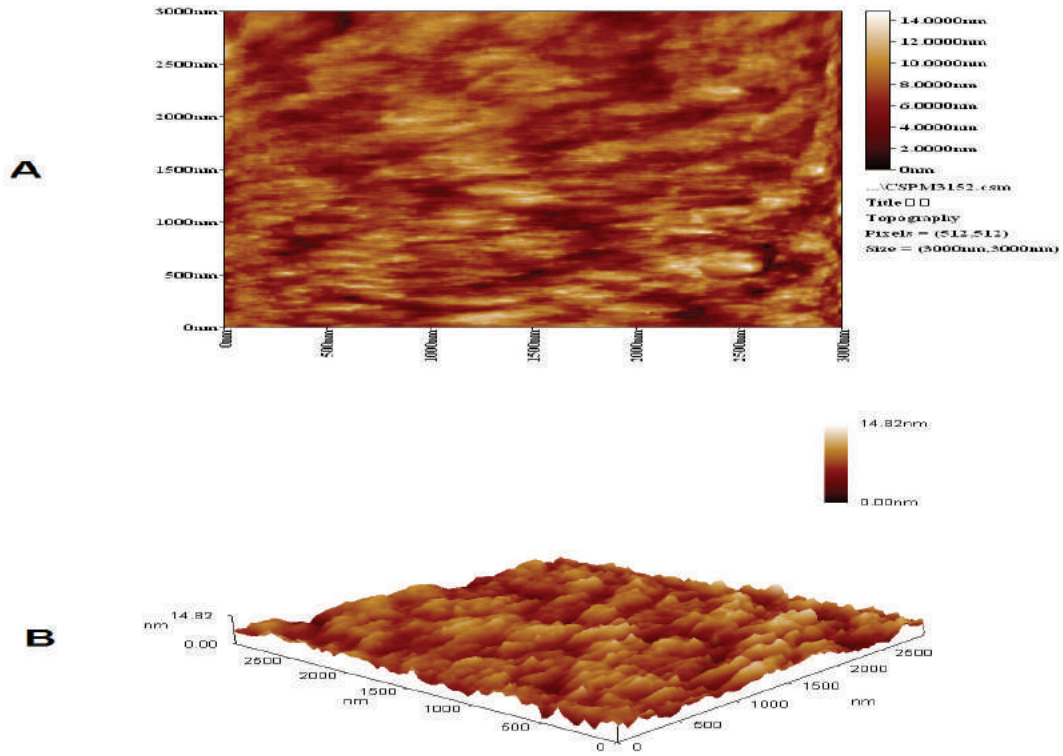


**Figure-1: AFM results for mixing of ZnO nanoparticle. A: The two-dimensional image of ZnO nanoparticle. B: The three-dimensional image of ZnO nanoparticle**

## Granularity Cumulation Distribution Report

| | |
|---|---|
| **Sample**:Sample Name | **Code**:Sample Code |
| **Line No.**:lineno | **Grain No.**:4051 |
| **Instrument**:SPM | **Date**:2021-01-25 |

| | |
|---|---|
| **Avg. Diameter:45.55 nm** | **<=10% Diameter:20.00 nm** |
| **<=50% Diameter:40.00 nm** | **<=90% Diameter:75.00 nm** |

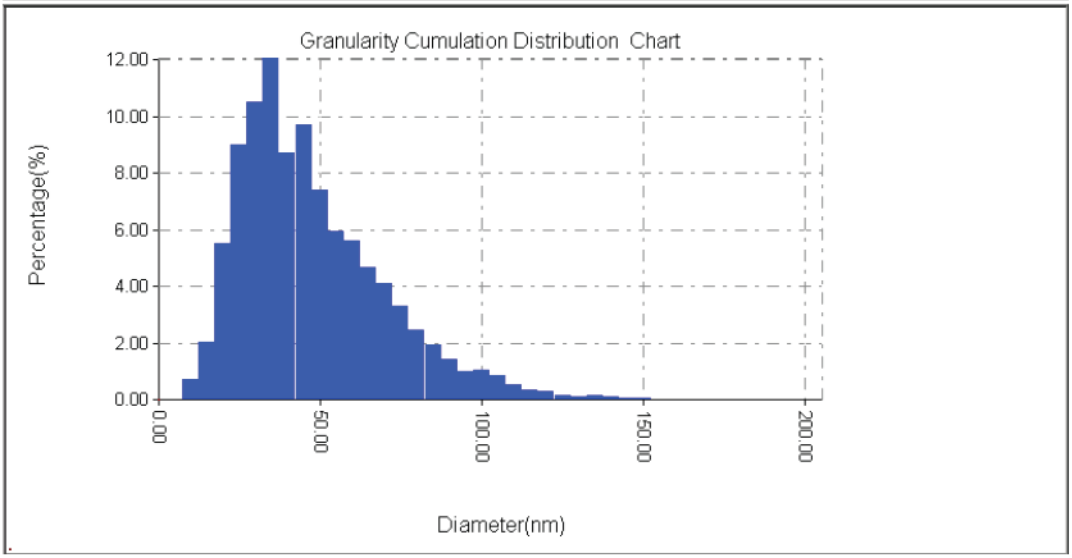


**Figure-2: AFM result report of ZnO nanoparticle**

ZnO Nanoparticle Sensitivity Test

On a Muller – Hinton agar, ZnO nanoparticles (with a concentration of 0.03 µg/µl) were put in wells and then the plates were inoculated with overnight bacterial culture. After examining the results on the next day, antimicrobial activity of ZnO NPs against *E. coli* isolates was tested with an inhibition zone of 13 mm. Dens Check machine were used for standardization the first Kahn tube to McFarland standard (1.5 X 108 CFU/ml).

**Ceftriaxone Sensitivity Test to *E. coli***

On Muller – Hinton agar, *E. coli* was allowed to grow overnight alongside a disk of ceftriaxone (35 µg. The results on the next day, it was found that *E. coli* is resistant to the ceftriaxone.

**Ceftriaxone MIC Test**

The use of the ceftriaxone antibiotic at a concentration of 100 µg/µl produce an obvious inhibitory effect against the tested isolates depending on the diameter of the inhibition zone compared with that of the antibiotic disc, while no antibacterial effect was detected for the antibiotic at the tested concentration of (10 µg/µl, 1 µg/µl, and 0.1 µg/µl as described in figure-3 which shows dilutions effect on *E. coli*.

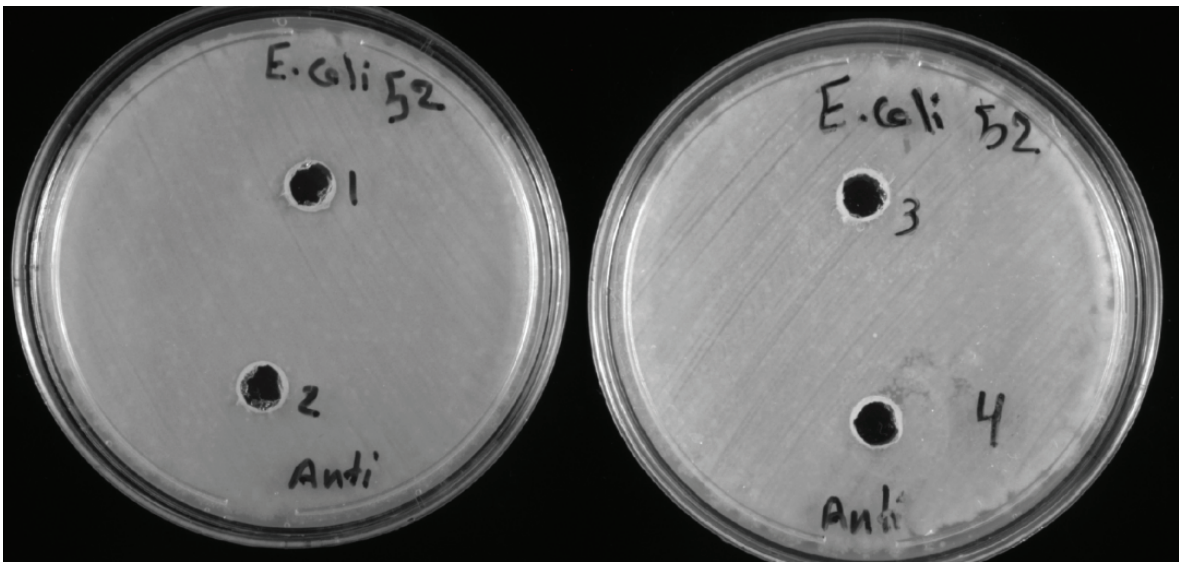


**Figure-3: Serial dilution of ceftriaxone. Dilution number 1 is showing no inhibition zone.**

Ceftriaxone Fourier transform infra-red Spectroscopy (FTIR) Test

The FTIR result of ceftriaxone carrier showed many characteristic bands at specific frequencies, which includes the bands at 3446.56 and 3259.47 $cm^{-1}$, which were caused by stretching vibration of O-H bond; the band at 1649.02 and 1502.44 $cm^{-1}$, which were as a result of the N-H bond; the bands at 1741.60 $cm^{-1}$, which was due to the C=O stretch; the two bands at 1398.30 and 1367.44 $cm^{-1,}$ which were due to C-H rock; and the band at 1033.77 $cm^{-1}$, which was due to the C-N stretch of aliphatic amines.

Hybrid Nanoceftriaxone Fourier transform infra-red Spectroscopy (FTIR) Test

The FTIR result of hybrid nanoceftriaxone showed the appearance of some characteristic bands as shown in figure-4, which includes the band at 3440.77 $cm^{-1}$, which was due the O-H alcoholic stretching; the bands at 1643.24 and 1627.81 $cm^{-1}$, which were caused by the aminic N-H bonds; and lastly the band at 513.03 and 441.67 $cm^{-1}$, which were due to the Zn=O group.

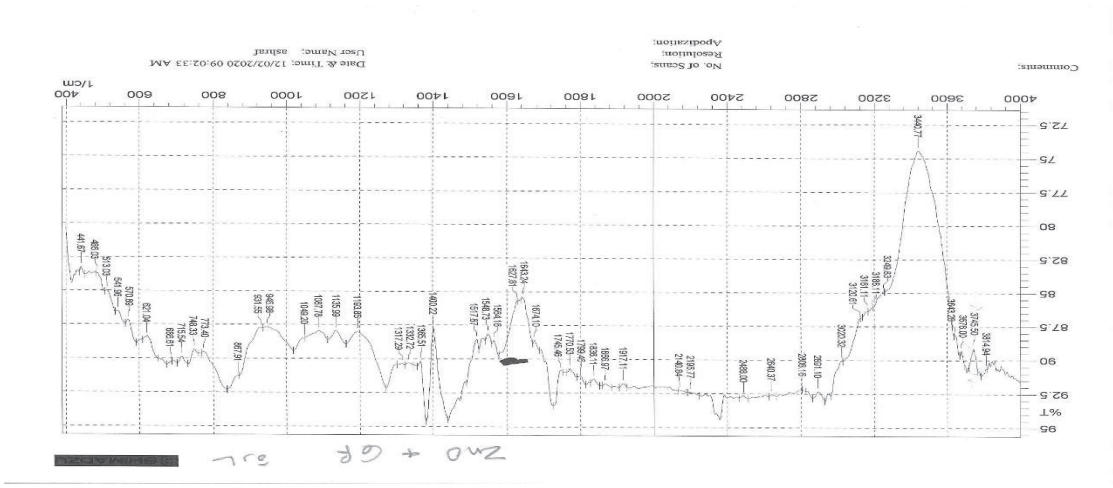

**Figure-4: FTIR result of hybrid nanoceftriaxone.**

The antimicrobial efficiency of ceftriaxone/ZnO NPs showed a remarkable inhibition activity with an inhibition zone of 19 mm compared with 13 mm for ZnO NPs when used alone. Figure-5 shows the inhibition zones resulted from the ceftriaxone-nanoparticles combinations.

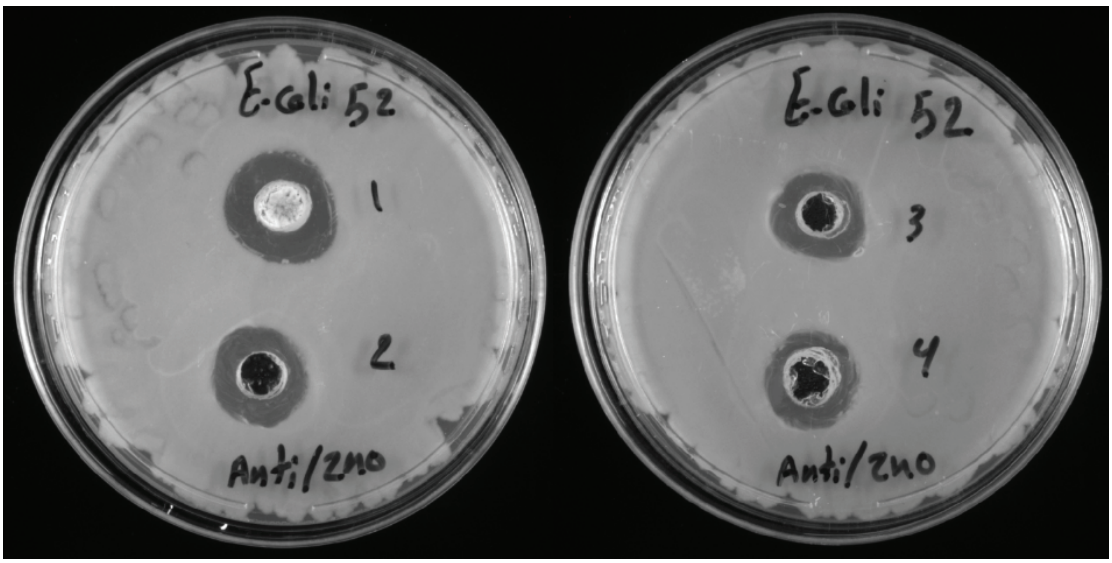


**Figure-5: Hybrid nanoceftriaxone concentrations on Muller – Hinton agar.**

According the results there is a greater capability of the hybrid nanoceftriaxone to inhibit *E. coli* growth on Muller – Hinton agar. As mentioned before, *E. coli* was able to resist free ceftriaxone concentration without any inhibition zone. The present study also showed that free ZnO nanoparticle had the ability to inhibit *E. coli* growth around the ZnO NP well, with an inhibition zone of 13 mm in diameter.

Creating the hybrid solution of ZnO NP with ceftriaxone has led to an increased killing ability of the ceftriaxone and ZnO NP to be 19 mm for the stock concertation of ZnO NP with dilution number 1 of ceftriaxone. Considering this area, the inhibition zone was increased by around 46% from what used in the stock concentration of free ZnO NP, and to activate dilution number 1 of ceftriaxone to able to act according to its mechanism of action. Table-1 shows each concentration of the Nanoceftriaxone with its killing zone diameter and the percentage of each increase.

**Table-1: Inhibition zones difference**

| | Free ZnO NP Diameter (mm) | Hybrid Nanoceftriaxone Diameter (mm) | Killing Elevation Percentage |
|---|---|---|---|
| Comparison #1 | 13 | 19 | 46.2 % |
| Comparison #2 | 13 | 17 | 30.8 % |
| Comparison #3 | 13 | 16 | 20.1 % |
| Comparison #4 | 13 | 14 | 7.7 % |

Antibiotic-tagged nanoparticles have been shown to increase antibiotic concentrations at the site of bacterium–antibiotic contact and promote antibiotic binding to microorganisms[22].

Luo *et al.,* (2013) showed ZnO nanorods could obviously achieve synergistic antibacterial effects with ceftriaxone against *Escherichia coli* (*E. coli*)[23], which agreed with the present result. Also, Cephalexin, other type of cephalosporins, also revealed an enhanced antibacterial activity against *E. coli* when mixed with ZnO NP according to Namasivayam *et al*., (2015)[24].

Our observations are in line with those of Banoee *et al.,* (2010), who observed that antibiotics (ciprofloxacin) have greater efficacy when combined with nanoparticles than when mixed with ampicillin. As ZnO nanoparticles were paired with ciprofloxacin, they reported a 27 percent and 22 percent rise in inhibition zone areas against *S. aureus* and *E. coli*, respectively[25].

The presence of an inhibition zone indicates that ZnO NPs biocidal action involves disrupting the membrane. It's likely that ZnO NPs antimicrobial effects are due to their tiny size, which is 250 times smaller than a bacterium cell. This makes it possible for them to bind to the microorganisms' cell walls, causing their degradation and, as a result, the cell death. Furthermore, the high rate of production of surface oxygen species from ZnO allows the bacteria to be killed[26]. As a result of the improved impact of nano-ZnO on ceftriaxone's antibacterial activity, the diameter of the inhibition region around the wells has increased significantly. This is thought to be due to the antibiotic-zinc nanoparticles combination's synergistic effect. Zinc nanoparticles greatly increased antibiotic effectiveness against *E. coli* at the concentrations tested. Ansari *et al*., (2012) published a paper on the antibacterial properties of ZnO nanoparticles as a potential new unconventional antibacterial agent that could be useful in fighting methicillin-resistant *S. aureus* and other drug-resistant bacteria[27], and our findings are considered to be in agreement.

Also, the current study's results were compared to those of Moodley, (2014), who paired gold nanoparticles GNPs with a particular antibiotic, ciprofloxacin, and investigated the antibacterial function of the ciprofloxacin-conjugated gold nanoparticles by exposing them to pathogenic bacteria such as *Staphylococcus aureus, E. coli, Klebsiella pneumoniae, Enterococcoccus spp*., and discovered that conjugate nanoparticles improve antibiotic concentrations at the site of bacterium-antibiotic contact, facilitating antibiotic binding and entry into bacteria, which could have significant consequences for infection treatment[28].

## Conclusion

Zinc oxide nanoparticle (ZnO NP) prepared from fresh leaf aloe vera plants showed a small antibacterial ability and can act as drug carrier to overcome

emerging antibiotic resistance. When mixing with ceftriaxone, ZnO nanoparticles showed a synergistic effect leading to a greater inhibition zone. In fucture, more elaborate experimental have been needed to elucidate the mechanism of synergistic antibacterial impact.

**Conflict of Interest:** Nil/None to declare.

**Source of Funding:** Self-funding.

**Ethical Clearance:** Samples were taken under the scientific ethics committee of the Iraqi Ministry of Health and the College of Applied Biotechnology, Al-Nahrain University, Baghdad, Iraq.

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# Effectiveness of Mixed Clove Flower Extract (Syzygium Aromaticum) And Sweet Wood (Cinnamon Burmanni) on the Growth of Enterococcus Faecalis

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## Background

Failed root canal treatment can cause problems such as recurring pain and leave persistent bacteria in the root canal. One of the bacteria that often causes root canal treatment failure is Enterococcus faecalis. The use of a root canal irrigation agent such as sodium hypochlorite (NaOCl) can help inhibit the growth of bacteria in the root canal. The use of materials derived from nature can be used as an alternative material for root canal irrigation because they inhibit growth and kill bacteria and have fewer side effects than chemicals. Several studies have tried to prove the use of ingredients of natural origin in the form of a mixture and the results obtained are higher when compared to single use. Natural ingredients that are often used as herbal medicine include cloves and cinnamon. **Objective.** To determine the effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) on the growth of Enterococcus faecalis. Methods. Using experimental laboratory methods, with a research design in the form of Post Test Only Control Design, sampling by random sampling using 5 treatments and 5 repetitions. Statistical test using One Way Anova. **Results.** This study showed the diameter of the inhibition zone of Enterococcus faecalis bacteria in a mixture of clove flower (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) extract at 5% concentration of 10.84 ± 0.73, 10% concentration of 15.00 ± 0.80 mm. The concentration of 15% was 16.32 ± 1.04 mm and based on statistical tests obtained a significant value of P <0.01. **Conclusion.** The alternative hypothesis of this study is accepted and this study shows that there is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) concentrations of 5%, 10%, and 15% in inhibiting the growth of Enterococcus faecalis.

***Keywords:*** *Enterococcus Faecalis, Extract Clove Flowers (Syzygium Aromaticum), Extract Cinnamon (Cinnamon Burmanni)*

## Introduction

Root canal treatment is a treatment option that can be used to treat pulp tissue infections. Failed root canal treatment can cause problems such as recurring pain and leave persistent bacteria in the root canal. From various research results, it was found that the microorganism that often causes root canal treatment failure is the bacterium Enterococcus faecalis. Enterococcus faecalis is a facultativeanaerobic gram-positive bacteria and is one of the most resistant microorganisms among the root canal microflora.

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The main goal of pulp and root canal treatment is to remove microbes from the pulp and periapical tissues. Root canal treatment is influenced by proper instrumentation, disinfection, and obturation of the root canals. During root canal preparation, irrigation is very important for several reasons, namely for cleaning the root canals, lubricating files during preparation, cleaning residual debris, removing dentin tissue or smear layers, and removing bacteria without affecting the periapical tissue. The ideal irrigation solution must have two main qualities, namely having an antibacterial effect and low toxicity to periapical tissue. [4],[5]

The use of a root canal irrigation agent such as sodium hypochlorite (NaOCl) can help inhibit the growth of bacteria in the root canal. NaOCl is inadequate in eliminating persistent bacteria. The concentrations commonly used range from 0.5% to 5.25%. The use of materials derived from nature can be used as alternative materials for root canal irrigation because they inhibit growth and kill bacteria. Traditional medicines are considered to have less side effects than drugs derived from chemicals. Indonesia is a country that is rich in biodiversity. Of the total 28,000 species of medicinal plants in Indonesia, 1,845 have been identified as having medicinal properties.[6]

Several studies have tried to prove the use of plants in the form of mixtures or combinations of several types of plants as reported by Miksusanti et al. (2012) with mangosteen rind extract and secang wood against antioxidant activity, Wajdi et al. (2017) with a mixture of Moringa seed extract and cherry leaves against Pseudomonas aeuroginosa and Bacillus subtilis, and the results obtained were higher when compared to single use. Therefore, research related to extract mixtures is important to do in order to increase the biological activity of several types of plants whose activity is known in the form of single use.[7]

Two types of plants that are known to have a single biological activity are cloves and cinnamon. The stems, leaves, and flowers of the clove plant have many benefits. Clove flower is used traditionally as an antimicrobial. Extracts from clove flowers have biological activities, such as antibacterial, antifungal, insecticidal, topical analgesics, sedatives and antioxidants. The content of antibacterial compounds in clove flowers, namely flavonoids, tannins, alkaloids, and eugenol.[8]

Cinnamon is known to be used as antibacterial, antifungal, anti-inflammatory, analgesic, antioxidant, inhibits the formation of dental plaque and periodontal disease, as well as other activities. Chemical compounds that are thought to act as antibacterials in cinnamon are known to be used as antibacterial, antifungal, anti-inflammatory, analgesic, antioxidant, inhibiting the formation of dental plaque and periodontal disease, as well as other activities. Chemical compounds that are thought to have an antibacterial role in cinnamon are essential oils of about 0.5–2% (such as eugenol, safrol, cinnamaldehyde and linalool), polysaccharides around 10% (such as diterpenes and coumarin), 4–10% phenolic components (such as tannins) and flavonoids.[9]

Based on the description above, the researcher is interested in conducting research on the effectiveness of a mixture of clove flower extract and cinnamon on the growth of the bacteria Enterococcus faecalis. “The researchers hope that these clove and cinnamon plants can complement each other so that they can have a strong effect.

## Methods

The research design used was true experimental research design with post test only control group design and sampling with random sampling using 5 treatments and 5 repetitions. Statistical test using One Way Anova. Testing the ability of the zone of inhibition of a mixture of clove flower extract and cinnamon using the agar diffusion method.

This research was conducted in the Microbiology Laboratory of the Hasanuddin University Medical

Faculty. The sample was Enterococcus faecalis bacteria from the pure bacterial stock of the Hasanuddin University Microbiology Laboratory. The independent variable (independent) studied was a mixture of clove flower extract and cinnamon made by maceration method using 96% ethanol solvent and made concentrations of 5%, 10% and 15% with positive control 5.25% NaOCl and sterile Aquades as negative control.

The materials used in this study were clove flowers (Syzygium aromaticum) and cinnamon (Cinnamon burmanni). Enterococcus faecalis bacteria, 96% ethanol, 5.25% NaOCl, DMSO (Dimethyl sulfoxide), Mueller Hinton Agar (MHA), and paperdisk. The tools used in this research are autoclave, incubator, analytical scale, petri dish, sterilizer / oven, flatware, round loop, tweezers, digital calipers, erlenmeyer tube, vial bottle, dropper, filter paper jar.

## Result

The results showed that a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5%, 10%, 15% had antibacterial power against Enterococcus faecalis as indicated by the presence of a clear zone formed around the paper disk.

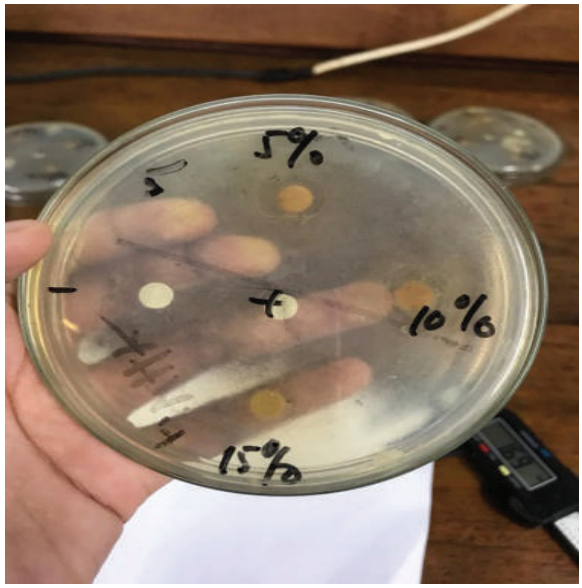


**Figure 1. Inhibition zone formed on MHA media**

The inhibition zone produced by each treatment has a different diameter. Observations are made by measuring the horizontal diameter and vertical diameter of the formed drag zone and then enter it into the formula to find the mean of the drag zone and then enter it in the worktable.

**Table 1. The average diameter of the zone of inhibition of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5%, 10%, 15%, on the growth of Enterococcus faecalis bacteria and the results of data normality test.**

| Type of Solution | | Mean ± SD | p-value Shapiro Wilk |
|---|---|---|---|
| Mixed solution of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) | Concentration 5% | 10,84± 0,73 | 0,872 |
| | Concentration 10% | 15,00 ± 0,80 | 0,731 |
| | Concentration 15% | 16,32 ± 1,04 | 0,529 |
| Positive Control | NaOCl 5,25% | 21,74± 3,26 | 0,979 |
| Negative Control | Aquades | 0,00±0,00 | - |

Note: Normality Test; Shapiro-Wilk test: p> 0.05, normal data distribution

* Anova One-way test: p <0.01: significant

## Discussion

This research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Hassanuddin University for two days, which was conducted in January 2021. This study was conducted with the aim of determining the effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) on the growth of Enterococcus faecalis. In this study using clove flower extract and cinnamon extract and then the extraction process was carried out using the maceration method. After obtaining the thick extract, then diluting it to make a concentration of 5%, 10% and 15%.

The effectiveness of the inhibition was carried out using the inhibition zone observation method by looking at the clear zone around the paper disk which was given a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) 5%, 10%, 1 5%, NaOCl, respectively. 5.25% as a positive control, and Aquades as a negative control that was incubated in the incubator for 1 x 24 hours.

The visible zone of inhibition indicates an inhibition of Enterococcus faecalis bacteria on agar medium. The agar medium used was Muller Hinton Agar (MHA) which contained the bacteria Enterococcus faecalis. Muller Hinton Agar (MHA) was chosen as the agar medium used in the study because it is the most selective medium for bacterial growth and is more optimal when incubation is incubated.

The results of this study indicated that a mixed solution of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) could inhibit the growth of Enterococcus faecalis at each concentration. The biggest zone of bacterial inhibition is in a solution of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 15% of 16.32 ± 1.04 mm, so it can be categorized as moderate in inhibiting Enterococcus faecalis bacteria, while the power zone The smallest inhibition was a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a 5% concentration of 10.84 ± 0.73 mm, and could be categorized as weak in inhibiting Enterococcus faecalis bacteria.

This result is supported by research conducted by Rastina et al. (2015) said that the ability of an antimicrobial to inhibit microorganisms depends on the concentration of the antimicrobial material and the type of antimicrobial material produced. The greater the concentration of an antimicrobial, the larger the clear zone is formed. This is because the higher the concentration of antimicrobial ingredients, the more active substances contained in it so that the effectiveness in inhibiting bacteria will increase and produce a wider clear zone. Conversely, at low concentrations, the less antimicrobial substances contained in an antimicrobial agent will reduce their activity.[10]

Lorain (2005) explains that the greater the antimicrobial concentration, the faster diffusion occurs, so that the antibacterial power will be greater and the resulting inhibition zone diameter will be wider. This result is supported by the statement of Prawata and Dewi (2008), that the effectiveness of an antibacterial substance is influenced by the concentration of the substance. Increasing the concentration of substances causes an increase in the content of active compounds that function as antibacterials, so that their ability to kill bacteria is also greater. However, the effectiveness of the antibacterial action of a substance can be influenced by several factors, including the antibacterial concentration, the number of bacteria, bacterial species, organic matter, temperature, and environmental pH. This is also supported by the statement of Jenie and Kuswanto (1994) which states that the effectiveness of an antibacterial substance in inhibiting growth depends on the nature of the tested bacteria, concentration and length of contact time. [11],[12],[13]

This study aims to determine that a mixture of clove flower extract and cinnamon can inhibit the growth of the bacteria Enterococcus faecalis. Which is the effectiveness of a mixture of clove flower extracts and cinnamon against the inhibition of Enterococcus faecalis bacteria as an antibacterial agent. This is in line with research conducted by Huda et al. (2018) which states that the content of antibacterial compounds in clove flowers, namely flavonoids, tannins, alkaloids, and euganol. Meanwhile, according to Muslim, et al (2018) stated that Cinnamon extract contains cinnamaldehyde compounds as antibacterials. Which is the mechanism of the cinnamaldehyde compound in cinnamon extract is to inhibit energy metabolism in bacteria. This is evidenced by the synthetic inhibition of L. monocytogenes bacterial cell walls and inhibiting the biosynthetic enzymes used for the formation of energy.[14],[15]

Based on the research of Suhendar et al (2019) regarding the antibacterial activity of clove flower extract (Syzygium aromaticum) against Streptococcus mutans bacteria, the results of the qualitative phytochemical test showed that the methanol extract of clove flowers contained alkaloid, flavonoids, terpenoids and phenolic compounds. The steroid compound was not identified in this test. Based on the results of the antibacterial activity test, clove flower extract produced its inhibition zone. The inhibition zone indicates that the clove flower extract has the potential to be antibacterial. Clove flower extract has antibacterial activity by inhibiting S mutans bacteria, inhibition zone diameter is 37 mm, and positive control is 28 mm. The formation of an inhibitory zone around the well / disc indicates the inhibitory activity of clove flower extract against S. mutans bacteria.[8]

The formation of bubbles after adding one drop of 0.1 HCl indicates that the sample contains saponins. The formation of a white precipitate after the addition of 10% gelatin proves that the sample contains tannins. The green-black color formed after the addition of 1% FeCl3 solution indicates the presence of polyphenols in the cinnamon extract. Meanwhile, the brown color formed after Mg and 1 ml of HCl were added, indicating that the sample contains flavonoids. Quinone was detected in the cinnamon extract due to the formation of a red color due to the addition of 1% NaOH. The formation of a red color after the addition of Carr Price's reagent indicates that the sample contains triterpenoids. From this study, it can be concluded that Cinnamon burmannii has antibacterial activity in the form of the ability to inhibit the growth of Enterococcus faecalis.[9]

## Conclusion

Based on the results of the research conducted, it can be concluded:

1. There is the effectiveness of a mixture of clove flower extracts (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5% with an average inhibition zone of 10.84 ± 0.73 mm in reducing Enterococcus faecalis bacteria.

2. There is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 10% with an average inhibition zone of 15.00 ± 0.80 mm in inhibiting Enterococcus faecalis bacteria.

3. There is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 15% with an average inhibition zone of 16.32 ± 1.04 mm in inhibiting Enterococcus faecalis bacteria.

4. The most effective treatment in inhibiting the growth of Enterococcus faecalis bacteria is a mixture of 15% concentration of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni).

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**Ethical Considerations:** Ethical clearance was obtained from Universitas Muslim Indonesia, Makassar; with number" 009/A.1/KEPK-UMI/I/2021. Just before the interview, written (or

thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia, Makassar guidelines.

**Conflicts of Interest**: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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# Efficacy of Serum Levels of Antioxidants in Oral Submucous Fibrosis Patients

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## Abstract

**Background and Aim:** Serum total protein, albumin and uric acid are plasma's potent antioxidant defense; it comes out convincing enough to be used as a reliable marker of oxidative stress in the body and could be helpful in the early identification of oral submucous fibrosis (OSMF), against the other invasive and not so cost effective diagnostic adjuncts. Hence the present study was aimed to compare the serum levels of total protein, albumin and uric acid in oral submucous fibrosis patients.

**Material & Methods:** Patients were assigned to one of three groups: Group one consisted of 25 healthy individuals, Group two consisted of 25 individuals with chewing tobacco habit without OSMF, Group three consisted of 25 patients with chewing tobacco habit with OSMF. Participants were instructed to fast overnight. On the following day between 8 and 10 AM, 5 ml of venous blood was drawn from each subject using a sterile disposable syringe taking care to prevent hemolysis. Assay of albumin & total protein were carried out using the bromocresol green method and that for uric acid was carried out by biuret method.

**Results:** Mean serum levels of Total protein decreased from group I to group III; difference was not found to be statistically significant. Serum levels of Albumin decreased from group I to group III; difference was found to be statistically significant ($p < 0.05$). Serum levels of Uric acid decreased from group I to group II; difference was found to be statistically significant ($p < 0.05$).

**Conclusion:** Our findings suggest that the degree of oxidative damage in OSMF can be assessed by estimation of serum Total Protein, Albumin & Uric Acid levels in affected patients. Underlying deficiency of antioxidants can be corrected by dietary supplementation of these antioxidants. This may be helpful for successful management of OSMF and for avoiding the consequences of malignancy.

***Key Words:*** *Serum, Albumin, Total Protein, Uric Acid, OSMF*

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## Introduction

With the increase in current globalization and coontinous transformation of metro cities into mega cities, surveys have found upsurge in the rate of use

of various habitual products in India and various other Southeast Asian countries. Of all the products betel quid Gutkha are one of the most commonly practised habits.[1] In India betel quids & Gutkha are socially accepted habits which are openly distributed, easily avaliable and popular among youngsters. Around 26% of Indian population consumes betel quid & Gutkha. Bihar and Jarkhand have the highest percentage whereas Goa and Himachal pradesh have the least amount of betel quid and Gutkha chewers. Betel quid is the combination of fresh betel leaf, fresh areca nut, slaked lime, catechu and tobacco; whereas Gutkha is industiral manufactured item contaning tobacco.[2]

Betel quid and Gutkha consumption are considered as the leading causative agents for oral submucous fibrosis, oral cancer and upto some extent liver diseases.[3] OSMF first described by Schwartz in 1952, is considered as one of the potentially malignant condition that hamperes the normal functional acitivity of oral cavity and sometime pharynx.[4] OSMF is multifactorial in origin, which varies from local irritants like capsaicin, tobacco, betel nut, punget and spicy food to other factors like iron and nutritional deficiencies, chronic candidiasis, genetic abnormalities, Herpes simplex virus (HSV), Human papilloma virus (HPV), autoimmunity. Various case control studies have shown the association of areca nut, a constituent of betel quid towards exaggerating the occurrence of OSMF.[5]

The International Agency for Research on Cancer has considered areca nut in betel quid as group I carcinogen to humans.[6] Aerca nut is known to contain many alkaloids and several polyphenols. Aercoline is the major alkaloid found in aerca nut. Several evidences conclude that aercoline is genotoxic and mutagenetic component for many cells and inihibts the growth of oral mucosal fibroblasts, gingival fibroblasts and keratinocytes.[7] Reactive oxygen species (ROS) implicated in the process of multistage carcinogenesis, are generated during chewing of areca nut. Chang et al. 2001 in the study concluded that areca nut ingredients are crucial in the pathogensis of OSMF & oral cancer.[1] Nair et al 1995 first demonstrated that aqueous extracts of areca nut and catechu were capable of generating superoxide anion and hydrogenperoxide (pH > 9.5).[2]

ROS is a collective term which include molecules like Hydroxyl radical ($OH^-$), Superoxide anion ($O_2$) and Hydrogen peroxide ($H_2O_2$). According to Pryor 1986, ROS causes tissue damage by different mechanisms which include protein damage, cancer-causing mutation, lipid damage, DNA damage and alter cellular antioxidant defence system.[8] Damage to the DNA and other cellular molecules, by reactive oxygen species are considered as major culprits in occurance of cancer.

Antioxidants are the substances which inhibits the adverse effect of ROS. They are present in all body fluids. Antioxidants can act by scavenging reactive oxygen species, by inhibiting their formation, by binding transition metal ions and preventing formation of OH and/or decomposition of lipid hydroperoxides, or by any combination of the above.(plant) Plasma is known to contain wide range of antioxidants including Albumin, Uric acid and Total protein. They possess antioxidant properties owing to their free thiol group present in them. Thiol group is known to act as important scavengers of various free radicals.[9,10] The imbalance between the oxidants – antioxidants results in formation of oxidative stress. Oxidative stress plays a major role in promotion of various pathological processes.[11-13]

Despite of advancement in therapy and diagnostic aids, the rate at which the prevalance of OSMF and its malignant transformation rate are spreading is alarming. According to the study done by Pillai et al. in 2005[14] malignat transformation rate of OSMF is recorded to vary from 7.6% to 15 %.(Teklal patel) This highlights that there is need to discover suitable biomarker for early detection of oxidative stress which can adjuvant in diagnosis of this potentially malignant disorder.[15] Moreover the role of oxidative stress in the initiation, promotion and progression of

various pathological entities has been subject of much speculation; hence the present study was conducted with the aim to evaluate Serum Total Protein, Albumin & Uric acid levels in normal individuals, patients chewing tobacco without Oral Submucous Fibrosis and patients chewing tobacco with Oral Submucous Fibrosis.

## Material & Methods

**Study population:**

The study was conducted at the Outdoor Patient Department of a dental college and the Indoor Patient Department of Oncology Department of a medical college for a period of 8 months. Patients were assigned to one of three groups: Group one consisted of 25 healthy individuals, Group two consisted of 25 individuals with chewing tobacco without OSMF, Group three consisted of 25 patients with chewing tobacco with OSMF.

**Collection of data:**

None of the study group was on a therapeutic regimen or suffering from any systemic conditions that could have affected the albumin level in the body. Also, chronic alcoholics were excluded from the study to rule out the possibility of altered liver function that could have altered serum levels of albumin, uric acid and total protein. Participants were informed in detail about the planned study and written informed consents were obtained. An ethical clearance certifi cate from the institution's ethical committee was obtained prior to the study. Participants were instructed to fast overnight. On the following day between 8 and 10 AM, 5 ml of venous blood was drawn from each subject using a sterile disposable syringe taking care to prevent hemolysis. Serum was separated by ultracentrifugation with care to prevent hemolysis. Assay of albumin & total protein were carried out using the bromocresol green method and that for uric acid was carried out by biuret method.

## Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS software for differences between groups. Values for $p < 0.05$ were considered statistically significant. The normality of data was checked before the statistical analysis was carried out.

## Results

Mean serum levels of Total protein decreased from 7.42 g/dl in normal individuals to 6.10 g/dl in individuals with chewing tobacco without OSMF and 5.83 g/dl in individuals with chewing tobacco with OSMF; difference was not found to be statistically significant ($p > 0.05$) (Table 1, Fig 1). Serum levels of Albumin decreased from 4.70 g/dl in normal individuals to 3.01 g/dl in patients diagnosed with chewing tobacco without OSMF and 2.30 g/dl in patients diagnosed with patients diagnosed with chewing tobacco with OSMF; difference was found to be statistically significant ($p < 0.05$). Serum levels of Uric acid decreased from 6.05 g/dl in normal individuals to 3.06 g/dl in patients diagnosed with chewing tobacco without OSMF and 2.70 g/dl in patients diagnosed with patients diagnosed with chewing tobacco without OSMF; difference was found to be statistically significant ($p < 0.05$).

**Table 1: Evaluation of Total Protein, between Normal Control group, Chronic Tobacco Users group and OSMF group**

| GROUPS | N | Mean | Std. Deviation | P value |
|---|---|---|---|---|
| 1 | 15 | 7.42 | 0.67 | 0.006 |
| 2 | 15 | 6.10 | 0.49 | |
| 3 | 15 | 5.83 | 0.36 | |

**Table 2: Evaluation of Albumin, between Normal Control group, Chronic Tobacco Users group and OSMF group**

| GROUPS | N | Mean | Std. Deviation | P value |
|---|---|---|---|---|
| 1 | 15 | 4.70 | 0.55 | 0.003* |
| 2 | 15 | 3.01 | 0.32 | |
| 3 | 15 | 2.30 | 0.52 | |

*** indicates statistically significant difference at P < 0.005**

**Table 3: Evaluation of Uric Acid, between Normal Control group, Chronic Tobacco Users group and OSMF group**

| GROUPS | N | Mean | Std. Deviation | P value |
|---|---|---|---|---|
| 1 | 15 | 6.05 | 0.88 | 0.002* |
| 2 | 15 | 3.06 | 0.34 | |
| 3 | 15 | 2.70 | 0.38 | |

*indicates statistically significant difference at P < 0.005

## Discussion

Oxygen is an important substance for life on earth especially for human life and act like double edged sword. Oxygen is required for its cellular function and about 5% of inhaled oxygen is converted into production free radicals through univalent reduction of O2.(plant) According to Halliwell, 2007 free radicals are constantly produced during the normal cellular metabolism, mainly in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).[16] These toxicity of oxygen molecule is itself beneficial in performing a function in inter and intracellular signalling, in therapeutics aid like hyperbaric oxygen therapy, and on other hand over production of free radicals causes formation of oxidative stress and further lead to damage the cellular metabolism, which would result in triggering or transforming normal cells into malignant ones.[10]

Substances that neutralize the oxidative stress and neutralize the ill effect of free radicals are grouped in Antioxidant Defense System.[17] Such system encompasses many substances which are often called as Antioxidants, Free radiacl scavengers, Chain terminators.[6,17] There are a wide variety of antioxidants which are different in their composition, physical and chemical properties, mechanisms and site of action. An ADS is classified into four categories based on their function; **First:** - Preventive antioxidants (suppress formation of free radicals). **Second:** - Scavenging antioxidants (suppress chain reaction). **Third:** - Repair and denovo antioxidants. **Fourth:** - Adaptation where the signal for the production & actions of free radicals induces formation and transport of the appropiate antioxidant.

Free radicals are highly reactive molecules containing one or more unpaired electrons in their outermost shell. Antioxidants neutralize free radicals by donating an electron, which ends the electron stealing reaction. Antioxidants get oxidized in this reaction but are stable in their reduced form.[18]

Betel quid one of the causative agent in occurance of OSMF is a mixture of Betel leaf, Areca nut, Slaked lime and tobacco pieces. When combination of areca nut and slaked lime occurs there is production of higher percentage of alkaloids. Arecoline is the major alkaloid and constitute about 0.15% to 0.67% of dry weight of betel quid.[6,19] In many studies the genotoxicity and mutagenicity of these alkaloids have been detected.[6,20] Tobacco consists of tar phase and smoke phase. Smokeless tobacco consists of tar phase. The tar phase of tobcco contains stable free radicals of which principle is quinone/hydroquinone. This stable free radical when combine with alkaloids produce active redox system which reduce molecular oxygen to produce hydroxyl radicals.[21,22]

Areca nut contain a high level of copper (mean 302 nmol/g) when compared to other commonly eaten nuts (22–173 nmol/g) and that soluble copper is released into whole mouth saliva following chewing areca for 5–30 min.[7] Copper has been implicated in the pathogenesis of several fibrotic conditions. Data from the study done by **Trivedy CR et al. 2000[23]** using the element spectrum obtained by analytical scanning electron microscopy, however, demonstrated a high content of copper in the oral biopsy of OSF tissues. The exact mechanism of copper-induced mutagenesis is not fully understood. Copper-induced DNA damage has been reported [24] and there is evidence to suggest that copper may bind to the protein product of p53, resulting in alteration of its conformation.[25-28] p53 aberrations is found in OSF tissues. P53 stabilization, which may be critical in the progression of potentially malignant lesions to squamous cell carcinoma (37), may thus arise from the DNA damage inflicted by chewing copper-containing areca nut. (Raised tissue copper levels in oral submucous fibrosis)[29-34]

Active redox systems along with copper content of areca contribute in generation of free radicals by Fenton & Haber – Weiss reaction.

Haber – Weiss reaction

$Cu^{2+} + O_2^- = Cu^+ + O_2$

Fenton Reaction

$Cu^+ + H_2O_2 = Cu^{2+} + OH + OH^-$

Copper combine with this oxygen radical & hydrogen peroxide to produce free hydroxyl radicals. These are quenched by body's second line of non enzymatic antioxidants like Total Protein, Albumin & Uric Acid.[35] Plasma is known to contain a wide range of important anti-oxidants including total protein, albumin & uric acid; posseses antioxidant property owing through free thiol group. Normal concentration range of each of these parameters in serum is as follows total protein is 6.6 – 8.3 gm/dl; Albumin: 3.5 – 5.0 gm/dl; Uric acid: 3.5 – 7.2 mg/dl.

Albumin is the most abundant plasma protein in humans. It accounts for 55 – 60% of total serum proteins. It is synthesized in the liver. Owing to the presence of abundance of sulfhydryl (-SH) and free thiol groups in the albumin molecule; it posseses antioxidant properties.[25,26] Albumin can provide ten times more antioxidant protection against various ROS than other plasma proteins and protect the body against ROS. Albumin helps in the scavenging action of already produced free radicals and inhibits the production of free radicals with the help of polymorphonuclear leukocytes. In the present study Serum Albumin Levels statistically decreased from **4.70 g/dl** in healthy control to **3.01 g/dl** in Betel Quid & Gutkha chewers without OSMF to **2.30 g/dl** in patient's Betel Quid & Gutkha chewers with OSMF. Our result is in consistent with various authors like **Nayyar A et al. 2012 and Shrestha R et al. 2012;[8,9]** found the level of serum albumin to decrease in premalignancy as compared to health individuals. Low serum albumin may be due partly to the effect

of cytokines such as interleukin-6 (IL6) and tumor necrosis factor (TNF). These infl ammation mediators which are produced by both tumor and host cells, act both by increasing the local trans-capillary escape of albumin in the tumor bed and by decreasing the hepatic synthesis of albumin.[25]

Total serum protein is the combination of albumin and globulin. In the present study Serum Total Protein Levels decrease from **7.42 g/dl** in healthy control to **6.10 g/dl** in Betel Quid & Gutkha chewers without OSMF to **5.83 g/dl** in Betel Quid & Gutkha chewers with OSMF. The difference was not found to be statistically significant. The result is in accordance with various other authors like Change Be et al. 2001,[1] Nayyar A et al. 2012;[25] and Hans et al. 2006,[30] found serum total protein level to decrease in premalignancy as compared to healthy individuals. One of the reasons for decrease in total protein is decrease in serum albumin level and other reason is tobacco effect on liver which results in decrease in synthesis of protein and ultimately total protein level decreases.

Uric acid the next demonstrated important antioxidant and a free radical scavenger in humans is one of the major radical trapping antioxidants in plasma and is reported to protect the erythrocyte membrane against lipid peroxidation.[3] Over half the antioxidant capacity of human blood plasma is derived from Uric Acid. Thus uric acid could be expected to protect against oxidative stresses.[6] In the present study Serum Uric Acid Levels statistically decreased from **6.05 mg/dl** in healthy control to **3.06mg/dl** in Betel Quid & Gutkha chewers without OSMF to **2.7mg/dl** in Betel Quid & Gutkha chewers with OSMF. This result is in accordance with Mazza A et al. 2001,[33] Lawal AO et al. 2012,[34]. Tumor necrosis factor and interleukins cause decrease in appetite of the patient which results into decreased intake of nutritious food which results in decreased level of uric acid in the body because major portion of uric acid in the body is derived from the diet. Malnutriton is associated with number of clinical consequences including risk of chemotherapy induced toxicity and a reduction in cancer survival.[3,4]

## Conclusion

Decrease in level of these important antioxidants in serum can be considered as an important event by which oxidative stress can cause toxic effects on antioxidant defense system in our body and initiate precancerous transformation and other oral diseases. Results of our study will not only be useful in understanding the pathogenesis of OSMF but it also focuses on antioxidant therapeutic approaches and in treatment of OSMF. However there is need for more studies to be conducted in this regard and to accept the utility of the antioxidants such as serum albumin, total protein and uric acid in providing a scientific ground for their use in monitoring the disease activity and use as prognostic marker.

**Ethical Clearance-** Taken from Institutional ethical committee

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# The Differential Pattern in Skeletal-Dental Age and Duration of Growth Spurt based on Chronological Age and Gender Types (A Comparison Study Between Indonesian and Malaysian Children Populations)

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## Abstract

**Context:** Growth and development is a continuous process that occurs intrauterine and continues until adulthood. In the process of reaching adulthood, children must go through various stages of growth and development, Growth is influenced by two main factors, namely internal (genetic) factors and external (environmental) factors. Internal factors include gender, obstetrics and race or ethnicity. Based on the background pattern above, the researcher wants to compare the difference in the duration of growth spurt between the Malaysian and Indonesian populations in terms of the Cervical Vertebrae Maturation (CVM) seen on the lateral cefalogram

**Aims:** to compare the differences in the duration of growth spurt between girl and boy in Indonesian and Malaysia Population based on Cervical Vertebrae Maturation (CVM) seen in the lateral cephalogram to determine the right time and orthodontic treatment plan in order to get maximum treatment results.

**Methods and Material:** The study was conducted in June 2020. The research variables were divided into 3 types, namely independent variables (chronological age), dependent variables (skeletal age and growth spurt duration) and controlled variables (Indonesian population children, Gender, CVM CS3 - CS4). Every sample that met the criteria was performed skeletal maturity analysis using Cervical Vertebrae Maturation.

**Results:** The boy sample required a duration of age to reach maturity, 17.93 months in Indonesia and 17.91 months in Malaysia. Meanwhile, the girs sample only required the duration of growth spurt, namely 6.59 months in Indonesia and 6.64 months in Malaysia

**Conclusion:** There is no significant difference between the duration of growth spurt in Indonesian Boy and Malaysian Boy and so does the duration of growth spurt for Indonesian and Malaysian girl.

***Keywords*** *: Cervical vertebrae maturation; Growth Spurt; Indonesian; Malaysian*

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## Introduction

The period of growth and development is a period of various changes, including in the oral cavity. Evidence of growth and development is the process

of replacing primary teeth with permanent teeth. Growth and development is a continuous process that occurs intrauterine and continues until adulthood. In the process of reaching adulthood, children must go through various stages of growth and development, including the adolescent stage. The adolescent stage is a transitional period between childhood and adulthood which begins with the maturation of the physical (sexual) organs so that they are able to reproduce, secondary sex characteristics arise, increased height and weight, skeletal growth accompanied by an increase in bone mass, changes in body composition, and psychological and cognitive changes [1]

The right time to treat class II malocclusion is when the growth spurt occurs and class III malocclusion treatment occurs before and during the growth spurt[2]The peak of growth (growth spurt) is the time of the fastest growth followed by slower growth[14]. All children will go through a growth spurt in early adolescence which is clearly visible with changes in height and weight[3].The occurrence of growth spurt depends on sex and varies with each chronological age. This variation determines the speed as well as the duration of the growth process. Growth spurt in women occurs at the age of 10 to 12 years, while in men aged 12 to 14 years[4]. pubertal growth spurt in women on average occurs at the age of 12 years, while in men at the age of 14 years[5]. Knowledge of when this growth spurt occurs can determine a person's morphological and dimensional end, namely by utilizing their growth potential and maturity[2]

Growth is influenced by two main factors, namely internal (genetic) factors and external (environmental) factors. Internal factors include gender, obstetrics and race or ethnicity. If these factors can interact in a good and optimal environment, it will result in optimal growth as well.

Socio-economic factors that affect children's growth include: education, employment, technology, culture and family income. These factors will interact with one another so that it can affect the intake of nutrients and infection in children. The availability of nutrients at a low cellular level which in turn will result in disrupted growth[4].

Based on the background pattern above, the researcher wants to compare the difference in the duration of growth spurt between the Malaysian and Indonesian populations in terms of the Cervical Vertebrae Maturation (CVM) seen on the lateral cefalogram. This information is important for determining the right time and orthodontic treatment plan in order to get maximum treatment results.

## Subjects and Methods

This research was conducted as an observational analytic study with a cross-sectional study design. The sample used in this study was the patient of RSGM FKG UNAIR (Universitas Airlangga Dental Hospital), Surabaya-Indonesia, and Universiti Sains Islam Malaysia (USIM), Nilai-Malaysia- . Sample data was collected by inclusively selected in children criteria as follow: aged 8-18 years, had never performed orthondontic treatment, had no history of systemic diseases that interfere with growth and bone and tooth development, have never experienced facial trauma or surgery on facial structures, have no congenital tooth abnormalities, do not have growth syndromes or anomalies from facial structures, are Javanese and are willing to do cephalometric photographs.

The sample used refers to the formula is 60.[4] This research was conducted at the Dental Hospital Airlangga University and USIM dental hospital. The study was conducted in June 2020. The research variables were divided into 3 types, namely independent variables (chronological age), dependent variables (skeletal age, tooth age and growth spurt duration) and controlled variables (Indonesian population children, Gender, CVM CS3 - CS4). Every sample that met the criteria was performed skeletal maturity analysis using Cervical Vertebrae Maturation (CVM. Skeletal analysis with Cervical Vertebrae Maturation (CVM) by looking at bone maturation and defined into six categories ranging

from CS1 to CS6. Cervical Vertebrae Maturation (CVM) analysis in the CS3 and CS4 phases for chronological age was also performed. Every data that has been analyzed is recorded and grouped according to chronological age and calculates the average based on gender and race. It was also conducted to calculate the interval between groups CS3 and CS4 in each sex and race. Furthermore, to analyze differences in children growth and development patterns using those indicators between the study samples in the two countries, a discriminant analysis was carried out using SPSS version 22.

## Results

For the entire sample and each individual group, a linear discriminant function analysis was used to discriminate and display which variables allow for the best classification between the groups. Wilks' Lambda analysis was used to identify the contribution of each variable and its significance in discriminating each group and the overall sample size. A canonical correlation analysis was then completed to determine the relationship among the variables and the discriminant function analysis. A test for normality was also conducted to determine which variables were normally distributed throughout the groups.

Each variable listed as indicators such: independent variables (chronological age), dependent variables (skeletal age, tooth age and growth spurt duration) and controlled variables (Indonesian population children, Gender, CVM CS3 - CS4) was tested for normality of data. All variables were within normal ($p>.05$) limits. All variables resulted in a 95% confidence interval with mean values falling between the upper and lower bounds. Here are the distribution analysis:

**Table 1. Average age of children (overall) in Indonesia and Malaysia based on cervical vertebra maturity level.**

| Country | Cervical vertebra maturity level (CVMS) | Mean Age ± SD | n | % |
|---|---|---|---|---|
| Indonesia | CVMS 3 | 127,40 ± 19,175 | 48 | 51,62 % |
| | CVMS 4 | 139,18 ± 25,693 | 45 | 48,38 % |
| Malaysia | CVMS 3 | 173,29 ± 24,395 | 34 | 56,66 % |
| | CVMS 4 | 185,08 ±25,887 | 26 | 43,34 % |

The peak of growth (growth spurt) is the time of the fastest growth followed by slower growth[14]. All children will go through a growth spurt in early adolescence which is clearly visible with changes in height and weight[2]. The occurrence of growth spurt depends on sex and varies with each chronological age. This variation determines the speed as well as the duration of the growth process. Growth spurt in women occurs at the age of 10 to 12 years, while in men aged 12 to 14 years. Pubertal growth spurt in women on average occurs at the age of 12 years, while in men at the age of 14 years[14]. Knowledge of when this growth spurt occurs can determine a person's morphological and dimensional end, namely by utilizing their growth potential and maturity[13]. This information is important to determine the right time and orthodontic treatment plan for children in order to get maximum treatment results.

Referring to the results of these previous studies, it is illustrated that in general, the duration of growth spurt in men tends to take longer than women. In this study, this tendency also has the same pattern, namely the duration of growth spurt in boys has a longer period when compared to girls.

**Table 2. Average age of children (boys and girls) in Indonesia and Malaysia based on the maturity level of the cervical vertebrae.**

| Country | Cervical vertebra maturity level (CVMS) | Gender | Mean Age ± SD | n | % |
|---|---|---|---|---|---|
| Indonesia | CVMS 3 | Boys | 134,36 ± 18,303 | 25 | 52,08 % |
| | | Girls | 119,83 ± 17,466 | 23 | 48,92 % |
| | CVMS 4 | Boys | 140,95 ± 23,230 | 20 | 44,44 % |
| | | Girls | 137,76 ± 27,899 | 25 | 55,56 % |
| Malaysia | CVMS 3 | Boys | 170,82 ± 27,992 | 17 | 50 % |
| | | Girls | 175,76 ± 20,759 | 17 | 50 % |
| | CVMS 4 | Boys | 188,73 ± 30,401 | 11 | 42,31 % |
| | | Girls | 182,40 ± 22,768 | 15 | 57,69 % |

**Table 3. One Sample Kolmogorov Smirnov Test Results (Normality) Age between boys and girls in both countries**

| Country | Gender | n | Nilai p (Sig.) |
|---|---|---|---|
| Indonesia | Boys | 45 | 0,066 |
| | Girls | 48 | |
| Malaysia | Boys | 28 | |
| | Girls | 32 | |

**Table 4. Mean increased value in duration of growth spurt (CVMS) between boys and girls in both countries**

| Country | Cervical vertebra maturity level (CVMS) | Gender | Mean Age ± SD | n | % | p value |
|---|---|---|---|---|---|---|
| Indonesia | CVMS 3 – CVMS 4 | Boys | 17,93 ± 10,433 | 45 | 48,38 % | 0.860 |
| Malaysia | | | 17.91± 2,409 | 48 | 51,62 % | |
| Indonesia | | Girls | 6,59 ± 4,93 | 28 | 46,66 % | 0.583 |
| Malaysia | | | 6,64 ± 2,009 | 32 | 53,34 % | |

**Table 5. Description of the difference in the duration of cervical vertebral maturation (CVMS) of boys between the two countries**

| Country | Cervical vertebra maturity level (CVMS) | Gender | Mean Age ± SD | n | % | P Value |
|---|---|---|---|---|---|---|
| Indonesia | CVMS 3 | Boys | 134,36 ± 18,303 | 25 | 55,56 | ,000 |
| Malaysia | | | 170,82 ± 27,992 | 17 | 60,71 | |
| Indonesia | CVMS 4 | | 141,26 ± 23,823 | 20 | 44,44 | ,000 |
| Malaysia | | | 188,73 ± 30,401 | 11 | 39,29 | |

In the cervical vertebrae maturation duration status (CVMS) with a CVMS level of 3, the cases analyzed were 40 respondents. 23 respondents were recorded as data for boys from Indonesia and 17 respondents were recorded as data for boys from Malaysia. The mean duration of cervical vertebrae maturation (CVMS) in the MAS - Boys (Malaysia) variable was 170.82, while the INA - Boys (Indonesia) variable was 119.83. In the cervical vertebrae maturation duration status (CVMS) with a CVMS level of 4, the cases analyzed were 36 respondents. 25 respondents were recorded as data for boys from Indonesia and 11 respondents were recorded as data for boys from Malaysia. The mean duration of cervical vertebrae maturation (CVMS) in the MAS - Boys (Malaysia) variable was 188.73, while the INA - Boys (Indonesia) variable group was at 137.76. The significance value of the mean duration of cervical vertebrae maturation (CVMS) 3 and 4 in the samples in the two countries above is at a sig <0.05 (0.000).

## Discussion

The growth and development period is the period in which various changes occur, including in the oral cavity. Evidence of growth and development is the process of replacing deciduous teeth with permanent teeth. Growth and development is a continuous process that occurs from intrauterine and continues into adulthood. Human growth consists of periods of accelerated growth where accelerated growth is followed by periods of slower growth. The time of accelerated puberty growth and the maximum speed of growth during this developmental phase differ in boys and girls, and also vary at each chronological age. Identification of periods of individual growth acceleration is important in orthodontic treatment because growth modification is best achieved during periods of accelerated adolescent (pubertal) growth.

Growth and development can be assessed through several parameters such as chronilogical age, increase in body weight and height, characteristics of sexual maturation, tooth maturation, skeletal maturation. Chronological age is the clearest and easiest method of calculating developmental age which can be seen through the child's birth date, but is not an accurate indicator of developmental levels. Skeletal age is determined by looking at skeletal maturity which refers to the level of development of ossification in the bone. The CVMS (Cervical Vertebrae Maturation Stage) method has proven to be clinically effective can be used to assess the growth of the mandible during a child's growth. CVMS is an assessment of the level of cervical vertebrae maturation based on the shape and size of the 2, 3, and 4 cervical vertebrae. Indicators used in cervical vertebral radiographic analysis can be seen from the hollowness of the lower edge of the corpus, corpus height and shape of the cervical vertebra.

The Malay race is the largest racial group found in Indonesia as well as Malaysia. This group is divided into the Old Malay race (Proto Melayu) and the Young Malay race (Deutero Melayu). Based on this racial pattern, the population of Malaysia and Indonesia were further defined into the same sub-race, namely the Malayan-Mongoloid sub-race. This sub-race has physical characteristics such as straight to wavy black hair, large eyes and olive to brownish skin. Its distribution includes Malaysia, Singapore, Indonesia, Brunei Darussalam and the Philippines. Race is one of the factors that can affect growth, this can be seen from the different facial types between the existing races. The same racial group will show the same craniofacial growth patterns so that they have a tendency to have similar skull and jaw shape patterns, although this kind of pattern is also influenced by individual variation[1].

Patterns of growth and development of children are reflected in height and can vary between races, sexes and ages. The variation in height between races can be seen from, for example, differences in the mean height of the Caucasians and Mongoloid races[2]. This can be due to genetic variation between races. In this study, the races that were the research subjects in both countries (Malaysia and Indonesia) came from the same race, namely the Mongoloid race (Deutero-Malay), thus implying that there are similarities in the growth and development patterns experienced by children in both countries[4]

On the other hand, differences in the geographic location of a country could theoretically affect this variation. Differences in growth and development patterns can also vary according to gender because they are influenced by the presence of sex hormones. After puberty, androgens and estrogens also play a role in growth. This could explain why the growth spurt in adolescent girls occurs earlier than in boys. Differences according to age can be caused because height growth occurs linearly from bottom to top, therefore height increase will be directly proportional to age increase. The growth rate of each age group varies. This is due to a growth spurt at certain ages[1]. This study will discuss whether the interaction of confounding variables such as geographic differences in countries and the influence of sex hormones

on different sexes is able to show differences or similarities in child development patterns in terms of growth spurt speed seen from The CVM analysis.

Recently, a series of investigations performed in different parts of the world have confirmed the validity of the cervical vertebral maturation (CVM) method, mostly by comparing it with the hand and wrist method[11]The CVM hgs proved to be effective to assess the adolescent growth peak in both body height and mandibular. The ossification events in the cervical vertebrae begin during fetal life and continue until adulthood. Therefore, maturational changes can be observed in the vertebrae during this interval, which covers the period when orthodontic treatment is typically performed in the growing patient.

From Table 5 data, it is concluded that the mean duration of cervical vertebrae maturation (CVMS) level 3 in the MAS - Boys (Malaysia) variable is higher than the INA - Boys (Indonesia) variable group. Furthermore, the mean duration of cervical vertebrae maturation (CVMS) level 4 in the MAS - Boys (Malaysia) variable is also higher than the INA - Boys (Indonesia) variable group. According to the comparison analysis, there is a significant difference in the mean duration of cervical vertebrae maturation (CVMS) 3 and 4 between the variable MAS - Boys (Malaysia) and the variable INA - Boys (Indonesia). Due to the significance value of the Wilk's Lambda Test by 0.000 (<0.05), it can be concluded that in each country, boys have significantly different cervical vertebral maturation (CVMS) duration for boys, both at CVMS level 3 or 4.

According to the published data, mean chronological age at menarche for Tehranian females is reported from 12 years to 12.6 years.Therefore, it can be concluded that the peak of the growth spurt occurs at 11-11.5 years of age in named population. The mean chronological age at pre-pubertal stages (CS3 and CS4), in this study, was 11.48 years, which is very close to calculated age of the growth spurt. In other words, the time interval between the age at peak skeletal growth and the average age of the first menstruation is about 6-12 months in these subjects that seem logical.[6]

The growth to adult shape and size is controlled by more than one gene, each gene having small effect. It is difficult to spot any single gene that determine morphological characteristic. For example, the genetic factors influencing bone mass will be different from the genetic factors that control skeletal growth, which all contributes to the child's weight.

From table 4, we could tell that duration of growth spurt between Indonesian and Malaysia on boys and girls, there are no significant differences. It causes, Malaysia and Indonesia has same race, Melanesia. Indonesia and Malaysia are two countries not only because of location geographic as a neighbor but has a diversity of cultures that almost the same. The one that differs Indonesia and Malaysia, that Malaysia has cultural diversity supported by skills that can drive the communication process effective. This cannot be separated from the role ethnic Malaysian origin, namely Ethnic Malays. In Malaysia, there are many cultural like chineese, indian, ethnic malays itself. From this data, we thought the data was taken from ethnic Malay because ethnic malays has many similarities with Indonesian people.

**Ethical Clearance**- Taken from faculty of dental medicine airlangga university committee

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**Conflict of Interest** - Niil

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*Original Article*

# Effects of Motivational Interviewing on the Self-Efficacy of Type 2 Diabetes Mellitus Patients

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## Abstract

Diabetes mellitus is a metabolic disease that cannot be fully cured, thereby needing continuous treatment. As poor lifestyles increase in number and variation, the prevalence of diabetes mellitus is increasing every year. The purpose of this study is to determine the effects of motivational interviewing on the self-efficacy of type 2 diabetes mellitus patients. This study is an experimental study using Non-Randomized Control Group Pretest Posttest Design. The population was type 2 diabetes mellitus patients receiving treatment at Polres Tuban Polyclinic. Sample collection was performed purposively with a sample size of 58 patients. This study utilized univariate and bivariate analyses using the t-test for two dependent means. The results of statistical analyses showed the effects of motivational interviewing on the self-efficacy of type 2 diabetes mellitus patients in which there was an increase in the mean score of the intervention group from 27.3 to 36.8 ($p$=0.000). We concluded that motivational interviewing has an effect on improving the self-efficacy of type 2 diabetes mellitus patients.

***Keywords:*** *diabetes mellitus, motivational interviewing, self-efficacy*

## Introduction

Diabetes mellitus (DM) is a disease where the level of blood sugar exceeds the normal threshold. This is linked to lifestyles such as poor diet and activity habits. The risk is elevated in individuals with hereditary diabetes mellitus, uncontrolled hypertension, lack of exercise and activity and poor diet and an unhealthy lifestyle[1]. It is currently the 6th deadliest disease in the world. In 2015, 1.58 million deaths are caused directly by diabetes (2.8% of all deaths in the world) and annually as many as 1.6 million deaths are directly attributed to diabetes mellitus[2]

WHO (2016) in 2015 revealed that Indonesia ranked seventh for country with the highest prevalence of diabetes mellitus after China, India, United States, Brazil, Russia, and Mexico[3]. Diabetes with complications is the third leading cause of death in Indonesia. 2013 Data of the Basic Health Research showed a high prevalence of diabetes mellitus in Indonesia, increased from 1.1% in 2007 to 2.4% in 2013 of the total population of 250 million[4].

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Type 2 DM is a chronic disease that cannot be fully cured, leading to increasing prevalence every year[5]. Study by Dante et al. (2020) expressed that without proper treatment, diabetes patients in general have worse quality of life than non-patients due to the risk of complications[6].

Diabetes treatment depends not only on how patients manage their health but also on support from health workers who provide diabetes management education about lifestyle changes in managing the disease[7]. The core purpose of the treatment is not to cure the disease but to improve patient's functional status, lessen symptoms of complications, prolong life through secondary prevention and improve quality of life[8]. Self-efficacy requires further study because by understanding self-efficacy, one can assist health workers to guide the determination of intervention in accordance with patient conditions[9].

The management of DM into 4 primary components namely education, nutritional therapy, physical activity, and pharmacological interventions[10]. One intervention to help transform patient behaviors by taking advantage of interpersonal relationship is motivational interviewing[11]. There is a difference between the group given motivational interviewing and the control group, where a significant difference of $p < 0.05$ was found for HbA1C measure, that is, the intervention is able to affect the quality of life of DM patients. Women with type 2 diabetes mellitus showed that medical nutritional intervention with motivational interviewing approach is proven to improve glycemic control and self-confidence of respondents[12].

The number of DM patients at the Polres Tuban Polyclinic, severe burden caused by DM and its complications, its effects to patient's quality of life, and the lack of studies focusing on interventions through counseling with the motivational interview approach combined with physical activity encourage the researchers to investigate the effects of motivational interviewing on the self-efficacy of type 2 diabetes mellitus patients in the region.

## Result and Method

*Location and Research Design*

The study was conducted at the Polres Tuban Polyclinic using Quasi Experimental design with Non-Randomized Control Group Pretest Posttest from March 1 to April 1, 2021.

*Population and Sample*

The population was all type 2 diabetes mellitus patients receiving treatment at the Polres Tuban Polyclinic in 2021. The sample size was 58 patients selected using purposive sampling technique.

*Data Collection Method*

Data collection began with secondary data which were type 2 DM patient data recorded in the medical records of the Polres Tuban Polyclinic. During the administration, data were obtained through direct interviews with the respondents during pretest and posttest using the GSE (General Self-Efficacy) questionnaire which has been tested for validity and reliability on the questionnaire in Indonesian. During the intervention stage, respondents in the intervention group were given motivational interviewing counseling by the counselor while the control group were be given a module containing the life guide of DM patients.

*Data Analysis*

Data analyses were performed univariately and bivariately. Univariate analysis was used to identify respondent characteristics presented in the table. Bivariate analysis was used to describe the differences between independent and dependent variables using Wilcoxon test with a level of significance (alpha-α) 5%. To determine the difference between mean values of two paired groups (two samples), manwithney test was performed.

## Results

*Respondent Characteristics*

Table 1 shows respondent characteristics based on age group, gender, employment, marital status, family history and routine blood checks. For age group, most respondents belong to age groups 51-60 years and 41-50 years, where 17 (58.6%) respondents from the intervention group belong to both age groups and 14 (48.2%) from the control group belong to both age groups. Of the total 29 respondents in the intervention group, 80% (24 respondents) of them are women, while 63% (19 respondents) of the respondents in the control group are women. In terms of employment, most of the respondents in the intervention and control groups are housewives with the proportions of 60% (18 people) and 46.7% (14 people), respectively. Based family history of DM, 33.3% (10 people) in the intervention group and 30% (9 people) in the control group have a history of DM. According to the status of routine blood sugar checks, 3.3% (1 person) of the intervention group respondents said they did not routinely check their blood sugar at the health care center, while in the control group, 23.3% of the respondents stated the same

**Table 1 Characteristics of Respondents**

| Characteristics of Respondents | Intervention Group | | Control Group | |
|---|---|---|---|---|
| | Frequency | % | Frequency | % |
| Age | | | | |
| 30-40 | 1 | 3.44 | 1 | 3.44 |
| 41-50 | 9 | 31.03 | 5 | 17.24 |
| 51-60 | 17 | 58.62 | 14 | 48.27 |
| 61-70 | 2 | 6.89 | 9 | 31.03 |
| Gender | | | | |
| Male | 6 | 20.68 | 11 | 37.93 |
| Female | 23 | 79.31 | 18 | 62.06 |
| Work | | | | |
| Civil servants | 3 | 10.34 | 1 | 3.44 |
| Private employees | 1 | 3.44 | 1 | 3.44 |
| Self employed | 3 | 10.34 | 5 | 17.24 |
| Farmer | 4 | 13.79 | 8 | 27.58 |
| Housewife | 18 | 62.06 | 14 | 48.27 |
| Family history of diabetes mellitus | | | | |
| Yes | 10 | 34.48 | 9 | 31.03 |
| No | 19 | 65.51 | 20 | 68.96 |
| Routine blood check | | | | |
| Yes | 28 | 96.55 | 22 | 75.86 |
| No | 1 | 3.44 | 7 | 24.13 |

Table 2 shows respondent distribution based on statistical values which are the minimum and maximum values of pretest and posttest scores, the average value (mean) and standard deviation. The mean value in the self-efficacy domain for the intervention group during the pretest was 27.3 with a standard deviation of 1.16, rising to 36.8 with a standard deviation of 1.28. The lowest score during the pretest was 24 and the highest was 29, while the lowest score during the posttest was 34 and the highest was 39. The control group's result shows that the mean value of the self-efficacy domain during the pretest was 28.5 with a standard deviation of 1.27, dropping to 28.3 with a standard deviation of 0.82. The lowest score during the pretest was 27 and the highest was 32, while the lowest score during the posttest was 26 and the highest was 30.

**Table 2 Distribution of respondents by self-efficacy statistical value**

| Statistical value | Pretest | Posttest |
|---|---|---|
| Intervention group | | |
| Minimum | 24 | 34 |
| Maximum | 29 | 39 |
| Mean | 27.3 | 36.8 |
| Standard deviation | 1.16 | 1.28 |
| Control group | | |
| Minimum | 27 | 26 |
| Maximum | 32 | 30 |
| Mean | 28.5 | 38.3 |
| Standard deviation | 1.27 | 0.82 |

Table 3 shows the difference in means for the pretest and posttest of the self-efficacy domain for both groups. There was a difference between the two groups, where the mean value for the intervention group increased from 27.3 at the pretest to 36.8 at the posttest. While the control group decreased from 28.5 at the pretest to 28.3 at the posttest. The results of statistical tests show a $p$ value of 0.000 ($p < 0.05$) for the intervention group, meaning that that there is a difference in the respondents' self-efficacy values before and after being given motivational interviewing. For the control group, the statistical test shows a $p$ value of 0.368 ($p > 0.05$), meaning that there is no difference in self-efficacy values during the pretest and posttest. The difference in the mean self-efficacy scores between the intervention and control groups at the posttest was 8.5.

## Discussion

Our results show an increase in the mean score of the self-efficacy variable at the pretest and posttest for the intervention group after being given motivational interviewing. The statistical test shows $p$ value < 0.05, demonstrating a significance difference of 8.5 in the

mean value of self-efficacy between the intervention and control groups, leading to a conclusion that motivational interviewing has an effect on the self-efficacy of type 2 DM patients.

This self-efficacy is related to individual probability to lead a healthy lifestyle. People who lack conviction that they can practice a behavior supportive of their health will tend to be reluctant to make an attempt[13]. The essential behavior for diabetes patients is medication adherence, where it is very closely related to the willingness and belief of the patient themselves. Self-efficacy varies from situation to situation depending on the competencies required for different activities and the presence of other people[14].

Our results are in line with the study by Renate et al. (2013) on type 2 diabetes mellitus patients in Netherlands, demonstrating that the experimental group who was given motivational interviewing through motivational and cognitive enhancement therapy showed a significant improvement in terms of quality of life physically and mentally compared to the control group that displayed no improvement throughout the research[14].

Study by Fathi (2018) in Sudan found a positive significant relationship between physical activities and the score of Health-Related Quality of Life on the dimensions of physical function, vitality and general health of type 2 DM patients[15]. However, these results are not in line with the study by Catherine (2014) in Toronto that found no significant intervention based a web to support self management, the self efficacy score did not improve (p value = 0.263)[9]. Furthermore, a quasi-experiment by Hasan et al (2021) on diabetes mellitus patients obtained similar significant the intervention group given a counselling the self efficacy score is 91 ($p<0.05$)[16].

Chen found that counselling positively affects domain self management, glycemics outcomes and psychological of type 2 DM patients where a significant mean the intervention groups was found with p value = 0.001 ($p < 0.05$)[17]. Li (2014) also found intervention through motivational interviewing to be effective in improving self-management (including activity, diet and medication aspects) and glycemic control in type 2 diabetes mellitus patients[18].

Study by Song et al. (2014) on diabetic patients aged >18 year in Chinese revealed that scores in all dimensions of Health-Related Quality of Life (HRQoL) were significantly higher ($p < 0.05$) in the moderate and high physical activity group compared to the love physical activity group, and also a significant correlation between the five dimensions in HRQoL and physical activity level ($p < 0.001$)[19]. The experimental study by Kamal et al. (2017) on 50 group intervention namely motivational interviewing arm the score was significant p=0.0001 patients with type 2 diabetes overweight and obesity to be effective in increasing weight[20].

## Conclusion

There is an increase in the self-efficacy of type 2 DM patients at the Polres Tuban Polyclinic (intervention group) before and after the administration of the motivational interviewing program. Consistency is the key for type 2 DM patients in carrying out physical activities (exercise) routinely and continuously along with innovations in the management of DM patients through effective counseling. The polyclinic needs to develop a program for applying the intervention method of DM management through the motivational interviewing approach which could facilitate the internalization of self-management and independence for better quality of life.

**Ethical Clearance :** Ethical clearance was taken from Faculty Medicine of Brawijaya University No.51/EC/KEPK-S2/02/2021

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# Is there a Relationship between the Characteristics and Attitudes of Adolescents with Premarital Sex?

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## Abstract

**Background**: Premarital sex is one of the high-risk behaviors of adolescents today. The characteristics of adolescents can also be a triggering factor for premarital sex. The purpose of this study was to analyze the relationship between characteristics and attitudes of adolescents towards premarital sex in East Java Province.

**Methods**: This is a quantitative research with a cross sectional research design. The data used were taken from Program Performance and Accountability Survey (2018) on adolescents aged 15-24 years in East Java Province. The sample of this study was 2796 adolescents after normal weight adolescents were carried out. The dependent variable in this study is the attitude of adolescents, while the independent variables are place of residence, age, gender, education, and economic status. Data collection using smartphones and instruments that refer to the IDHS and PMA. The data were analyzed descriptively and tested using chi-square with a significance level of 0.05.

**Result and Conclusion**: The results showed that the characteristics associated with the attitude of female adolescent was the place of residence with a significance value of 0.026. Age was related to the attitude of male adolescent with a significance value of 0.014. Education was related to the attitude of female adolescent (p-vaue = 0.000) and attitudes of male adolescent (p-value = 0.016). Economic status was related to the attitude of female adolescent (p-value = 0.000) and attitudes of male adolescents (p-value = 0.035). Characteristics had a relationship with adolescent attitudes in conducting pre-primary sex behavior. Therefore, it is necessary to have appropriate efforts to prevent adolescent risk behavior in accordance with the characteristics and needs of today's youth

***Keywords****: Adolescent, attitude, premarital seks, characteristic, good health, well-being*

## Introduction

The World Health Organization (WHO) defines adolescents based on 3 (three) criteria, namely: biological, psychological, and socio-economic. Adolescence in terms of biological conditions is a period when an individual develops from the first time he shows secondary sexual signs until he reaches sexual maturity. Adolescents are viewed psychologically as the period when individuals experience psychological development and identification patterns from childhood to adulthood. Adolescents are viewed from the aspect of socio-economic conditions during the

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transition period to become more independent from socio-economic dependence.

Adolescence is a unique period and full of various transitional periods. This transitional condition often causes various problems in adolescents. Adolescence is a period of transition in which physical and psychological changes from childhood to adulthood [1]. Psychological changes that occur in adolescents include intellectual, emotional life, and social life. Physical changes include sexual organs, namely the reproductive organs have reached maturity and are starting to function properly[2]. All aspects of development in adolescence globally take place between the ages of 12-21 years, with the age division of 12-15 years being early adolescence, 15-18 years being middle adolescence, 18-21 years being late adolescence[3].In his teenage years his curiosity about all things is very big. Including sexuality. Desire to try new things and curiosity about sexuality as adults do[4].

In the current era of 4.0, the ease of access to information and developing technology makes it easier for teenagers to find information. Various risky behaviors carried out by adolescents reduce the health status of adolescents. Whether we realize it or not, the increase in risky behavior among adolescents causes the emergence of various diseases. Since 2013 the prevalence of smoking among adolescents (10-18 years) has continued to increase, namely7,2% [5], 8,8%[6]dan 9,1% [7].In addition, the problem of early marriage is also one of the concerns of adolescent reproductive health problems. Unicef released data that Indonesia ranks seventh for early marriages that occur at the age of less than 17 years. Based on statistical data from BPS, it is stated that the percentage of East Java women aged 10 years and over who marry underage (less than 17 years), in 2014 it was 27.11%, in 2015 it was 8.99% and in 2016 it was 21.16%.

According to the results of the 2017 IDHS Survey, it can be seen that 59% of girls received lessons on reproductive health, 55% of boys had knowledge about HIV and Aids, 48% of girls and 46% boys got knowledge about HIV. And they first got information about reproductive health at junior high school age [8]. The condition of information about sexuality without assistance from parents will have a bad impact. They can access pornography so that it has great potential for sexual behavior outside of marriage. This condition is quite worrying considering that such behavior can lead to Unwanted Pregnancy Cases which in turn triggers unsafe abortion practices, transmission of STDs and HIV/AIDS, and even death [9].(IDHS) 2017 revealed that around 2 percent of female adolescents aged 15-24 years and 8 percent of male adolescents in the same age range, had had sexual relations before marriage. As many as 11 percent of them admitted to having an unwanted pregnancy[8]. Premarital sex behavior can be prevented by one of its efforts, namely the formation of positive attitudes of adolescents in assessing premarital sex behavior [10].

According to Eaglyand Chaiken in Albaracin (2005), attitude is the degree of a person's psychological tendency to judge something whether they like it or not. Attitudes are influenced by one's beliefs and behavior in the past as well as affection arising from environmental reactions [11].Many studies stated that one of the factors that influence attitudes is the characteristics of the person. Based on the data and descriptions of reproductive health problems and adolescent risk behavior, the purpose of this study was to analyze the characteristics of adolescents on pre-primary sex behavior.

## Methods

This research is a quantitative with a cross-sectional research design. The population of this study were adolescents aged 15-24 years in East Java Province. The sample of this study was 2796 adolescents after normal weight adolescents were carried out. The data used in the form of secondary data from Program Performance and Accountability Survey (2018) of East Java Province in 2018. The

dependent variable in this study is the attitude of adolescents, while the independent variables are place of residence, age, gender, education, and economic status. Data collection used a smartphone and the use of instruments that refer to the IDHS and PMA. Descriptive data analysis and tested using chi-square with a significance value of 0.05.

## Results and Discussion

**Table 1 Relationship between Adolescents' Sexual Attitudes and Residence, Gender, Age, Education, and Economic Status**

| No. | Characteristic | Girls Attitude against Premarital Sex | | Total | Boys Attitude against Premarital Sex | | Total |
|---|---|---|---|---|---|---|---|
| | | Agree | Disagree | | Agree | Disagree | |
| Based on Living Place Classification | | | | | | | |
| 1 | Urban | 78.3% | 55.1% | 55.2% | 73.1% | 55.1% | 55.2% |
| 2 | Village | 21.7% | 44.9% | 44.8% | 26.9% | 44.9% | 44.8% |
| Total | | 100% | 100% | 100% | 100% | 100% | 100% |
| Sig | | 0.026 | | | 0.066 | | |
| Based on Sex | | | | | | | |
| 1 | Male | 73.9% | 58.1% | 58.3% | 76.9% | 58.1% | 58.3% |
| 2 | Female | 26.1% | 41.9% | 41.7% | 23.1% | 41.9% | 41.7% |
| Total | | 100% | 100% | 100% | 100% | 100% | 100% |
| Sig | | 0.126 | | | 0.052 | | |
| Based on Age (in years) | | | | | | | |
| 1 | 15 | 27.3% | 16% | 16.1% | 0.0% | 16.2% | 16.1% |
| 2 | 16 | 22.7% | 14.3% | 14.3% | 20.8% | 14.3% | 14.3% |
| 3 | 17 | 13.6% | 13.0% | 13.0% | 12.5% | 13.0% | 13.0% |
| 4 | 18 | 22.7% | 13.6% | 13.6% | 25.0% | 13.6% | 13.7% |
| 5 | 19 | 13.6% | 10.2% | 10.2% | 16.7% | 10.1% | 10.2% |
| 6 | 20 | 0.0% | 8.2% | 8.2% | 0.0% | 8.2% | 8.2% |
| 7 | 21 | 0.0% | 8.1% | 8.0% | 0.0% | 8.1% | 8.0% |
| 8 | 22 | 0.0% | 6.6% | 6.6% | 0.0% | 6.6% | 6.6% |
| 9 | 23 | 0.0% | 6.6% | 6.5% | 12.5% | 6.5% | 6.5% |
| 10 | 24 | 0.0% | 3.4% | 3.4% | 12.5% | 3.3% | 3.4% |
| Total | | 100% | 100% | 100% | 100% | 100% | 100% |
| Sig | | 0.233 | | | 0.014 | | |

***Cont...*** **Table 1 Relationship between Adolescents' Sexual Attitudes and Residence, Gender, Age, Education, and Economic Status**

| No. | Characteristic | Girls Attitude against Premarital Sex | | Total | Boys Attitude against Premarital Sex | | Total |
|---|---|---|---|---|---|---|---|
| | | Agree | Disagree | | Agree | Disagree | |
| **Based on Education Level** | | | | | | | |
| 1 | Did not school | 0.0% | 0.1% | 0.1% | 0.0% | 0.1% | 0.1% |
| 2 | Elementary School | 21.7% | 2.3% | 2.4% | 21.7% | 2.4% | 2.4% |
| 3 | JHS | 39.1% | 23.3% | 23.5% | 39.1% | 23.2% | 23.4% |
| 4 | SHS | 39.1% | 60.1% | 59.9% | 39.1% | 60.2% | 59.9% |
| 5 | Diploma | 0.0% | 1.8% | 1.7% | 0.0% | 1.8% | 1.7% |
| 6 | Bachelor | 0.0% | 12.4% | 12.3% | 0.0% | 12.3% | 12.4% |
| Total | | 100% | 100% | 100% | 100% | 100% | 100% |
| Sig | | 0.000 | | | 0.016 | | |
| **Based on Economic Status** | | | | | | | |
| 1 | Lowest | 26.1% | 5.6% | 5.7% | 20.0% | 5.6% | 5.7% |
| 2 | Mid-Low | 34.8% | 15.5% | 15.7% | 12.0% | 15.7% | 15,7% |
| 3 | Mid | 0.0% | 21.1% | 21.0% | 20.0% | 21.0% | 20.9% |
| 4 | Mid-High | 39.1% | 30.8% | 30.9% | 32.0% | 30.9% | 30.9% |
| 5 | Highest | 0.0% | 27.0% | 26.7% | 16.0% | 26.9% | 16.0% |
| Total | | 100% | 100% | 100% | 100% | 100% | 100% |
| Sig | | 0.000 | | | 0.035 | | |

Adolescents' Attitudes towards Premarital Sex and Residence

Based on Table 1, it can be seen that the place of residence is related to the attitude of girls in assessing premarital sex behavior in male and female with a significance value of 0.026. Girls who lived in urban areas tend to have an agreeable attitude if female and male adolescents engage in premarital sex. The results of this study were in line with Umaroh's research (2015) which stated that place of residence has a significant relationship with adolescent premarital sexual behavior, namely adolescents who lived in urban areas tended to be more at risk for premarital sexual behavior 1,340 times compared to adolescents who did not live in urban areas. One form of juvenile delinquency was premarital sex behavior [12].Adolescents' premarital sex behavior can be influenced by lack of affection and attention from parents and inappropriate family conditions [13]. Adolescents who lived in urban areas tended to have busy parents. Parents who paid less attention, were less involved in the maturation of their children and feel proud of their children tended to form children who deviate [14]. Besides that, parents tended to give a negative response to adolescents who ask questions related to sexuality which can cause curious teens to

look for answers on their own in obscure sources. The shift in adolescent attitudes towards premarital sex was currently influenced by western culture that entered Indonesia [15]. This was because adolescents who lived in urban areas were more open to the influence of information and culture from the west. Self-disclosure to western culture if not accompanied by strong self-restraint will result in juvenile delinquency [16].

Adolescents' Attitudes towards Premarital Sex and Gender

According to Table 1, it can be seen that there is no relationship between the attitudes of female and male adolescents in assessing premarital sex behavior. This was in accordance with research conducted by Kiptiyah (2017) which stated that the gender of adolescents did not have a relationship with adolescents' attitudes towards premarital sex [17]. However, this finding was different from the research conducted by Yolanda, (2020) which stated that boys tended to have negative attitudes towards premarital sexual behavior, namely attitudes that support premarital sexual behavior [18]. Thus, it can be said that gender is one of the characteristics of adolescents related to adolescent attitudes towards premarital sex. Males were usually more involved in deviant behavior that was influenced by psychosocial influences, such as limited rational thinking patterns, weak emotional management, and strong peer influence [19]. Public perception of boys and girls was different if both of them have premarital sex. Girls were seen as bad if they have premarital sex, in contrast to boys who have premarital sex. If a man has premarital sex, it will increase his reputation [20]. Research conducted by Ahrold and Meston (2010) stated that there was a shift in sexual attitudes in the adolescent group and there were differences in the attitudes of female and male adolescents. Men have more liberal attitudes towards sex than women [21].

Adolescents' Attitudes towards Premarital Sex and Age

Based on Table 1, it can be seen that there was a relationship between age and male adolescent attitudes in assessing premarital sex behavior in male and female with a significance value of 0.014. Boys tend to have an attitude of agreeing if girls and boys have premarital sex. The results of this study were supported by research by Fauziah (2017) which stated that there was a strong relationship between adolescent age and premarital sexual behavior [22]. According to the Indonesian Ministry of Health (2009), age categorization was divided into 9, namely toddlerhood (0-5 years), childhood (5-11 years), early adolescence (12-16 years), late adolescence (17-25 years), early adult (26-35 years), late adult (36-45 years), early elderly (46-55 years), late elderly (56-65 years), and seniors (65 years and over). Adolescents at the age of 18 fell into the category of late adolescence. In this phase, the accomplishment in regards of self-identity is prominent, the way of thinking is more logical, abstract, and idealistic and more time is spent outside the family [23]. At this time, adolescents experience the development of sexuality: physical and hormonal changes during puberty. The impact of those leads to great social and psychosocial consequences for adolescents. This causes adolescents to look physically mature and more likely to imitate the behavior of adults, including sexual behavior. In the late adolescence phase, their curiosity in attempting new things in life makes them commit deviance, including committing sexual intercourse [24].

In line with Azinar's research (2013) that there is a significant correlation between attitudes and premarital sexual behavior towards risk of Unintended Pregnancy (UP) [25]. This means that adolescents' permissive attitudes towards sexuality is four times greater risk or tendency to engage in sexual behavior at risk of adverse event compared to respondents who are less permissive. The National Family Planning Coordinating Board stated that 40% of those with permissive attitude did not mind dating by hugging each other, 20% did not mind dating by kissing each other, 35% of boys did not need to keep their virginity, 10% of girls did not need to keep their

virginity. Furthermore, 95% of them said chatting is the old style of dating. Meanshile, 60% of adolescents with non-permissive attitude objected to the courtship style holding on to each other [26].

Adolescents' Attitudes towards Premarital Sex and Education

Based on Table 1, it can be seen that there is a correlation between adolescents' education and their attitudes in perceiving premarital sex behavior. This applied in male and female as well. In his research, O'Donnel (2020) discovered that most of the unreported cases of premarital pregnancy and premarital sex occurred among people with low education. [27]. Generally, education affects a person's knowledge. The higher a person's education, the easier it is to perceive information from various sources. The higher a person's education, the better his knowledge. This knowledge has an impact on determining one's attitudes and behavior. A person with higher education is more likely to represents a positive attitude and behavior [28].

Adolescents' Attitudes towards Premarital Sex and Economic Status

Based on Table 1, it can be seen that there is a correlation between economic status and male and female adolescents' attitudes in perceiving premarital sex behavior. Those who are in the upper middle economic status tend to have an agreeable attitude in perceiving their fellow committing premarital sex. Adolescents with upper middle economic status have a sufficient life and can access various facilities or suspicious places. Adolescents with upper middle and upper economic status have higher exposure of premarital sex and have a modern lifestyle. Ease of access to information from various media and lack of parental control can be the cause of teenagers with upper and upper middle economic status committing premarital sex. In contrast to adolescents with low economic status, they are more likely to find it difficult to access information from various media and establish friendly relations with the opposite sex [29]. Research from Cofie (2010) stated that adolescents with upper middle economic status have more opportunities to commit premarital sex compared to those with lower middle economic status [30].

This results analysis differ from research by Anjarwati (2009), which states that adolescents with low economic status tend to commit premarital sex compared to adolescents with high economic status [31]. Low economic status relates to premarital sex behavior in adolescents, particularly among female adolescents. Their low economic status and inability to meet their basic needs are often tempted them to seek financial rewards from young or old men, substituted with sexual rewards. [32] [33]. Therefore, it is imperative to provide educational efforts that are in accordance with the needs of adolescents through the suitable media. This is since many media that has produced remains involve teenagers partially, thus there is a gap between the existing media and the youth needs [34]. Cooperation from across sectors is one of the keys to succeed in organizing youth health programs [35].

## Conclusion

Characteristics correlates with adolescents' attitudes in perceiving premarital sex behavior. Male and female adolescents have different attitudes of premerital sex behavior. They have their respective point of view in perceiving primary sex behavior. Female adolescents are more likely to disapprove the behavior of sex offenders compared to men. There are aspects of adolescent characteristics related to disapproval of premarital sex behavior in female adolescents: place of residence, gender, age, education, and economic status. Furthermore, there are aspects of male adolescents related to disapproval of premarital sex behavior: age, education and economic status. Therefore, it is necessary to prevent premarital sex in accordance with the needs and characteristics of current youth.

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# Inappropriate Use of Antibiotics among Children Under Five in Rural and Urban Communities of Cambodia

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## Abstract

***Background:*** Inappropriate use of antibiotics is a major treat especially in low- and middle-income countries with antibiotic resistant. Cambodia with high prevalence of infectious diseases among children under five, antibiotics use in urban and rural setting has not been clearly identified. Therefore, this study aimed to investigate the situation of inappropriate antibiotic use among children under five in urban and rural communities of Cambodia.

***Methods:*** An analytical cross-sectional study was carried out in both urban and rural communities in Cambodia from September to November 2020 by structured questionnaire interviewed among 512 Cambodian caregivers on antibiotic use for the under 5 children.

***Results:*** Among 512 children, majority were males both in urban (55.6 %) and rural (50.3%) settings, with the median age of 29.5 (3:59) months in urban and 30.0 (3:59) months in rural. The prevalence of inappropriate use of antibiotics among children under five was 79.3% (95% CI = 75.55 to 82.60), of which it was 26.0 % in urban and 53.3% in rural. Most of the rural respondents (82.1%) use antibiotics for bacterial infection diagnosed by doctors. However, it was only 52.6% in rural areas. Similar proportion of 58.6 % in urban and 61.1 % rural used antibiotics for fewer days than prescription.

***Conclusions:*** Inappropriate use of antibiotics were found in more than 3 quarters of children under five, of which the proportion in rural areas were much higher than urban. The further investigation on factors contributing to inappropriate use of antibiotics should be conducted.

***Keywords:*** *Antibiotics, antibiotic resistance, caregivers, children under five, and inappropriate use of antibiotics*

## Introduction

Infectious disease is one among the major reasons driven to great morbidity as well as mortality ([1]). Consequently, this made consumption of antibiotics rose remarkably from 21.1 to 34.8 billion daily dose (DDDs) from 2000 to 2015 ([2]). However, antibiotics were not always prescribed, dispended or used in a rational way ([3, 4]). This inappropriate use of antibiotic contributing to the escalation of global antibiotics resistance as well as antimicrobial resistance ([4-6]). Antibiotic resistance could impact on many aspects such as the augmentation of adverse drug

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reaction (ADR), re-consultations, complications of disease, and increase in healthcare cost ([7]), delay in hospitalizations, the increase in the length of recovery time and death rate in severe infection ([8-11]). Centers for Disease Control and Prevention (CDC) reported that the serious form of antibiotic resistance was developed in more than 2 million people annually, which led to 23,000 deaths with the cost of health care around $20 billion globally ([12]). It has been considered as a global threat.

In most countries about 20 percent of antibiotics were used in healthcare facilities, and 80 percent were used in the community ([13]) of which about 30 % is used inappropriately ([14]) because community colonizes with freely antibiotic selling, widespread of self-medication of antibiotics and other improper forms of antibiotic use. Many findings highlighted the association between outgrowth of antimicrobial resistance with the improper use of antimicrobials in the community setting ([15]).

Importantly, inappropriate use of antibiotics seemed exceptionally grave among the pediatric group of population in the developing countries ([16]). In the similar context, Cambodian children population younger than 5 years old which accounted for 1.8 million ([17]) living in one of the developing countries, was considered as in the risk situation ([18]). In many countries there were significant different of inappropriate use of antibiotics between rural and urban settings. Meanwhile, the extent of inappropriate use of antibiotics in children under five in the urban and rural communities has not yet been studied in Cambodia.

Therefore, this study aimed to investigate the situation of inappropriate antibiotic use among children under five in urban and rural communities in Cambodia.

## Materials and Methods

### *Study design*

This analytic cross-sectional study was carried out in Cambodia from September to November 2020. Data collection was carried out in four urban and eight rural communities covering one capital city and two provinces (Phnom Penh capital city, Kampong Chhnang and Prey Veng Province) in Cambodia. The participants were caregivers of children under five.

### *Study variables*

Dependent variable (outcome) was appropriateness / inappropriate use of antibiotics.

Independent variables consisted of gender of caregivers, age of caregivers, marital status of caregivers, education level of caregivers, occupation of caregivers, sex of children, age of children, family size of children, average monthly income of children's family, financial status of children's family, and relationship between caregivers and children.

Sample size determination and sampling technique

A sample size was calculated by proportion formula with reference from a previous study done in Northwest Ethiopia (19).

5 percent of participants (24) were added original sample size (488) because we spared 5 percent in case of error or incomplete information in some questionnaire. The final sample size was 512 (488 +24).

A multistage sampling method was used to select samples based on the 2014 National Census retrieved from the Ministry of Planning in Cambodia for observing the distribution of children under five. The study area consisted of both urban and rural settings which were stratified into 3 levels of city/ province, district, and commune (community). The study population was caregivers aged from 18 years old and older, lived in the selected study areas, caring, and clearly aware of their children administered of antibiotics at least once in the previous six-month counting from the date of interview backward. Caregivers who critically ill or had communication

problems were excluded from this study.

**Data collection tool**

A face-to-face structured questionnaire interview was conducted among the participants by three well-trained pharmacy students. The questionnaire was pretested by 3 experts for content validity and tested in the actual context of the population on suitable for data collection. The structured questionnaire consisted of three parts as the following (supporting file 1):

Part 1: Demographic and socioeconomic characteristics of caregivers and children with 12 items

Part 2: Practice in antibiotic use with 10 items to measure the outcome. The novel tool was developed by following the definition of inappropriate use of antibiotic of World Health Organization (20).

Operational definition

***Inappropriate use of antibiotics***

According to WHO, the inappropriate use of antibiotics is defined as using in the incorrect purpose/ indication, inappropriate dosage, inappropriate timing, incorrect duration, over-prescription, omission of prescription/self-medication, incorrect selection, incorrect route of administration, requesting antibiotic from health care providers and unnecessary expense (20).

**Data quality management**

Content validity index (CVI) of questionnaire was assessed by 3 experts in pharmacy, medical, health literacy and statistics field. Pretest was tried out in 30 participants outside the study area in order to check the reliability of the tool. Some modifications were made after CVI assessment and pretest. Data collectors were given training and supervised daily by principal investigator to collect quality data. Double data entry validate check and exploratory data analysis were done to check the completeness of data. For the rest of minimal missing data was solved by using listwise data deletion, mean substitution, hot deck imputation, expectation maximization approach and raw maximum likelihood methods or multiple imputation.

**Data analysis procedure**

All analyses were performed by using the Stata program version 10.0. The baseline characteristics and other variables were analyzed and presented as frequency and proportions for categorical data and mean, standard deviation, median, maximum, minimum for continuous data. To estimate the prevalence of inappropriate use of antibiotics, it was calculated with frequency having value as percentage and confidence interval at 95%.

**Ethical considerations**

This study was conducted after receiving permission from the office of the Khon Kaen University Ethics Committee in Thailand for human research for endorsement of ethical approval of this research with approval reference number HE632210 and ethic approval from National Ethics Committee for Health Research in Cambodia with the approval reference number 196 NECHR.

## Result

***Demographic and Socioeconomic Characteristics of Caregivers***

Out of 512 households, 350 were in rural. Majority of caregivers were female, 96.9 % in urban and 94.6 % in rural. Their median age was 32.5 (20:66) years old in urban and 33.0 (18:75) in rural. More than three quarter of both urban (85.8%) and rural (88.8%) were married/ living with a partner. About half of caregivers (43.8) % in urban and only 1.7% in rural completed bachelor's degree, whereas only 6.8 % urban and 12.0% rural caregivers were illiterate. Around 40.7% of caregivers in urban, and 20% in rural were employee while only 0.1% but nearly 40% of participants in urban and rural were farmer, respectively.

***Demographic and Socioeconomic Characteristics of Children Under Five***

Among children under five, the proportion of male and female gender both in urban and rural was comparable. Their mean age was 30.8 (±15.8 S.D) years old in urban and 30.7 (±15.7S.D) in rural. Children from both urban (58.6%) and rural (57.5%) areas were mostly from 4 to 5 members-family while the median family sizes were 5, both in urban and rural. The median monthly family income was 750 (22.5:10,000) for urban, whereas it was only 150 (15:1,000) US$ in rural. As regarding to financial status, 38.9% urban and only 4.6% rural children's family had enough money with saving while the proportion of those which having not enough money with debt was higher in rural (42.9%) than in urban (16.7%). Caregivers of children under five both in urban and rural areas were mostly mother/father with a similar proportion of (79.6% vs 78.3% respectively).

***Situation of antibiotic use in children under five years of age***

**Table 1 Situation of antibiotic use in children (n= 512)**

| Antibiotic use | Urban (n=162) | | Rural (n=350) | |
|---|---|---|---|---|
| | Number | % | Number | % |
| **Source of antibiotics for children** | | | | |
| Doctor's clinic | 81 | 50.0 | 182 | 52.0 |
| Health center | 20 | 12.4 | 74 | 21.2 |
| Hospital | 41 | 25.3 | 46 | 13.1 |
| Pharmacy shop | 20 | 12.3 | 43 | 12.3 |
| Shop | 0 | 0.0 | 5 | 1.4 |
| **Purpose of using antibiotics for children** | | | | |
| Fever | 120 | 74.1 | 256 | 73.1 |
| Bacterial infection diagnosed by doctors | 133 | 82.1 | 184 | 52.6 |
| Common cold/running nose | 85 | 52.5 | 196 | 56.0 |
| Cough | 83 | 51.2 | 114 | 32.6 |
| Sore throat | 107 | 66.1 | 75 | 21.4 |
| Diarrhea | 17 | 10.5 | 47 | 13.4 |

***Cont...*** **Table 1 Situation of antibiotic use in children (n= 512)**

| Antibiotic use | Urban (n=162) | | Rural (n=350) | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Wound with pus | 4 | 2.5 | 17 | 4.9 |
| Swelling, red wound or other types of injuries | 2 | 1.2 | 19 | 5.4 |
| All types of infections (bacterial, viral, parasite or other microorganisms) | 5 | 3.1 | 2 | 0.6 |
| Route of antibiotic administration | | | | |
| Different from recommendation by physician or instruction in the prescription | 20 | 3.9 | 34 | 6.6 |
| As recommendation by physician or instruction in the prescription | 142 | 87.7 | 316 | 90.3 |
| Dosage of antibiotics | | | | |
| Less than dosage prescribed by doctor | 28 | 17.3 | 41 | 11.7 |
| Same as dosage prescribed by doctor | 134 | 82.7 | 301 | 86.0 |
| Higher than dosage prescribed by doctor | 0 | 0.0 | 8 | 2.3 |
| Number of times per day of antibiotics | | | | |
| Less frequent than the instruction by doctor | 44 | 27.2 | 51 | 14.6 |
| Same as the instruction by doctors | 116 | 71.6 | 295 | 84.3 |
| More frequent than the instruction by doctor | 2 | 1.2 | 4 | 1.1 |
| Number of days of using antibiotics | | | | |
| Shorter than the instruction by doctor | 95 | 58.6 | 214 | 61.1 |
| Same as the instruction by doctor | 64 | 39.5 | 130 | 37.2 |
| Longer than the instruction by doctor | 3 | 1.9 | 6 | 1.7 |

***Cont...*** **Table 1 Situation of antibiotic use in children (n= 512)**

| Antibiotic use | Urban (n=162) | | Rural (n=350) | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Over-prescription of antibiotics | | | | |
| Not use for any other purpose than prescribed by doctor | 130 | 80.3 | 308 | 88.0 |
| Used for any other purpose than prescribed by doctors | 32 | 19.7 | 42 | 12.0 |
| Using the leftover antibiotics (for children antibiotics when they had a similar sign to the previous illness take antibiotics from family members/friends when children had a similar sign to those relatives/ friends without having to see a medical doctor): | | | | |
| No | 120 | 74.1 | 272 | 77.7 |
| Yes | 42 | 25.9 | 78 | 22.3 |
| Using non-prescribed antibiotics/self-medication for children | | | | |
| Never | 145 | 89.5 | 296 | 84.6 |
| Rarely | 2 | 1.2 | 18 | 5.1 |
| Sometimes | 3 | 1.8 | 16 | 4.6 |
| Often | 9 | 5.6 | 15 | 4.3 |
| Always | 3 | 1.8 | 5 | 1.4 |
| Requesting antibiotics from physicians/doctors or other healthcare providers even children had mild disease that you were not sure whether it was caused by bacteria or not. | | | | |
| No | 153 | 94.4 | 306 | 87.4 |
| Yes | 9 | 5.6 | 44 | 12.6 |

Most of caregivers both in urban and rural setting received antibiotics for their children under five from health care facilities such as health center, hospital, doctor's clinic rather than pharmacy or general shops. Those caregivers mostly brought their children to see doctor before getting antibiotics, which was a appropriate practice. However, the most common reasons antibiotics used for children were fever (74.1% in urban and 73.1% in rural), bacterial infection (82.1% in urban and 52.6% in rural) and common cold/running nose (52.5% in urban and 52.6%). Almost all children both in urban (87.7%) and rural (90.35) were given antibiotics as its correct pharmaceutical form. Most of their caregivers both in urban (82.7%) and rural (86.0%) gave them the antibiotics in the amount as prescription. Noticeably, the percentage of caregivers in urban who were likely to give antibiotics less frequent than the

instruction was double, compared to those in rural. Approximately 80 per cent of caregivers in urban and rural administered antibiotics to their children with the same frequency times as physician prescribed. In concern with number of days using antibiotics, the proportion of the right period of course of treatment was only 39.5% in urban and 37.2 %, which was the most concerning issue. Caregivers in urban (19.7%) prone to use antibiotics for any other purpose than prescribed by doctor, compared with those in rural (12.0%). Approximately, one third of caregivers in Cambodia were more likely to practice wrongly in using leftover antibiotics to their children, 25.9% in urban and 22.3 % in rural, see Table 1.

***Prevalence of inappropriate use of antibiotics (IUA) by urban vs rural and overall prevalence of IUA (n=512)***

**Table 2 Prevalence of inappropriate use of antibiotics (IUA) by area (n=512)**

| Antibiotic Use | Number | % | 95% Confidence Interval |
|---|---|---|---|
| Urban (n=162) | | | |
| Appropriateness (10 scores) | 29 | 5.7 | 3.96 to 8.04 |
| Inappropriateness (<10 scores) | 133 | 26.0 | 22.35 to 29.96 |
| Rural (n=350) | | | |
| Appropriateness (10 scores) | 77 | 15.0 | 12.19 to 18.41 |
| Inappropriateness (<10 scores) | 273 | 53.3 | 48.97 to 57.62 |
| Overall prevalence | | | |
| Appropriateness (10 scores) | 106 | 20.7 | 17.40 to 24.45 |
| Inappropriateness (<10 scores) | 406 | 79.3 | 75.55 to 82.60 |

Overall, almost 80% of those children (95% CI: 75.55 to 82.60) faced problem of inappropriate use of antibiotics, of which children residing in rural tended to face almost two-folds of inappropriate use of antibiotics (53.3%: 48.97 to 57.62) when comparing with those in urban settings (26.0%: 22.35 to 29.96) see table 2.

## Discussion

Inappropriate use of antibiotics (IUA) among under five children in Cambodia was extremely high (79.30%).This rate was higher than a study among Chinese children (35.12%) ([21]) and another study among adult of Northwest Ethiopia (30.9%) ([19]). However, it was similar with a study carried out among children in Saudi (41-92%) ([22]). This inconsistent in prevalence of inappropriate use of antibiotics might be due to variation on level of awareness, education level, belief, cultural preference, accessibility to health facilities, and other socio-economic characteristics of participants in the studies.

Interestingly, prevalence of IUA tended to be high among children who residing in rural areas (53.3%), compared to those in urban settings (26.0%). The possible reasons might relate to limited accessibility to antibiotics and information on antibiotic use, which might be in those with low family income, inconvenience in traveling to health facility, few health facilities and licensed healthcare providers as well as low level of education. These factors seemed to be better in urban settings. In contrast, a study in Northwest Ethiopia did not prove any great controversial regarding the proportion of IUA between urban (33.1%) and rural (29.2%). The reason to explain this situation by researcher was that those communities were in the close proximity leading to exchange of information ([19]). The disparity in these findings might be due to different context of study areas and population. Similar finding was reported in a study in China showing that respondents in less developed areas tended to use of antibiotics inappropriately ([23]). The reason might be associated with socio-demographics characteristics.

The high prevalence of inappropriate use of antibiotics in this study might related to caregiver factors. The situation of antibiotic use was improper in all criteria. Many Cambodians had wrong perceptions concerning antibiotics. For instance, antibiotics were believed to be effective for treating common cold, fever, general pain, malaria, and inflammation. In addition, left-over antibiotics could be used. Mostly, inappropriate use of antibiotic is related to unrestricted accessibility of antibiotics. People could easily get them from pharmacies, shops, nurses as suppliers and unlicensed medical providers in villages ([24]), which increased of self-medication practices. However, laws and regulations to control non-prescription antibiotics seemed to remain ineffective ([25]). Remarkably concerning the period of treatment, about two third of them did not completed the full course of antibiotics recommended. This was the most common inappropriate use of antibiotics found in this study. Low level of awareness about the significance of correct indication and dosage of purchasers and dispensers and limited affordability on medication expense fostered the practice of buying a few doses of antibiotics ([26]), as well as self-medication in developing countries ([27]). Other issues involving sources of antibiotics, routes of administration, dosage, number of times, and over-prescriptions did not show any seriously wrong practices among Cambodian caregivers. However, it could be noticed that rural participants were more likely to have inappropriate practices than urban ones. The underlying reasons might be due to level of education of caregivers in rural was still lower than those in urban as evidenced in findings on the characteristics of caregivers in this study. Level of education was more likely to relate with knowledge on antibiotic use. Appropriately, 94.4 % of urban and 87.4 % of rural participants did not request antibiotics from physician for their children without diagnosis, of which they might not know about medication that good for the children illness.

## Conclusion

Inappropriate use of antibiotics among Cambodian children under five was very high and was much higher in rural than urban communities. Only about half of the rural respondents used antibiotics for bacterial infection. Both urban and rural tended to use antibiotics only for a few days. The situation could increase antibiotic resistance and leading to not only increase health expenditure but also shortage of effective antibiotics in curing bacterial infections in the future**.** The finding from this was emphasize the prominence in conducting further studies to discover determinants of inappropriate use of antibiotics and more possible and efficient approaches that antibiotic resistance can be combated in communities both in urban and rural Cambodia.

### Limitation

This study used information reported by the participants which could be subjected to recall bias. However, the potential of recall bias was minimized as much as possible by decreasing time frame of

retrieving information dated back to six-month before data collection.

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# Healthcare Facilities Choice for Maternity Care in Indonesia: Do Socioeconomic Factors Affects?

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## Abstract

The government encourages maternity care in health facilities to reduce maternal mortality. The study aims to analyze the impact of socioeconomic factors on healthcare facilities' choice for maternity care in Indonesia. The study used secondary data from the official report of the Indonesia Ministry of Health. The study takes all provinces as samples. Moreover, the study used the proportion of maternity care in health facilities as a dependent variable. On the other hand, the research analyzed four other variables as independent variables: percentage of the poor population, percentage of the population having health insurance, literacy percentage of population >15 years, and the unemployment rate for population >15 years. The study analyzed the data using a scatter plot. The study results show the lower the poor population in the province, the higher the proportion of maternity care in health facilities in that province. The higher the percentage of the population having health insurance in an area, the higher the proportion of maternity care in health facilities in that area. Meanwhile, the higher the literacy percentage of population >15 years in a province, the higher the proportion of maternity care in health facilities in that province. Moreover, the higher the unemployment rate for population >15 years in a province, the higher the proportion of maternity care in health facilities in that province. The study concluded that the four independent variables analyzed ecologically were associated with maternity care in health facilities.

***Keywords****: maternity care, maternal care, socioeconomic, ecological analysis, public health.*

## Introduction

Maternal mortality is a health problem that has become a global issue. Indonesia has not achieved the sustainable development target by 2030 to reduce the international maternal mortality ratio (MMR) to less than 70/100,000 live births[1]. World Bank data states that the MMR ratio in Indonesia has shown a trend that has continued to decline since 2000[2]. The MMR in Indonesia in 2017 was 177 deaths per 100,000 live births; this achievement is still far from the SDGs target[2]. In the ASEAN region, Indonesia is a country with the 3rd highest MMR after Myanmar and Laos. With an average decline of around 3% per year, Indonesia must work harder to achieve the SDGs target by 2030[3].

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Indonesia has implemented a strategy to reduce maternal mortality by increasing the availability of midwives. However, the decline in MMR in Indonesia has not been significant. A study of women giving birth in Indonesia recorded 76% using health facilities[4]. However, studies in other countries show that the proportion of women who give birth in health facilities is lower than those who give birth at home. Research in Guinea-Bissau put more than three-fifths of the population, and in Guinea, more than three-quarters of women do not give birth in a health facility. Women of lower socioeconomic status are significantly less likely to use health facility services[5,6]. Other factors that support childbirth at home include low family income, living in a slum area, and not having a history of delivery in a health facility[7]. This phenomenon provides information that factors related to the value of childbirth at a comfortable home, work, and family income are still the reasons for choosing a birth.

Women who give birth at home and experience childbirth complications are at risk of receiving substandard assistance. Complications can include fetal distress, prolonged labor, and bleeding. They handled complications unproperly will increase the risk of maternal death. Several studies on economic factors and access to health services have provided empirical evidence regarding delivery in health facilities[8,9]. Women with lower income levels have a higher risk of maternal death within six weeks and within one year[10]. Another study states a relationship between a partner's job, per capita income, and the choice of place to give birth[11].

Access and characteristics of health services in Indonesia accounted for 23% of the difference in maternal mortality ratio between high and low performing provinces. Increasing access to hospitals outside Java is predicted to prevent better maternal mortality[12]. Based on the background, the study aims to analyze the impact of socioeconomic factors on healthcare facilities' choice for maternity care in Indonesia.

## Materials and Methods

### Study Design

The study employed an ecological analysis approach. The ecological analysis focuses on comparisons not individually but between groups. In ecological research, the data analyzed is aggregate data at a specific group or level; in this study, it is at the provincial level. The variables in an ecological analysis can be aggregate measurements, environmental measurements, or global measurements. The purpose of ecological study in epidemiology is to make biological inferences about individual risk effects or ecological inferences about effects on groups[13,14].

### Data Source

The study uses secondary data from the 2018 Indonesia Basic Health Survey and the 2018 Indonesia Health Profile report. Both reports are official publications from the Ministry of Health of the Republic of Indonesia. The unit of analysis in this study is the province. The study analyzed all areas in Indonesia (34 provinces).

## Data Analysis

The dependent variable in this study is the proportion of maternity care in health facilities. Health facilities in this study include hospitals, maternity hospitals, and health centers. The study analyzes four independent variables, which include: percentage of the poor population (as of September 2018), percentage of the population having health insurance, literacy percentage of population >15 years, and the unemployment rate for population >15 years (as of August 2018).

The study analyzed the data in a bivariate manner using a scatter plot. The study used the linear fit line to determine the relationship between the prevalence of hypertension and the independent variable. The research carried out analysis with the help of the IBM SPSS 21 software.

**Findings**

Table 1 is a descriptive statistic of maternity care in health facilities by the province in Indonesia, and other variables analyzed. The information presented informs that the lowest proportion of maternity care in health facilities is 30.10%, while the highest proportion of maternity care in health facilities is 98.50%. The range of the ratio of maternity care in health facilities between provinces in Indonesia is quite broad.

**Table 1. Descriptive statistics of the proportion of maternity care in health facilities by the province in Indonesia, 2018**

| Variables | N | Range | Min | Max | Mean | Std. Deviation |
|---|---|---|---|---|---|---|
| The proportion of maternity care in health facilities | 34 | 68.40% | 30.10% | 98.50% | 71.78% | 17.08215 |
| Percentage of the poor population (as of September 2018) | 34 | 23.88% | 3.55% | 27.43% | 10.61% | 5.70346 |
| Percentage of the the population having health insurance | 34 | 46.71% | 46.01% | 92.72% | 65.99% | 11.15727 |
| Literacy percentage of the population >15 years | 34 | 23.08% | 76.79% | 99.87% | 95.99% | 4.57075 |
| The unemployment rate for the population >15 years (as of August 2018) | 34 | 7.15% | 1.37% | 8.52% | 4.86% | 1.64399 |

Source: The 2018 Indonesia Basic Health Survey and the 2018 Indonesia Health Profile

Figure 1 shows the scatter plot of maternity care in health facilities and the percentage of the poor population by the province in Indonesia. Figure 1 shows the tendency for a negative relationship between the two variables. The situation means that the lower the poor population in the region, the higher the proportion of maternity care in health facilities in that region.

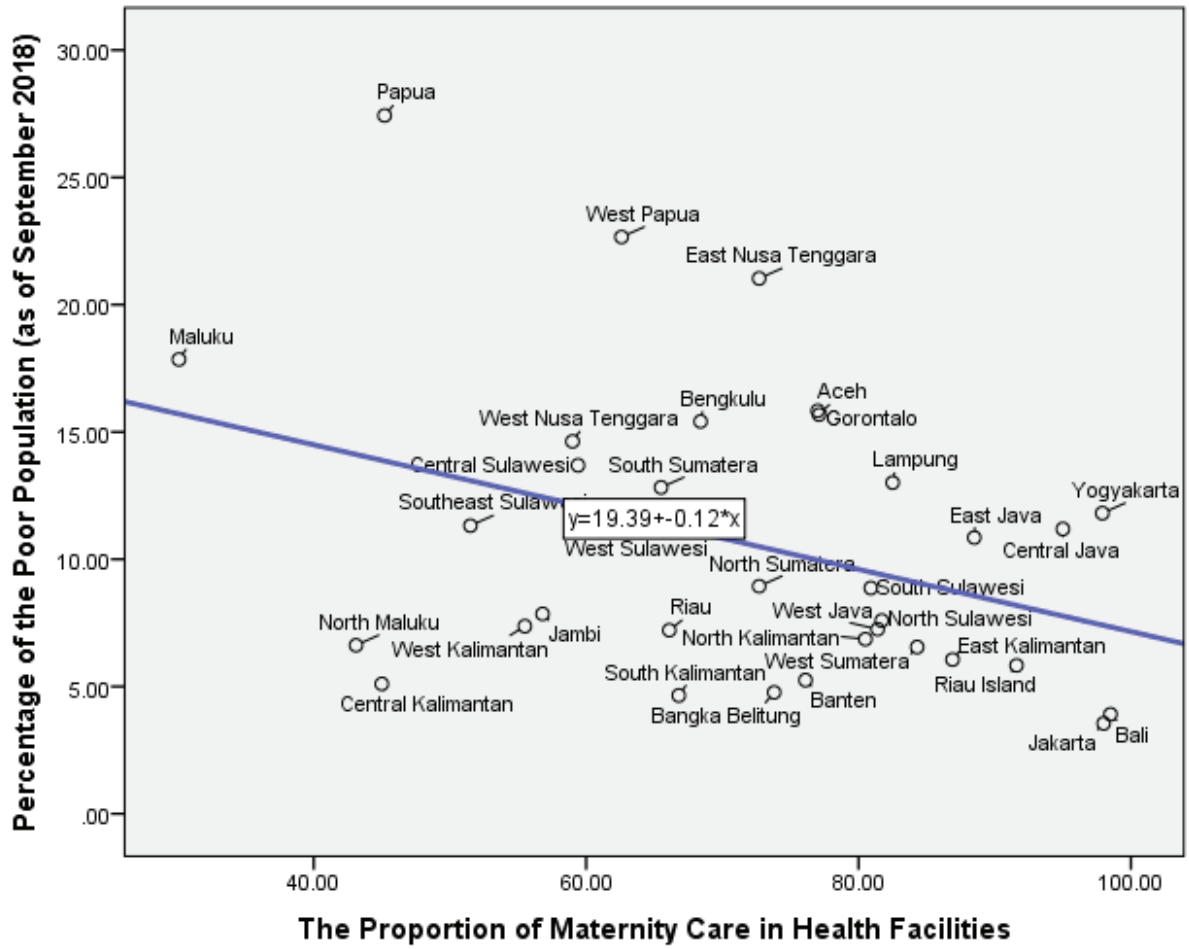


**Figure 1. Scatter plot of the proportion of maternity care in health facilities and the percentage of the poor population by the province in Indonesia, 2018**

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Figure 2 is a scatter plot of the proportion of maternity care in health facilities and the percentage of the population having health insurance by the province in Indonesia. Figure 2 shows the tendency for a positive relationship between the two variables. The condition means that the higher the percentage of the population having health insurance in an area, the higher the proportion of maternity care in health facilities in that area.

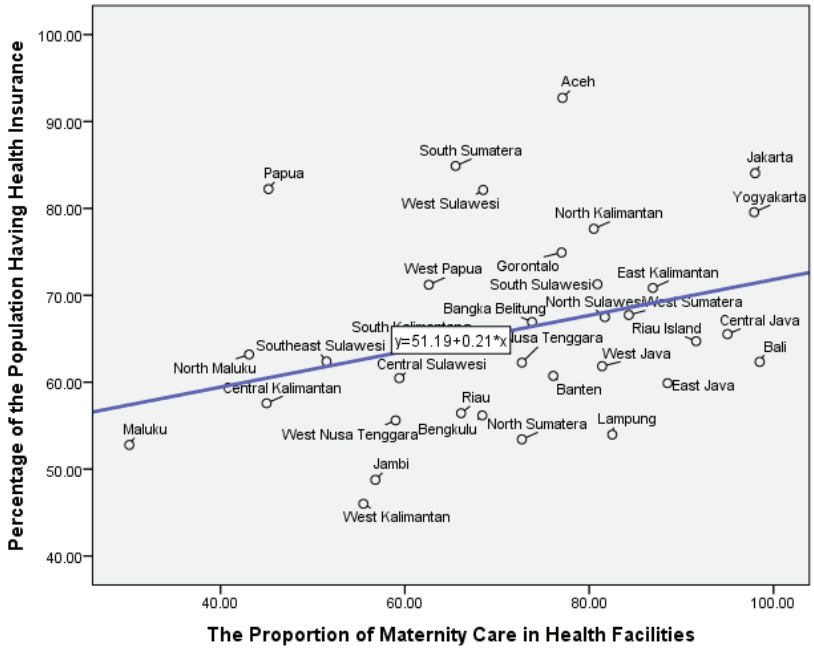


**Figure 2. Scatter plot of the proportion of maternity care in health facilities and the percentage of the population having health insurance by the province in Indonesia, 2018**

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Figure 3 is the scatter plot of maternity care in health facilities and the literacy percentage of the population >15 year by the province in Indonesia. Figure 3 shows the tendency for a positive relationship between the two variables. The situation means that the higher the literacy percentage of the population >15 year in a province, the higher the proportion of maternity care in health facilities in that province.

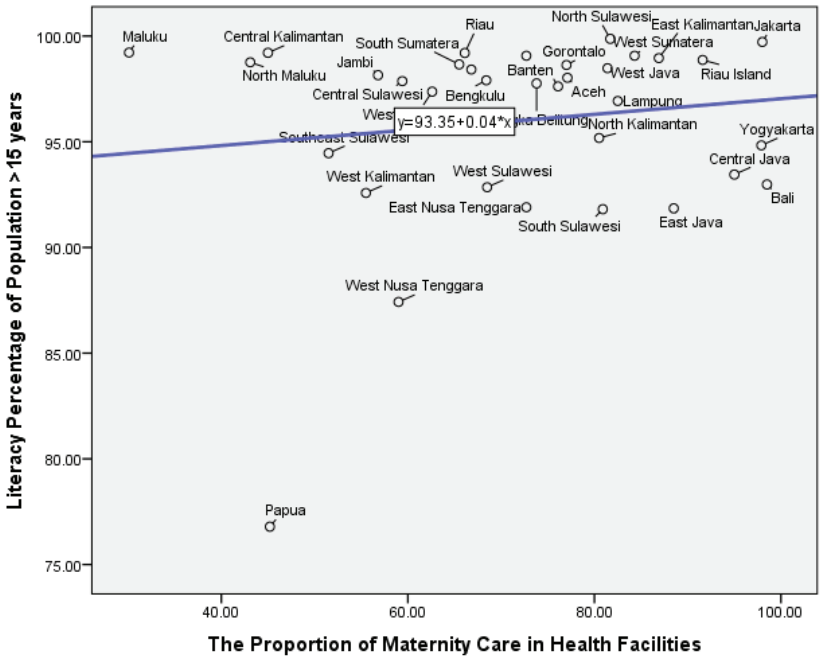


**Figure 3. Scatter plot of the proportion of maternity care in health facilities and the literacy percentage of population >15 years by the province in Indonesia, 2018**

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Figure 4 is the scatter plot of the proportion of maternity care in health facilities and the unemployment rate for the population >15 years by the province in Indonesia. Figure 4 shows the tendency for a positive relationship between the two variables. The condition means that the higher the unemployment rate for the population >15 years in a province, the higher the proportion of maternity care in health facilities in that province.

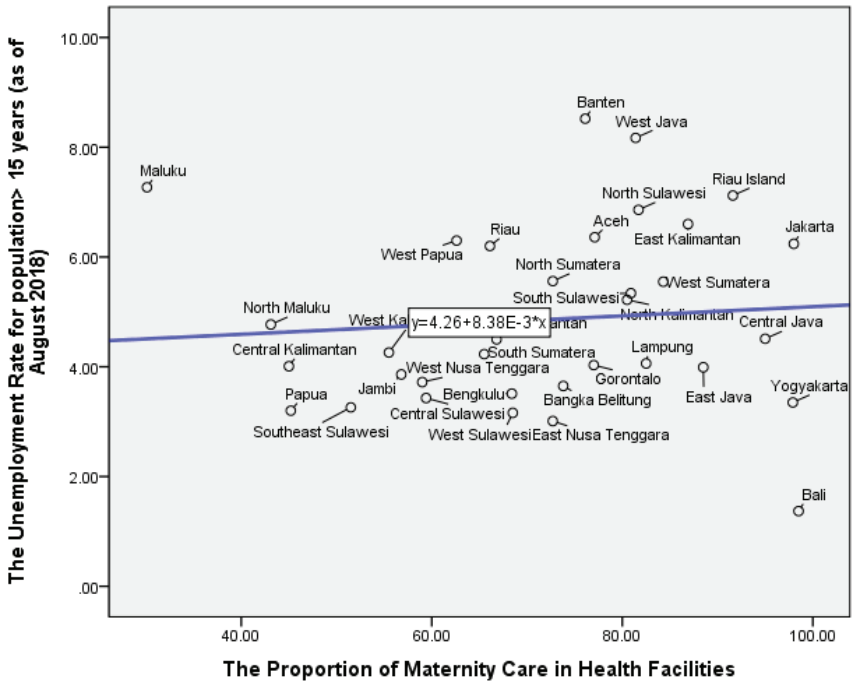


**Figure 4. Scatter plot of the proportion of maternity care in health facilities and the unemployment rate for population >15 years by the province in Indonesia, 2018**

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

## Discussion

The result finding is in line with the conclusions in several previous studies. The better the wealth status, the more likely it is to do maternity care in a health facility[15–18]. Moreover, several studies often found poverty a barrier to achieving better performance in the health sector[19,20].

The results of this study support the Indonesian government's policy that seeks to remove health financing barriers to access to health services[21,22]. Meanwhile, studies in several countries also show the same trend[16,23,24]. In the Indonesian context, the barrier to health financing is the cost of services and travel costs to reach health facilities. This situation is a consequence of Indonesia's topography as an archipelago with a relatively extreme geographical condition[25–27].

We can use the literacy condition in a country to measure education success in that country, especially in developing countries. The higher the level of education, the higher the opportunity to do maternity care in a health facility. Several previous studies have also found similar results. The more educated a woman is, the more she will understand the risks of maternity care outside of a health facility[17,28,29]. In general, better education is a strong determinant to produce higher quality performance in the health sector[30–33]. Meanwhile, the analysis result slightly contradicts a previous study that found that employment was

not related to the choice of maternity care in health facilities[15].

## Conclusion

Based on the results, the study concluded that the four variables analyzed showed an association with maternity care in health facilities. The association between maternity care in health facilities and the percentage of the poor population shows a negative trend. Meanwhile, the association between the proportion of maternity care in health facilities with three other variables (percentage of the poor population, percentage of the population having health insurance, literacy percentage of the population >15 years, and the unemployment rate for population >5 years) shows a positive trend relationship.

**Conflict of Interests:** Nil

**Source of Funding:** Self-funding

**Ethical Clearance:** The study was conducted by utilizing secondary data from published reports. For this reason, the study not required an ethical clearance in the implementation of this research.

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# Echocardiographic Study in Preterm Infant with Hemodynamic Significant Patent Ductus Arteriosus

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## Abstract

**Background:** Potential complications of hemodynamic significant patent ductus arteriosus (hsPDA) after birth include heart failure, need for respiratory support, renal disfunction, intraventricular hemorrhage, as well as long term altered growth and development. Nevertheless, clinical signs of patent ductus arteriosus (PDA) are not sensitive and specific enough. Therefore, echocardiography still remains the preferred method to evaluate the ductal patency in preterm infant. The present study aimed to evaluate the echocardiography characteristic in preterm infant with hsPDA.

**Methods:** A cross-sectional study was conducted on preterm infants aged 3-7 days with 24-33$^{6/7}$ weeks of gestation. Data taken were demographic, clinical and echocardiography. Diagnosis of hsPDA was carried out by echocardiography; defined as >1.5mm diameter of ductus and >1.4 left pulmonal artery and aorta (La/Ao) ratio. The statistical analysis was undertaken using SPSS 21.0.

**Results:** There were 11 out of 52 preterm infants diagnosed hsPDA. Mean birth weight was 1213±293 gram; Mean gestational age was 30.72±2.01 weeks. In hsPDA group, mean ductus diameter was 2.84±0.93 mm, mean La/Ao ratio was 1.56±0.26, and mean ejection fraction (EF) was 71.55±5.72%.

**Conclusion:** Echocardiographic evaluation is important for addressing hsPDA in preterm infants.

***Keywords:*** *echocardiographic, preterm infant, hemodynamic significant patent ductus arteriosus*

## Introduction

The birth rate of premature babies continues to increase year after year. Complications of preterm birth are still the leading cause of infant mortality, reaching one million deaths in 2015.[1] Patent ductus arteriosus (PDA) is the most common cardiovascular disorder in premature infants.[2] The ducts of arteriosus will normally shrink after birth in a reasonably month-old baby and are functionally closed at the age of 72 hours. The likelihood of spontaneous closure of the ductus arteriosus (DA) in full-term infants without congenital heart disease is very high; but in the preterm infant, closure rates are poorer.[3,4]

Some studies linking uncorrected hsPDA to infant morbidity for less than a month include

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intraventricular bleeding, necrotizing enterocolitis (NEC) and renal insufficiency that can be caused by decreased systemic blood flow and tissue ischemic consequences.[5] Decreased brain oxygenation in infants less than a month can occur in infants with hsPDA resulting in brain damage and developmental disorders.[6] Clinical assessment and echocardiography are reliable methods of diagnosing an hsPDA. Therefore a timely echocardiographic evaluation is needed. The aim of this study was to define the basic functional echocardiographic characteristic of preterm infant with hsPDA.

## Methods

A cross-sectional study was conducted in all preterm infants born between November 2019 and May 2020 at the tertiary level neonatal intensive care unit of Dr.Soetomo General Hospital whose oxygen support devices (i.e. high flow nasal canula, continuous positive airway pressure, invasive and non-invasive ventilator) were eligible for inclusion. Patients with multiple congenital anomaly, ductal dependent cyanotic heart defect, early onset of septicemia, and incomplete consent from parents were excluded.

Echocardiography screening was performed between 3rd and 7th postnatal day by pediatric cardiology consultant using Sonoscape Portable Digital Color Doppler Ultrasound System Model S9 (SonoScape, Shenzhen). hsPDA was considered existing if there were a ductus arteriosus with diameter > 1.5 mm on constriction phase, pulmonal perfusion seen in the left pulmonal artery diameter and aorta diameter ratio (LA/Ao) was > 1.4, and left to right shunt were present. While DA that was already closed and did not fulfill the requirement for hsPDA was considered non-hsPDA.

Echocardiographic characteristics of babies with hsPDA were described as mean (M) and standard deviation (SD). Differences in clinical characteristics of the two sample groups of hsPDA and non hsPDA were analyzed using Chi square test, Fisher exact test, and exact probability test. IBM SPSS 21.0 was used for all statistical analyses.

The ethical clearance was issued by the Ethical Committee of Dr. Soetomo General Hospital (No.1766/105/XI/2019). Before the subject recruitment, the researchers had explained to the parents about the general research information and the consent.

## Result

Echocardiography evaluation were performed in 52 out of 191 preterm infants treated in NICU during study. Four babies with early onset of septicemia, three babies with multiple congenital anomalies, and babies without complete data and consent were excluded from the study. There were 25 (48%) and 27 (52%) preterm male and female babies, respectively, as shown in Table 1. There were 11 infants meet the criteria of hsPDA based on echocardiography.

**Table 1. Subject characteristic**

| | **hsPDA n (11)** | **non-hsPDA n (41)** | **Total n (52)** | **P** |
|---|---|---|---|---|
| Sex<br>Male<br>Female | <br>5 (9.6)<br>6 (11.5) | <br>20 (38.5)<br>21 (40.4) | <br>25 (48.1)<br>27 (51.9) | 1.000* |
| Gestational age<br>*Extremely Preterm*<br>(<28 weeks)<br>*Very Preterm*<br>(28 - <32 weeks)<br>*Moderate Late Preterm*<br>(32 - <37 weeks) | <br><br>1 (1.9)<br>6 (11.5)<br>4 (7.7) | <br><br>3 (5.8)<br>23 (44.3)<br>15 (28.8) | <br><br>4 (7.7)<br>29 (55.8)<br>19 (36.5) | 1.000** |

***Cont...*** **Table 1. Subject characteristic**

| | | | | |
|---|---|---|---|---|
| Birth weight<br>ELBW (<1000 g)<br>VLBW (<1500 g)<br>LBW (<2500 g) | <br>2 (3.9)<br>7 (13.5)<br>2 (3.9) | <br>6 (11.5)<br>24 (46.1)<br>11 (21.1) | <br>8 (15.4)<br>31 (59.6)<br>13 (25) | 0.902** |
| Age during recruitment<br>3 day<br>4 day<br>5 day<br>6 day<br>7 day | <br>3 (5.7)<br>6 (11.5)<br>1 (1.9)<br>1 (1.9)<br>0 (0.0) | <br>21 (40.5)<br>10 (19.3)<br>5 (9.6)<br>4 (7.7)<br>1 (1.9) | <br>24 (46.2)<br>16 (30.8)<br>6 (11.5)<br>5 (9.6)<br>1 (1.9) | 0.118 |
| Infant's comorbidity<br>Respiratory distress synd.<br>Perinatal Asphyxia<br>PPHN<br>Hyperbilirubinemia<br>Others | <br>6 (11.5)<br>2 (3.8)<br>3 (5.7)<br>4 (7.7)<br>1 (1.9) | <br>12 (23.1)<br>11 (21.1)<br>3 (5.7)<br>6 (11.5)<br>9 (17.3) | <br>18 (34.6)<br>13 (24.9)<br>6 (11.4)<br>10 (19.2)<br>10 (19.2) | 0.086 |

Note: Sample characteristic using Chi square test; *Chi squre corrected; **Fisher's exact test

The echocardiography characteristics of hsPDA group research subjects presented in table 2 include ejection fraction (EF), arteriosus duct diameter (DA), left pulmonary artery diameter (La), aortic diameter (Ao), as well as La compared Ao ratio. In determining the status of hsPDA used parameters in the form of DA diameter and La/Ao ratio as a marker of the presence of pulmonary hyperperfusion. In this study obtained the smallest DA diameter of 1.5mm and the largest 4.8mm in the hsPDA group. Of the eleven hsPDA group samples, a diameter of duktus was obtained with an average of 2.84±0.93. The comparison between La and Ao obtained an average score of 1.56±0.26. Ejection fraction in hsPDA group was 71.55±5.72 and the EF in non hsPDA group was 73.94±0.26 (range 56.5-93.12%).

**Table 2. Echocardiographic characteristic in hsPDA group**

| **Echocardographic Characteristic** | **Patients (n)** | **hsPDA Mean ± SD** |
|---|---|---|
| Ejection fraction (%) | 11 | 71.55 ± 5.72 |
| DA diameter (mm) | 11 | 2.84 ± 0.93 |
| Left pulmonary artery diameter (La) | 11 | 11.30 ± 1.97 |
| Aorta diameter (Ao) | 11 | 7.19 ± 1.35 |
| La/Ao ratio | 11 | 1.56 ± 0.26 |

## Discussion

This study showed no significance between gestational age (p = 0.981), birth weight (p = 0.832), single birth of twin mapun (p = 0.322) between hsPDA and non hsPDA groups. Based on gestational age the most to smallest age group was the 28-<32-week group (55.8%), followed by 32-33$^{6/7}$ weeks (36.5%) and gestational age <28 weeks (7.7%). In contrast to other studies where there was an increase in the incidence of hsPDA inversely proportional to the gestational age. Studies in Italy by the Italian Neonatal Network showed higher incidence of PDAs at younger gestational ages: 88.9% and 81.5% at gestational ages of 23 and 24 weeks. PDA incidence decreased at gestation age of 25 weeks by 70.3% and 29 weeks by 32%.[7]

In this study, there were 2/11 neonates with hsPDA having a birth weight of 1000-<1500 grams and as much as 6/41 in the non hsPDA group. The very low birth weight (VLBW) group with a birth weight of 1000-1499 grams in both the hsPDA (7/11) and non hsPDA (24/41) groups had the highest frequency compared to the extremely low birth weight (ELBW, < 1000grams) and low birth weight (LBW, 1500-<2500 grams) groups. These findings are in accordance with previous studies in Palembang, where the majority of babies with PDAs have a birth weight of ≤1500gram and are a risk factor for the occurrence of PDA.[8] Different results were obtained in the study by Su et al., where the incidence of PDA was higher in ELBW (79%) compared to VLBW (65%) neonatal age of 4 days.[9] This difference in proportion can be due to the spread of different samples between birth weight groups where the number of ELBW samples is only 8 infants (15.4%) from the total sample.

The duct of arteriosus normally shrinks after birth in a term infant and is functionally closed at the age of 72 hours. The closure of the ducts was delayed to 4 days in 10% of the babies born with 30 to 37 weeks of gestational age, 80% of babies born gestational age 25 to 28 weeks, and 90% in infants born less than 24 weeks.[3,10] The persistent ductus arteriosus can cause hemodynamic disturbance where the systemic shunt to the pulmonary resulting in pulmonary hyperperfusion and systemic hypoperfusion.[11]

Although ductus arteriosus evaluation is focused on the functional echocardiography, the baby should always undergo a complete echocardiogram first. An initial echocardiogram enables structural congenital heart disease to be excluded. When performing functional echocardiography, it is also important to bear in mind the difficulties and challenges associated with scanning a premature infant. Some babies are very small, they can be agitated or unstable, have a high heart rate, and sometimes a poor echo window, particularly if they are ventilated and have severe underlying lung disease.[4]

In the early hours of life, relatively high pulmonary pressures result in a balanced pulmonary to systemic circulation. A heart murmur, hyperactive precordium, bounding pulses, and a widened systolic to diastolic pulse pressure amplitude are recognized clinical signs attributed to the presence of a hsPDA. Most clinical signs lack sensitivity in the first days of life and hence during this period, a PDA is usually diagnosed by echocardiography.[12] In this study, the pulsation rate between hsPDA and non hsPDA babies is not differ significantly.

This study obtained an average diameter of ductus of 2.84±0.93mm. This result is higher than the study by Visconti et al, in which a spontaneous closed duct with diameter of 1.63mm, ductus that required pharmacological therapy with diameter of 2.24mm, and ductus diameter which require operative therapy of 2.39mm.[13] Determination of hsPDA is generally given when transductal diameter of >1.5mm is obtained. Based on previous studies the value of this intersection indicates the presence of perfusion disorders of the target organ. The La/Ao ratio indicating pulmonary hyper perfusion showed an average value of 1.56±0.26. This figure meets the criteria made by Sehgal and McNamara where the

ducts with an L/Ao ratio of > 1.4±1.6:1 give the effect of moderate hemodynamic disorders on neonates.[14] One baby gave a manifestation of heart failure that was exacerbated by late onset of septicemia that ended in mortality. Three babies in hsPDA group had persistent pulmonary hypertension of the newborn, thus requiring vasodilator treatment prior to ductal closure.

The limitation of this study is focused solely on evaluating the presence of the ductal and left ventricle function. The sample limitation is also a shortcoming in this study. Therefore, future study is needed.

## Conclusion

In conclusions, doppler echocardiographic assessment of ductal flow in preterm infant is useful to predict the development of hsPDA. Clinicians need to perform simple echocardiography screening after 3 days of life in preterm infant.

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**Ethical Clearance**

This study had got permission from the ethics committee of The Faculty of Medicine, Airlangga University Before the subject recruitment, the explanation was done to the parents.

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**Conflict of Interest** – Nil

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# Laser Application for Management of Traumatic Ulcers Following Local Anesthesia in Children

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## Abstract

Prolonged local anaesthesia in soft perioral tissues may sometimes lead to accidental lip biting in small children forming a traumatic ulcer. Healing of such ulcers has been reported to enhance with treatment using low level laser therapy (LLLT). This case report describe two cases of traumatic ulcers due to lip bite following inferior alveolar nerve block caused during post anaesthesia period that were treated by LLLT. It resulted in better healing and lower pain, thus helping in healing and repair of the tissue.

***Keywords*** *– Children, Low level light therapy, oral ulcer*

## Introduction

Traumatic ulcers are common lesions having yellowish white necrotic pseudo membrane with borders that are raised and erythematous.[1] College et al [2] found that 13% of children from 2 to 18 years experienced soft tissue trauma following unilateral or bilateral mandibular nerve block anesthesia. The incidence of soft tissue trauma was 18% among children < 4 years of age, 16% in 4-7 years, 13% in 8-11 year old children, and 7% in ≥12 years of age. This report presents two cases of lip bite injury following inferior alveolar nerve block treated with low level laser therapy (LLLT).

**CASE 1:** A 10-year-old boy reported to the Department with decayed teeth. The right mandibular primary second molar was found to be grossly carious and showed furcation involvement therefore extraction was planned for the tooth following inferior alveolar nerve block. The patient was sent with appropriate postoperative instructions. The following day, the patient reported with swelling of the right lower lip with a large traumatic ulcer of the size of 1.5 by 1 cm. (Fig 1) The child had bit on his lower lip during the post extraction period owing to the peculiar feeling of numbness and tingling sensation which resulted in the traumatic ulcer.

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**Figure 1: Preoperative for Case 1**

**CASE 2 :** An 8 year old boy reported to the Department with pain in carious, left mandibular first and second primary molars. Intraoral periapical radiograph were taken and based upon the clinical and radiographic interpretations, pulpectomy for 74 and extraction of 75 was planned. Inferior alveolar nerve block was given with 2% lignocaine with vasoconstrictor in the concentarion of 1:100000 and the procedures were carried out. The patient came the next day with pain on the lower lip and left buccal mucosa due to the formation of traumatic ulcer 5mm by 15 mm in size. ( Fig 2 )



**Figure 2: Preoperative for Case 2**

For both the cases, a diagnosis of traumatic ulcer secondary to lip biting due to local anaesthesia was made. It was decided to treat the ulcer with soft tissue Diode laser (Picaso, 810+ 10 nm) (Fig 3) with LLLT aimed at alleviating the pain and achieving epithelization of the wound .




**Figure 3: Picaso Laser**

The treatment consisted of multiple sittings. The application of the Laser was done in the non-contact mode with a distance of 2-3 mm between the Laser tip and the ulcer surface. The laser beam was applied in a continuous, circular motion, so as to cover the entire ulcer surface. Each sitting consisted of four sessions of low level laser application, lasting forty five seconds each with a gap of thirty seconds between each application, for a total laser application time of about five minutes per visit. These precautions were taken to prevent overheating of the adjacent tissues, which can lead to necrosis. The LLLT was carried out on four consecutive days after which the ulcer was found to be approximately healed. (Fig 4).



**Figure 4: Postoperative healing after laser treatment for Case 1**

There was absence of pain and erythema after the fourth day and complete healing occurred in one week. ( Fig 5)



**Figure 5: Postoperative healing after laser treatment for Case 2**

## Discussion

Local anaesthesia blocks last longer than infiltration and soft tissue anesthesia lasts longer than pulpal anesthesia.[3] Sof tissue anaesthesia may be present for up 3-5 hours after local anaesthesia administration giving rise to minor soft tissue traumatic injuries.[4] The most common area of trauma is the lower lip and to a lesser extent the tongue, followed by the upper lip· seen in pediatric patients.[5]

Patients with traumatic ulcers are prescribed medication depending on the severity of the disease. In mild cases topical protective emollient such as orabase is advised whereas in severe cases topical corticosteroids preparation are helpful.[1]

De souza et al, showed that healing of aphthous ulcers following low level laser application was achieved in four days by once daily application. Also, the pain intensity was relieved after the first laser application itself.[6] Lalabonova H used low energy laser irradiation (LELT) in treating traumatic ulcers of oral mucosa and he found that pain was rapidly managed with the usage and epithelization of the ulcers was accelerated thus eliminating the use of drugs.[7] Agarwal H et al assessed clinically the efficacy of LLLT on recurrent aphthous ulcers for reduction of pain, lesion size, and healing time and concluded that LLLT is an effective modality for the treatment of aphthous ulcers as it lessen the healing time, and also provides immediate pain relief.[8]

Similar such findings were reported in the present cases where there was reduced pain, size of the lesion and erythema after the very first sitting. The bio stimulating effect of lasers accelerates the process of regeneration with predominating analgesic effect by causing alteration of the electrical activity in the nerve cells [8], anti-inflammatory effects and accelerates healing in inflamed and odematous tissue .

Several authors [9,,10,11] conducted similar studies using LLLT in the treatment of Aphthous Ulcers and stated that LLLT lead to spontaneous reduction of symptoms leading to decreased healing time, pain intensity, size, and recurrence of the lesion in patients with RAS with greatest clinical effectiveness .

LLLT has been found to act on mitochondria,thus enhancing the synthesis of ATP and promoting tissue repair.[12] It also has neuropharmacologic effects and cause release of a range of neurochemicals including histamine, serotonin and acetylcholine that produce an analgesic effect and anti- inflammatory effects[13] It has been

observed that LLLT decreases the permeability of the lymph vessels and can also stimulate lymph vessel collaterals.[14]

The present case reports shows that lasers are effective, rapid, simple and bloodless procedure although the routine treatment includes anti-inflammatory like corticosteroids, amlexanox and metalloprotease inhibitors and/or symptomatic therapy. Present case report show the effectivenss of lasers, though its disadvantages must be kept in mind i.e. it's a sensitive procedure. During laser procedure safety of the skin & eyes and control of temperature to prevent the damage to adjacent tissues should be taken into account.

## Conclusion

It can be said from the outome of above mentioned two case reoprts that laser is a good tool to treat traumatic ulcers and may reduce psychological trauma and fear during the dental visit.

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# Critical Success Factor For E-learning in the Covid -19 Pandemic: A Case Study in Jordan

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## Abstract

The questionnaire was in three parts. Part A was Availability of E-learning requirements related to technical, administrative and financial aspects Part B was Disadvantages of E-learning and Part C E-learning obstacles. And It was written using Google Forms, and the link was shared through multimedia. The reliability of instrument was determined. Data collected were analyzed using simple percentage and t_test and Anova was used to test the hypotheses. The result collected and analyzed showed that; the majority of study (84.0%) respondents were in the middle age between 29 to 48 years old. Males were accounted for less than one fifth (18.0%) of sample. And we found out through the results teachers with 12 to 22 years of experience found of obstacles of E- learning in comparison with those with the lowest number of years of experience, and that state schools have more obstacles than private schools.

***Key Wards:*** *E-learning, COVID-19/Coronavirus pandemic, Teachers, Students, Classroom, Education, Challenges, Jordan.*

## Introduction

The definition of e-learning is the rapid development of computer technologies and programs. The increasing influence of the Internet on our lives and the use of various educational applications and platforms have affected the learning process. This demonstrates that teachers and students have recently begun to rely heavily on the use of PowerPoint files, PDFs, and emails to share notes, homework and sometimes take exams online, by using computers and web-based platforms. It has been argued that this term does not have a common definition [(1)]. E-learning can be referred to as virtual learning, digital learning, or web-based learning. It can be used partly as part of the traditional teaching / learning process or entirely, depending on the materials delivered to students [(2)]

**Advantages and disadvantages:**

E-learning is currently considered one of the best methods of learning, especially in higher education [(3)]. For a number of advantages, including: Flexibility regarding time and space: Students are free to choose when and where to learn using technology, and in some cases, students are able to foster communication using forums and discussion groups in order to exchange different points of view. There is also another advantage of e-learning is that it facilitates distance learning, as many students can learn without the need to travel abroad in addition to that it allows to get the largest possible number of students without the need for more expenses and facilities. Others that

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e-learning also has a negative impact on the learning process [4] One of the most prominent disadvantages of e-learning is the lack of personal interaction between students with each other and between teachers and students on the other hand. Hence, school students find it very difficult to cope with this method of learning. Electronic testing is also considered, as there are materials where we cannot apply all kinds of questions, such as essay questions [5].

Before the COVID-19 pandemic, e-learning was increasing by about 15.4% annually in educational institutions around the world [6] However, the research was conducted in July 2020, as educational institutions provided most of their services online, as this education was applied to more than 60% of students around the world due to global restriction measures to limit the spread of COVID-19 [7] Countries have also closed schools and universities, and switched to full e-learning mode during the spread of the Coronavirus, to avoid expected waves in the future [8] This procedure was followed in line with the rules of social distancing, which were strongly recommended by the World Health Organization to prevent the spread of COVID-19 [9] The closures began in mid-March 2020, and it was not planned in advance, and not all educational institutions had the ability to smoothly deal with the closure plan as it had not been implemented before, and a difference was observed between educational institutions that were applying e-learning before and those who They did not implement this system. Since it was possible during typical times for students to visit the library, and to be able to go to places with good internet connection speed, before the COVID-19 pandemic, but during the COVID-19 pandemic everyone was on curfew, and the materials provided in the classroom were taught through E-learning is so well prepared before COVID-19, unlike during COVID-19 where no training sessions were planned.[6] Habits of mind are assessed as part of the educational process, as the teacher›s role is essential in developing habits of mind by focusing on learning specific concepts through clear teaching strategies [10]) The teacher is responsible for managing the learning process, which in turn facilitates students' learning in a meaningful way. The digital revolution has given educators a growing set of digital tools to achieve these results. (11) Teachers can provide direct directions to students when using Internet face-to-face technologies simultaneously, which has resulted in superior results for students compared to various other teaching methods, including traditional teaching (12,13)

The Field Survey and Public Opinion Department at the Center for Strategic Studies at the University of Jordan carried out its sixteenth survey in a series of surveys "The Jordanian Indicator - The Pulse of the Jordanian Street" during the period from 3/22/20 /. 2020, in a sample representative of the Jordanian community and from all governorates, with the result that (89)% strongly support the suspension of schools and universities until the end of the crisis.(14)

The aim of our study is to find the success and failure factor in e-learning among school students during the Covid-19 pandemic from the teachers 'opinion.

## Method

### A. Participants

A total of 200 participants were recruited in the current study, the majority of study (84.0%) respondents were in the middle age between 29 to 48 years old. Males were accounted for less than one fifth (18.0%) of sample. The majority of respondents (76.5%) were bachelor degree holders. About one third of the sample had experience between 12 to 17 years.

### B. Constructs of the study

We have three constructs Availability of E-learning requirements related to technical, administrative and financial aspects, Disadvantages of E-learning, and E-learning obstacles. Each category includes several variable: age, gender, educational level, Type of

school and experience.

## Statistical Analysis

Data were analyzed by the Statistical Package for Social Sciences (SPSS) software version 11.0 (SPSS®: Inc., Chicago, IL, USA). Means, standard deviations, frequencies and percentages were produced. Independent t- test, one-way ANOVA test with Scheffe post hoc were used to compare between subgroups as appropriate. Furthermore, Pearson correlation coefficient used to test the correlation between overall mean of the outcomes. The level of significance was set at ($P \leq 0.05$).

## Results

### A. Sample description

A total of 200 participants were recruited in the current study, the majority of study (84.0%) respondents were in the middle age between 29 to 48 years old. Males were accounted for less than one fifth (18.0%) of sample. The majority of respondents (76.5%) were bachelor degree holders. About one third of the sample had experience between 12 to 17 years.

### B. Reliability coefficient

The reliability coefficient (Cronbach's Alpha) of the study is 0.829, which is a good value reflecting a reliable measure of the study tool.

### C. Study of items' means

The following part shows responses to the levels of the variables. Here, mean and standard deviation are calculated for each item. Higher mean value indicates more agreement on that item.

i. Availability of E-learning requirements related to technical, administrative and financial aspects

From table 1, only Item 3 and item 6 " The school administration encourages teachers to use the e-learning and computerized curriculum in teaching" and "The computer lab official works with teachers and students constantly" have the highest mean values of 3.43(±SD=1.07) and 3.38 (±1.17), respectively, with "agree" attitude. In general respondents` attitude was neutral regarding the availability of E-learning requirements related to technical, administrative and financial aspects.

ii. Disadvantages of E-learning

From table 2, the most prominent disadvantages of E-learning which respondents strongly agreed focused on financial, educational and values issues. These issues were summarized in the following; item 11, item 5, item 2, item 6, item 7, and item 10. On the contrary, the E-learning didn't affect the nature of relationship between the teachers and their students in term of effective communications, encouragement and answering questions and inquiries. Generally, respondents` attitude was agree regarding to the disadvantages of E-learning.

iii. E-learning obstacles

iv.

From table 3, the difficulty to apply E-learning in practical and laboratory materials, in addition to the frequent interruption of internet and the difficulty for parents to subscribe to the internet were considered to be the main strongly agree reported obstacles by respondents to using E- learning. In general, respondents` attitude was agree regarding the presence of obstacles of E-learning.

### D. Correlation analysis

From table 4, the relation between construct 2 " Disadvantages of E-learning " and construct 3 " E-learning obstacles " is (r=0.691) which is a strong value. But the relation between construct 1 " Availability of E-learning requirements related to technical, administrative and financial aspects " and construct 2 " Disadvantages of E-learning "is negative with (r=-0.616 ) and the relation of construct 1 " Availability of E-learning requirements related to

technical, administrative and financial aspects " with construct 3 " E-learning obstacles " which is negative (r=- 0.657).

### E. T tests and Analysis of variance

Are there significant differences in the levels of the study constructs that can be attributed to age, gender, Educational level, Experience and Type of school? Independent samples t-test will be used to test for gender while, analysis of variance (ANOVA) will be used to test for other personal variables. Age, gender, Educational level, Experience and Type of school.

From table 5, there is a moderate positive significant relationship between the disadvantages of E-learning and the presence of obstacles (r=0.69; P<0.0001), whilst there were a moderate negative relationship between availability of E-learning requirements with either disadvantages or obstacles of E-learning.

From table 6, there is a significant difference between type of school regarding the availability of E-learning, private schools tend to have higher score in reporting positive answers regarding the availability of resources (3.3 ±0.71) compared with state schools (P<0.0001).

From table 7, there is a significant difference between type of school regarding the disadvantages of E-learning, state schools tend to have higher score in reporting more disadvantages of using E-learning (3.6 ±0.46) compared with private schools (P=0.001).

Teachers in the age group between 36 and 48 years old tend to report higher mean scores in presence of E- learning obstacles compared with the youngest age group (P=0.018). Also, teachers with 12 to 22 years of experience reported higher mean scores that confirm the existence of obstacles of E- learning in comparison with those with the lowest number of years of experience.

From table 8, there is a significant difference between type of school regarding the obstacles of E-learning, state schools tend to have higher score in reporting more obstacles of using E-learning (3.8 ±0.52) compared with private schools (P<0.0001).

**Table 1. Part A. Availability of E-learning requirements related to technical, administrative and financial aspects**

| Item | Mean | SD | Attitude |
|---|---|---|---|
| Item 1-The prevailing educational system continuously supports e-learning | 2.64 | 1.14 | Neutral |
| Item 2-School administration allocates part of the school's budget to support e-learning at the beginning of the school year | 2.665 | 1.27 | Neutral |
| Item 3-The school administration encourages teachers to use the e-learning and computerized curriculum in teaching | 3.425 | 1.07 | Agree |
| Item 4-E-learning goals doesn't branch into more commercial goals than educational | 2.375 | 1.11 | Neutral |
| Item 5-The speed of the internet is suitable to take advantage of the website services at all times | 2.28 | 1.17 | Neutral |
| Item 6-The computer lab official works with teachers and students constantly | 3.38 | 1.17 | Agree |

***Cont...*** **Table 1. Part A. Availability of E-learning requirements related to technical, administrative and financial aspects**

| | | | |
|---|---|---|---|
| Item 7-The software used for e-learning is easy to handle | 2.9 | 1.15 | Neutral |
| Item 8-Students respond positively to the offered subject | 2.7 | 1.10 | Neutral |
| Item 9-Availability of educational material on the platform all the time increases the ability to understand the educational material | 3.1 | 1.18 | Neutral |
| Item 10-The student will benefit from the educational platform services and continuously follow them | 2.7 | 1.15 | Neutral |
| Item 11-The Ministry holds training courses for teachers on using computerized curricula | 2.6 | 1.19 | Neutral |
| Item 12-There are centers for receiving student complaints when there is any defect | 2.8 | 1.16 | Neutral |
| Item 13-The calendar is used to determine the dates and schedules of daily and final exams | 3.2 | 1.05 | Neutral |
| Overall | 2.85 | 0.74 | Neutral |

**Table 2. Part B. Disadvantages of E-learning**

| Item | Mean | SD | Attitude |
|---|---|---|---|
| Item 1-E-learning lacks effective communication between students and the teacher | 3.9 | 1.05 | Agree |
| Item 2-E-learning is a heavy burden on the head of the family when pursuing his children | 4.4 | .79 | Strongly agree |
| Item 3-The student doesn't convinced of the necessity of e-learning as an alternative to traditional learning | 3.8 | 1.07 | Agree |
| Item 4-E-learning lacks confidentiality and honesty when answering tests | 4.3 | 1.02 | Agree |
| Item 5-Cheat prevention is difficult to control during electronic tests | 4.5 | .86 | Strongly agree |
| Item 6-E-learning weakens students 'attitudes and educational values, which are stipulated in the philosophy of education | 4.3 | .91 | Strongly agree |
| Item 7-It reduces students' skills in cooperative learning and learning by playing | 4.3 | .95 | Strongly agree |
| Item 8-The teacher feels that e-learning reduces their control over the course of the educational process | 4.1 | 1.06 | Agree |
| Item 9-The teacher hasn`t technological skills that enable him to deal with the system | 2.9 | 1.13 | Neutral |
| Item 10-Students and their parents lack experience in using computer software | 4.2 | .93 | Strongly agree |
| Item 11-The lack of a monetary factor affects the guardian's ability to provide computers and smart phones | 4.5 | .71 | Strongly agree |
| Item 12- Reducing the communication between teachers and students and answering their questions | 2.0 | .89 | Disagree |

***Cont...*** **Table 2. Part B. Disadvantages of E-learning**

| | | | |
|---|---|---|---|
| Item 13-Reducing the encouragement by Teachers for their students to enter the platform and follow up on assignments and tests | 2.0 | .88 | Disagree |
| Item 14-Lessons are not recorded for students using multimedia so the student can follow them | 2.1 | .93 | Disagree |
| Item 15-Students are not encouraged with rewards and certificates of appreciation for their continuous follow up | 2.4 | 1.06 | Disagree |
| Item 16-There is no phone number or email to communicate with school instructions and student inquiries | 2.2 | 1.11 | Disagree |
| Overall | 3.5 | .48 | Agree |

**Table 3: Part C. E-learning obstacles**

| Item | Mean | SD | Attitude |
|---|---|---|---|
| Item 1-Some e-learning programs are expensive | 3.89 | .91 | Agree |
| Item 2-E-learning is difficult to apply in materials that require practical application and laboratories | 4.37 | .79 | Strongly agree |
| Item 3-The connection to the system is frequently interrupted due to internet pressure | 4.36 | .73 | Strongly agree |
| Item 4-Students don`t follow the periodic program to broadcast lessons on your lesson channel | 3.53 | 1.03 | Neutral |
| Item 5-There are deficiencies in the services provided by the system and the mechanism for answering questions | 3.74 | .98 | Agree |
| Item 6-Students can`t see their ratings and scores | 2.42 | .98 | Disagree |
| Item 7-There is no coordination between the Ministry of Communications and the Ministry of Education in supporting distance learning | 3.14 | 1.09 | Neutral |
| Item 8-Discourage continuous use of E-learning | 4.04 | 1.15 | Agree |
| Item 9-It is difficult for all parents to subscribe to the Internet | 4.26 | .94 | Strongly agree |
| Overall | 3.75 | .54 | Agree |

**Table 4. Correlation analysis .**

| Construct | Part A | Part B | Part C |
|---|---|---|---|
| Part A | 1 | - 0.616*<br>P<0.0001 | -0.657*<br>P<0.0001 |
| Part B | - 0.616*<br>P<0.0001 | 1 | 0.691*<br>P<0.0001 |
| Part C | -0.657*<br>P<0.0001 | 0.691*<br>P<0.0001 | 1 |

**Table 5: Mean, standard deviation, t value, P-value and F –value according to part A, part B and part C.**

| PART | Variable | | Mean (SD) | P- value* |
|---|---|---|---|---|
| A. Availability of E-learning requirements related to technical, administrative and financial aspects | Type of school | State<br>Private | 2.6(.68)<br>3.3(.71) | t=-5.49<br>P<0.0001 |
| B. E-learning disadvantages | Type of school | State<br>Private | 3.6(.46)<br>3.3(.50) | t=3.39<br>P=0.001 |
| C. E-learning obstacles | Age | 22-28 years<br>29-35 years<br>36-42 years<br>43-48 years<br>≥49 years | 3.2(.62)<br>3.7(.54)<br>3.7(.55)<br>3.8(.48)<br>3.7(.51) | F=3.063<br>0.018 |
| | Experience | <5 years<br>6-11 years<br>12-17 years<br>18-22 years<br>>22 years | 3.5(.67)<br>3.6(.46)<br>3.8(.56)<br>3.8(.51)<br>3.6(.35) | F=2.79<br>P=0.027 |
| | Type of school | State<br>Private | 3.8 (.52)<br>3.4 (.49) | t=4.15<br>P<0.0001 |

## Discussion

E-learning includes the use of digital tools for teaching and learning. It uses technological tools to enable learners to study anytime, anywhere. It involves training, providing knowledge and feedback, so the fact that some school administrations encourage teachers to use e-learning and computerized curricula in teaching and provide the necessary assistance from laboratory evaluators to teachers and students constantly increases the success of e-learning. [16]

E-learning can be cost effective for many universities and schools because when learning

platforms are set up, they can be reused in many sessions. (2)

The Opinion Polls and Field Survey Department at the Center for Strategic Studies at the University of Jordan carried out 4-7 / 4/2020 on a sample representative of the Jordanian society and from all the governorates of the Kingdom, where it was noticed that 80 % of those who follow the e-learning platform and the educational TV channels provided by the Ministry of Education believe that these The platforms are not as good as school education, as half of the "Darsak" platform students encountered technical problems while registering. And the follow-up process for this statute (17).

E-learning in the study area constitutes a heavy burden on the head of the family when pursuing his children, as students and their parents lack experience in using computer programs in addition to the parent's inability to provide computers and smart phones for all children in the same family.

Teachers believe that it is difficult to control the prevention of cheating during electronic exams, which in turn weakens the ability to evaluate the outcomes of the educational process, in addition to the difficulty of monitoring students 'attitudes and educational values stipulated in the education philosophy in addition to that e-learning reduces students' skills in cooperative learning and learning through play. since Younger students want more contact with other students and communicate with the teacher (18)

There are also some technical problems related to the interruption of communication with the system due to the Internet or the lack of financial ability for parents to subscribe to the Internet So these obstacles can be avoided through government financial support, and IT infrastructure development (19)

Through media and electronic resources, which in turn can improve students' attitude towards e-learning

We agree with others around the world that teachers need more training courses to develop their expertise in software applications (18)

Jordanian teachers found that "continuing with the online learning model is unacceptable because it is socially and psychologically unhealthy measures of closures, closures and quarantines imposed by the Coronavirus, which have caused tension, frustration and depression. [11] We can also summarize the impact of e-learning on university students and their teacher, In the students 'opinion, studying using e-learning is not equal to direct education [12] Also, most people cannot imagine a "classroom without walls" in addition to some seeing that not going to work and direct teaching will make it impossible for them to feel that they are academic citizens. [18]

## Conclusion

The researchers noted that the availability of e-learning requirements related to technical, administrative and financial aspects for private school teachers more than for public school teachers. Therefore, the barriers to e-learning for public school teachers are greater than for private schools. It has been observed that teachers between the ages of 22 and 28 are less likely to see obstacles to e-learning than individuals between the ages of 36 and 48. These teachers who run online lessons can persuade their colleagues to adopt online teaching and link the positive incentive for e-learning and discuss their experience with them. Teachers who are reluctant to embrace the technology.

### Recommendation

We propose to develop the IT infrastructure and facilitate through media and electronic resources, and to provide the Internet in general as a tool to engage students in improvement. Their techniques, knowledge and skills. We suggest encouraging teachers to participate in training to acquire informed knowledge about new technological changes. We suggest that the government direct all school principals in Balqa Governorate to create academic plans that help

students cover missed lessons. Because education is one of the most important sectors that must be given priority in a while Corona time.

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**Declaration of Competing Interest**

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# Relationship between Media Access and Social Support with Contraception Plans in East Java, Indonesia

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## Abstract

**Objectives:** The percentage of contraceptive prevalence rate (CPR) and total fertility rate (TFR) of woman were indicators of the 2015-2019 National Medium Term Development Plan (RPJMN). Total fertility rates in East Java had decreased significantly from 1971 to 2018. The achievement of the prevalence of modern contraceptive use (CPR) in East Java was 63.1 percent based on the results of the Program Performance and Accountability Survey (PPAS, 2018). This achievement was far better than the national achievement which only reached 57 percent. However, this target cannot be achieved by all districts and cities in East Java. According to Green's theory, the factors of predisposing, enabling and reinforcing are factors that can be related to behavior. The purpose of this study was to analyze the relationship between enabling factors (media access) and reinforcing factors (social support) related to contraceptive plans in women of childbearing age in East Java, Indonesia.

**Study Design:** This research was a non-reactive study using data from the IDHS (Indonesia Demographic and Health Survey) 2017. The unit of analysis in this study was all teenage respondents in East Java who met the requirements of the 2017 IDHS respondents.

**Results:** The results showed that the enabling factor associated with contraception selection plans was listening to information about contraception through radio (sig = 0.038), television (sig < 0.001) and newspapers (sig < 0.001). While the reinforcing factor associated with contraceptive selection plans was supports from health facilities (sig = 0.000) and from farmacy (sig = 0.023). The role of health workers was very minimal in providing information access to woman in the process of selecting contraception. It can be seen that almost all of the woman said that they did not get information either from field officers (41.91%), from health facilities (39.45%), from village clinics (42.52%), and from pharmacists (38.00%).

**Implication to the fields:** Based on the results of this study, there is a need for equal distribution of education in the selection of contraceptive methods to woman through optimizing the role of various health facilities, one of them through contraception officers, optimizing the use of mass media, especially television, radio and newspapers and magazines in disseminating information related to family planning.

***Keywords*** *: media access, social support, contraception plan, good health, well being*

## Introduction

Indonesia is the fourth country with the most population in the world. Indonesia's population from year to year continues to increase. Indonesia's population in 2015 was 258.2 million, in 2016 as many as 261.1 million, and in 2017 as many as 264 million[1]. Government efforts in suppressing the high rate of growth in Indonesia by designing a Family Planning program that serves to balance between needs and population[2].

Indonesia is one of the developing countries in the world with various types of problems faced, one of which is in the field of population, namely: population is still growing. Government in an effort to improve the welfare of the nation has been and is carrying out development in all to solve the population problem. One of the necessary efforts What the government does to solve the population problem is a program family planning. The family planning program is an effort to increase care and community participation through maturing the age of marriage, birth control, family development, welfare improvement small family, happy and prosperous[3].

Family Planning was the four pillars with antenatal care, safe delivery, and postnatal care introduced by Safe Motherhood in 1987 to reduce maternal mortality in developing countries[4]. The government had carried out various promotions with the aim of encouraging people to realize the family planning program. The government was trying to influence the community through family planning program advertisements in various media. The increasing availability of media in the form of radio, television and print media in developing countries can be effectively used to influence people's behavior[5].

The family planning program promoted by the government had become very important as control of the population explosion. Data from the National Population and Family Planning Agency in 2013 received 8,500,247 fertile age couples who were new family planning participants, with details of injectable contraceptive users 4,128,115 participants (48.56%), pills 2,261,480 participants (26.60%), implants 784,215 participants (9.23%), condoms 517,638 participants (6.09%), uterine contraception 658,632 participants (7.75%), MOW (female surgery method) 128,799 participants (1,52%), MOP (male surgery method) 21,374 participants (0.25%), the above data shows injection contraception method was the most used method[6].

In accordance with Green (1980) theory in Notoatmodjo (2003) that health behaviour including the selection of contraceptives was influenced by three factors namely predisposing factors (Knowledge, attitudes, Education, family economy), supporting factors (availability of medical devices, sources of information) and driving factors (family and community leaders support). The study analyzed anabling (media access) and reinforcing factors (social support) related to contraceptive plans in women of childbearing age[7].

Some factors that influence the use of contraception include knowledge, information by family planning officers and husband's support. Knowledge was a significant relationship with the use of contraception, the better one's knowledge about contraception the more rational in using contraception. In addition, the high level of education of a person also support accelerating the reception of family planning information in couples of childbearing age. Good information from the officer helps the client in choosing and determining the type of contraception used. Good information will provide client satisfaction which has an impact on longer use of contraception so that it helps the success of family planning. In addition, husband's support also influences the use of contraception. Clients who are given support by their husbands will use contraception continuously while those who do not get husband support will use contraception less[8].

Mass media was the main strategy to increase demand for health services. This was a process that

helps communities to identify their own needs and to respond to and address needs[9]. The previous research showed that there was a significant p-value = 0.012 (< 0.05) relationship between the source of information and the use of contraception[10]. Previous research also showed factors related to the use of the Contraceptive Method from environmental factors, including the role of partners, the role of family and friends, the role of officers, the role of community leaders and the role of the mass media [11].

Apart from the mass media, the factors of staff, both Family planning field workers, health workers, village clinic officers, and pharmacists also greatly influenced the use of contraception. The knowledge gained by the community can be used as a material consideration in determining the contraception to be used[12]. The purpose of this study was to analyze the relationship between enabling factors (media access) and reinforcing factors (social support) related to contraceptive plans in women of childbearing age in East Java, Indonesia.

## Methods

This research was a non-reactive study using data from the Indonesia Demographic and Health Survey (IDHS) 2017. The unit of analysis in this study was all teenage respondents in East Java who met the requirements of the 2017 IDHS respondents. Three types of questionnaires used were the Household, Women's, and Men's Questionnaire. The IDHS sample includes 1,970 census blocks covering urban and rural areas. Respondents of women of childbearing age aged 15-49 were 59,100, 24,625 respondents were unmarried young men aged 15-24 years, and 14,193 married men aged 15-54 years. The IDHS sample frame uses the Master Census Block Sample from the 2017 Population Census data in this study.

## Result and Discussion

Based on this research, most respondents have plans to use injection contraception. Respondents with plans to use injection contraception get information from radio and newspapers / magazines (Table 1). The media in the form of radio, television and newspapers / magazines are related to contraceptive use plans[13]. The findings show that mass media impacts positively on family planning practice and using radio proved to be most effective means of informing people about family planning with 64.7%. Majority (62.7%) of the women were not practicing family planning, 63.08% had primary level of education, 82.78% were rural women, majority were married(81.09%), the biggest percentage of 49.97% of the women were self-employed and nearly 100% half mark of the respondents were poor (46.22%), As regards using contraceptives, most respondents were using Injections (16.75%), followed by implants (6.61%). Analysis shows that mass media in addition to other five socio-economic variables were significant thus can be used to explain variations in the family planning practices. These are income status, marital status, education level, religion and age. The multivariate regression results indicate that place of residence and occupation (women who never responded) are not statistically significant in the model. We recommend continued use of mass media since it proved an important factor in practice of family planning and that facilities providing family planning services are made more user friendly to accommodate all age groups[14].

Radio was a good mass media strategy in providing program information and using contraceptive methods[15]. Radio reached a wide and diverse audience. Radio station specialization based on the age, taste and even gender of the listener allows more selectivity in reaching the audience segment. In addition, because placement and production costs are less for radio than television, radio can deliver public health messages in more detail. Thus, radio is always considered to be more comfortable and efficient[16].

**Table 1. Percentage of contraceptive use plans for women of 15-49 years of age based on enabling factors**

| | | IUD | Women's Sterilization | Men's Sterilization | Periodic absence | Implant | LAM | Injection | Total | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Knowing Contraception from the Radio** | | | | | | | | | | |
| Yes | N | 619 | 1.719 | 644 | 492 | 318 | 7 | 1.885 | 6.683 | 0.038 |
| | % | 90.36 | 90.34 | 85.96 | 89.45 | 89.44 | 100 | 91.81 | 90.42 | |
| No | N | 66 | 184 | 105 | 58 | 38 | 0 | 257 | 708 | |
| | % | 9.64 | 9.66 | 14.04 | 10.55 | 10.56 | 0 | 8.19 | 9.58 | |
| Total | N | 685 | 1.903 | 749 | 550 | 356 | 7 | 3.143 | 7391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| **Knowing Contraception from TV** | | | | | | | | | | |
| Yes | N | 234 | 782 | 289 | 252 | 145 | 1 | 1.450 | 3.152 | 0.001 |
| | % | 34.17 | 41.08 | 38.62 | 45.78 | 40.71 | 10.16 | 46.13 | 42.64 | |
| No | N | 451 | 1.121 | 460 | 298 | 211 | 6 | 1.693 | 4.239 | |
| | % | 65.83 | 58.92 | 61.38 | 54.22 | 59.29 | 89.84 | 53.87 | 57.36 | |
| Total | N | 685 | 1.903 | 749 | 550 | 356 | 7 | 3.143 | 7391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| **Knowing Contraception from the Newspaper/magazine** | | | | | | | | | | |
| Yes | N | 565 | 1724 | 640 | 495 | 291 | 4 | 2.895 | 6.614 | 0.001 |
| | % | 82.46 | 90.62 | 85.44 | 89.98 | 81.91 | 60.16 | 92.12 | 89.48 | |
| No | N | 120 | 179 | 109 | 55 | 65 | 3 | 248 | 777 | |
| | % | 17.54 | 9.38 | 14.56 | 10.04 | 18.09 | 39.84 | 7.88 | 10.52 | |
| Total | N | 685 | 1.903 | 749 | 550 | 356 | 7 | 3.143 | 7391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |

Note: Total N is the total number of samples of women of childbearing age 15-49 years who were weighted

Respondents who did not get information from television could be caused by restrictions on the display of public service announcements about family planning specifically on contraception. Public service announcements about family planning were only aired on certain television stations that are not in great demand by a wide audience. One of family planning idea dissemination in massive and wide coverage is television ads. Ads could influence the change of people's knowledge, attitude, belief dan behavior who watches the ads. This article discusses effects of television ads toward (1) cognitive, (2) affective, (3) behavior level. This study shows that exposure of television ads has affected knowledge level. Meanwhile, exposure of television ads does not have any effect on attitude and behavioral level. Respondents have positive attitude toward family planning program and strong willingness to adopt the program in the future. There is also indication that family planning program acceptance is not affected by only televion ads, but it could be influenced by other aspects such as economy, family, environment and etc. Therefore, family planning television ads role as stimulator that could persuade people to adopt the program[17]. Television was a strong media to attract a wide audience and can reach people regardless of age, gender, education level, apparently not all families are able to buy and operate it because they need electricity. The findings from El-Bakly and Hess, state that in Egypt television contributes to an increase in contraceptive use. Television as the first source in obtaining family planning information[18].

The previous research showed that there were no significant differences in magazines or newspapers, for the use of family planning. Reading the newspaper can positively influence adherence to family planning programs. Method selection is an effort made by a woman to find a method of choice that fits her situation, this is because the choice of choice is determined by the variety of existing methods[19].

**Table 2. Percentage of contraceptive use plans for infertile women aged 15-49 years based on reinforcing factors**

| | | IUD | Women's Sterilization | Men's Sterilization | Periodic absence | Implant | LAM | Injection | Total | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Field Officers Visited in the Last 12 Months** | | | | | | | | | | |
| Yes | N | 7 | 29 | 5 | 15 | 7 | 0 | 45 | 108 | 0.482 |
| | % | 0.93 | 1.52 | 0.73 | 2.73 | 2 | 0 | 1.43 | 1.46 | |
| No | N | 678 | 1,874 | 743 | 535 | 349 | 7 | 3,098 | 7,283 | |
| | % | 99.07 | 98.48 | 99.27 | 97.27 | 98.00 | 100 | 98.57 | 98.54 | |
| Total | N | 685 | 1,903 | 749 | 550 | 355 | 7 | 3,143 | 7,391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| **Obtaining information from Health Facilities** | | | | | | | | | | |
| Yes | N | 66 | 77 | 36 | 46 | 41 | 0 | 227 | 492 | 0.001 |
| | % | 9.57 | 4.03 | 4.84 | 8.31 | 11.41 | 0 | 7.23 | 6.65 | |
| Yes | N | 619 | 1,826 | 712 | 504 | 315 | 7 | 2,916 | 6,899 | |
| | % | 90.43 | 95.97 | 95.16 | 91.69 | 88.59 | 100 | 92.77 | 93.35 | |
| Total | N | 685 | 1.902 | 747 | 550 | 356 | 7 | 3.143 | 7,391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| **Obtaining information from the Village Clinic** | | | | | | | | | | |

***Cont...*** **Table 2. Percentage of contraceptive use plans for infertile women aged 15-49 years based on reinforcing factors**

| | | IUD | Women's Sterilization | Men's Sterilization | Periodic absence | Implant | LAM | Injection | Total | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| Yes | N | 4 | 5 | 0 | 0 | 0 | 0 | 0 | 9 | 0.116 |
| | % | 0.59 | 0.29 | 0 | 0 | 0 | 0 | 0 | 0.13 | |
| No | N | 681 | 1,897 | 745 | 550 | 356 | 7 | 3,143 | ,.381 | |
| | % | 99.41 | 99.71 | 100 | 100 | 100 | 100 | 100 | 99.87 | |
| Total | N | 685 | 1,903 | 749 | 550 | 356 | 7 | 3,143 | 7,391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| **Obtaining Information from the Pharmacist** | | | | | | | | | | |
| Yes | N | 48 | 159 | 55 | 41 | 26 | 0 | 334 | 663 | 0.023 |
| | % | 7.04 | 8.33 | 7.41 | 7.41 | 7.32 | 0 | 10.64 | 8.98 | |
| No | N | 637 | 1,744 | 693 | 509 | 330 | 7 | 2,808 | 6,727 | |
| | % | 92.96 | 91.67 | 92.59 | 92.59 | 92.68 | 100 | 89.36 | 91.02 | |
| Total | N | 685 | 1,905 | 749 | 550 | 356 | 7 | 3,143 | 7,391 | |
| | % | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| Note: Total N is the total number of samples of women of childbearing age 15-49 years who were weighted | | | | | | | | | | |

Based on table 2 it can be seen that most respondents had plans to use injection contraception. However, some of the respondents did not get family planning information from Family Planning Field Officers, from health facilities, from village clinics or pharmacists. In other research, showed that there was a correlation with education (p value = 0.001), knowledge (p value = 0.036) and the role of health workers with (p value = 0.034) with the use of IUDs. Advice for health workers by increasing counseling about contraception on an ongoing basis, either individually or in groups, on various types of contraceptives to increase knowledge[20]. Another research, showed that role of health workers in providing family planning counseling with the use of contraceptives p=0.009 ($\alpha$<0.05) means that there was a relationship between the role of health workers in providing family planning counseling with the use of contraception in women at Primary health care of Rafae. There was also found r = 0.348, which means the level of correlation between the variables role of health workers in providing counseling of family planning with the use of contraception in women have low cohesion[21].

## Conclusion

The use of mass media and social support related to the choice of contraceptive plans. Therefore, it is necessary to optimize the role of health workers and media access in the successful implementation of contraception programs.

**Conflict of Interest** : The authors declare that there is no conflict of interests.

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**Ethical Clearance:** Procedures and questionnaires for standard DHS surveys have been reviewed and approved by ICF Institutional Review Board (IRB). Additionally, country-specific DHS survey protocols are reviewed by the ICF IRB and typically by an IRB in the host country. ICF IRB ensures that the survey complies with the U.S. Department of Health and Human Services regulations for the protection of human subjects (45 CFR 46), while the host

country IRB ensures that the survey complies with laws and norms of the nation. DHS Program has collected, analyzed, and disseminated accurate and representative data on population, health, HIV, and nutrition through more than 400 surveys in over 90 countries

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# Successful Treatment of Cerebral Tuberculoma and Tuberculous Lymphadenitis in an HIV/AIDS Patient: A Case Report

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## Abstract

Tuberculosis is one opportunistic infection that may occur in HIV/AIDS patients. Its clinical presentation may depend on infected organs. A 37-years old man complained of left limb weakness followed by mild fever, weight loss of more than 10 kgs within two months, and swelling in his left submandibular region. Laboratory tests showed mild anemia, hypoalbuminemia, and positive serology HIV test. Histopathological examination of neck swelling showed inflammation of chronic granulomatous tuberculous with acid-fast bacilli positive in Ziehl Nielsen staining. Head CT scan with contrast, showed multiple isodense lesions with rim contrast enhancement accompanied by perifocal edema. Anti-Tuberculosis Drug and Anti-Retroviral Therapy were given as a combination treatment for this patient. Clinical and radiological improvement in the patient indicated good outcome and successful treatment.

***Keywords:*** *Cerebral Tuberculoma, Tuberculosis, HIV/AID*

## Introduction

Tuberculosis (TB) is the most common opportunistic infection in HIV/AIDS patients. HIV-TB co-infection increases the frequency of extra-pulmonary TB involvement, such as respiration, digestive, lymphatic, and neurologic localization.[1] Clinical presentation of TB may depend on infected tissue.[2]

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Lymphadenopathy presents about 2-5% among all cases in Mycobacterium tuberculosis infection of lymphatic tissue, as cervical nodes are the most frequently involved.[3]

While the central nervous system (CNS) TB has been reported ten times higher in patients with HIV/AIDS infection, its mortality exceeds 50%. The focal form of CNS TB could be tuberculoma or an abscess.[4]

Due to the clinical manifestation of HIV-TB co-infection may vary and atypical with high mortality and morbidity, we present this case report emphasized in Cerebral Tuberculoma and Tuberculous Lymphadenitis in HIV/AIDS patients.

### Case Description

A 37-years old man came to the emergency department of Dr. Soetomo Hospital with complaints

of left limb weakness 1 week before admission. He also complained of decreased appetite followed by weight loss of more than 10 kgs within 2 months and mild fever.

Other complaints were swelling in his left submandibular region, 3 months before admission. The swelling was initially small then gradually increasing its size until the supraclavicular region and produced amounts of pus. The swelling had been treated by surgical procedure and the sample had been done with a histopathological examination. It is reported tuberculous lymphadenitis.

He had been given an Anti-Tuberculosis Drug (ATD) in the form of 4 tablets of Fixed Drug Combination (FDC) that consist of Rifampicin, INH, Pyrazinamide, and Ethambutol every 24 hours. However, this patient only took those medications for about 2 months as no improvement felt by the patient.

On physical examination, the blood pressure was 110/70 mmHg, pulse 105 times/ minute, respiratory rate 20 times/ minute, axillary temperature 36.5$^{o}$C, bodyweight 55 kgs. General appearance weak, anemic, oral thrush (+), Examination of neck region ulcer in the left side (5 x 8 cm), irregular, surrounded by redness, slough (+), pus (-). Neurological examination revealed GCS 456, negative meningeal sign, normal cranial nerve examination, normal reflexes with negative Babinski signs, motoric strength examination of right upper-lower limb is 5 and left upper-lower limb is 2.



**Figure 1. Ulcer appearance in**

**Left supraclavicular region**

Laboratory tests showed Hb 8.1 g/dL, MCV 82.6 fL, MCH 28 pg, MCHC 26.8 g/dL, leukocytes 6.150/mm$^3$, neutrophils 61.6%, platelets 265.000/mm$^3$, AST 16 U/L, ALT 22 U/L, albumin 2.4 g/dl, BUN 9 mg/dL, creatinine 0.7 mg/dL, sodium 140 mmol/L, Potassium 3.7 mmol/L, positive HIV serology, and negative IgG/IgM Toxoplasma.

Histopathological examination of ulcer showed inflammation of chronic granulomatous tuberculous, with acid-fast bacilli positive obtained from Ziehl Nielsen staining technique.

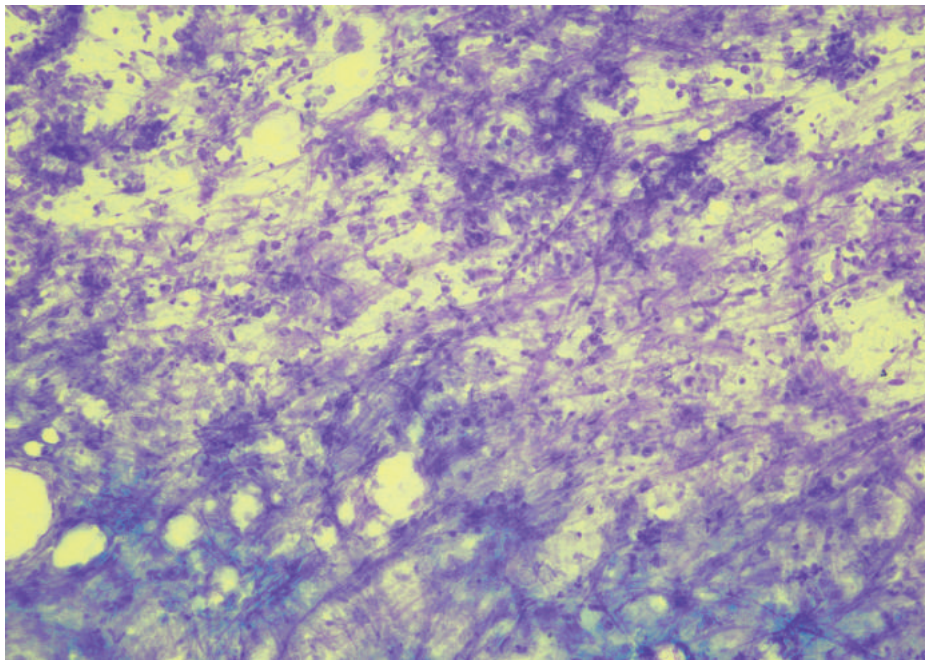

**Figure 2. Chronic granulomatous tuberculosis inflammation from histopathologic examination of neck ulcer**

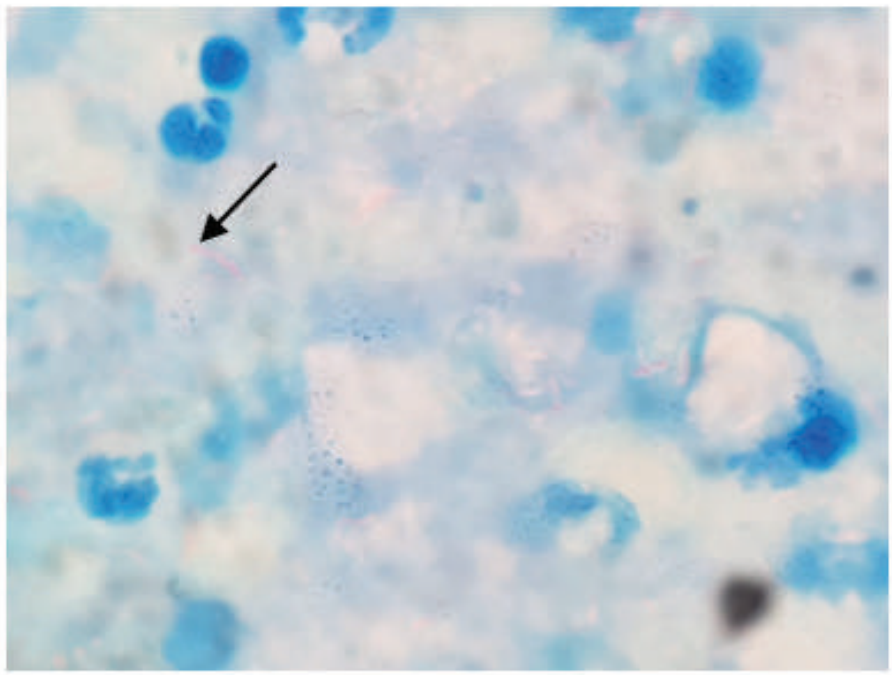

**Figure 3. Acid Fast Bacilli from**

**Ziehl Nielsen Staining**

This patient had been performed a head CT scan with contrast, it showed multiple isodense lesions with rim contrast enhancement accompanied by perifocal edema. The size approximately 1.5 x 1.5 x 1.7 cm in the posterior side of the right parietal lobe and 0.7 x 0.5 cm in the right lentiform nucleus leading to the appearance of tuberculoma.

Regarding the clinical presentation and other examination, this case is reported as HIV/AIDS and lost to follow-up (default) tuberculous lymphadenitis and cerebral tuberculoma.

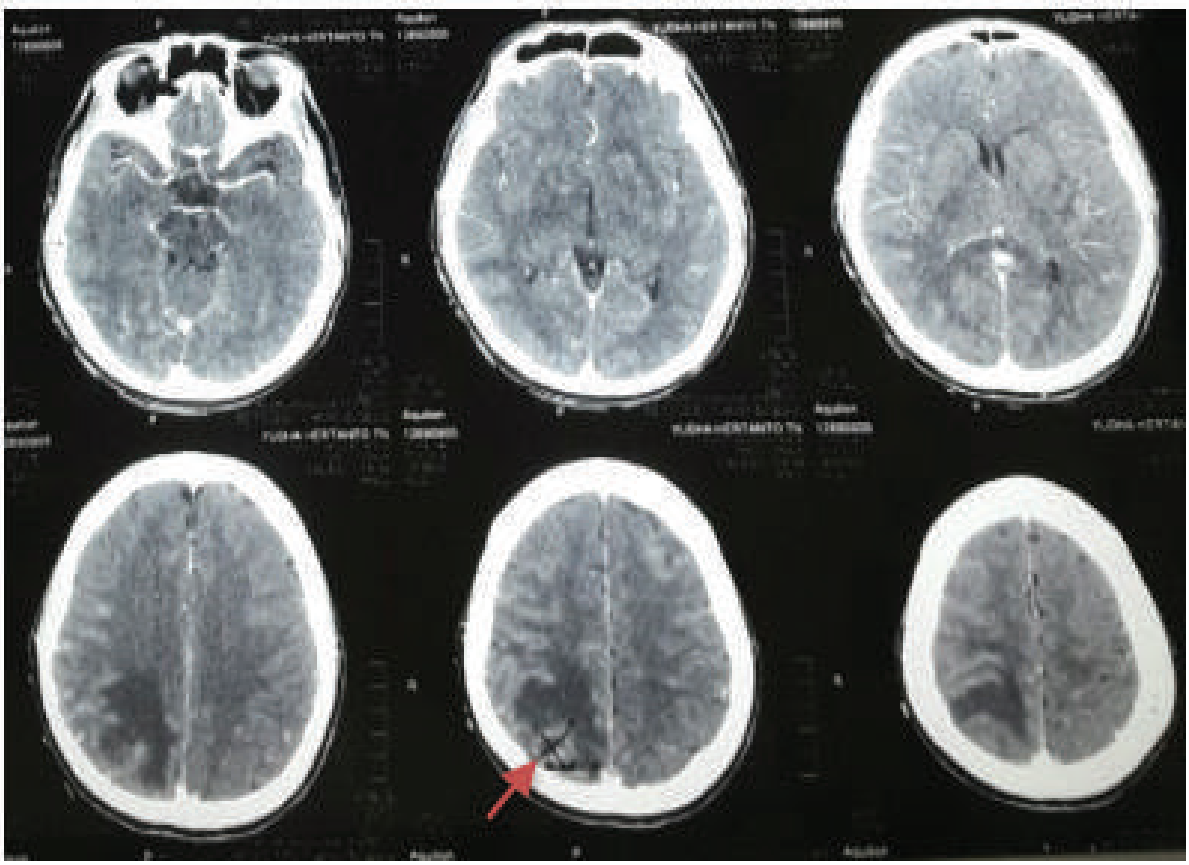

**Figure 4. Rim contrast enhancement accompanied by perifocal edema**

This patient was given Streptomycin injection 1 gram intramuscular every 24 hours for 2 months, ATD 4 Fixed Drug Combination (4FDC) (Rifampicin 150 mg, INH 75 mg, Pyrazinamide 400 mg, Ethambutol 275 mg) 4 tablets every 24 hours for 3 months, Cotrimoxazole 960 mg every 24 hours orally, done wound care three times a week, and medical rehabilitation.

The patient was started to get Anti-Retroviral Therapy (ART) in the form of FDC (Tenofovir 300 mg, Lamivudine 300 mg, Efavirenz 600 mg) fourteen-day after the first day of ATD administration. On the 30$^{th}$ day of treatment, any progress occurred to this patient: improvement of neck ulcer and left motoric strength.



**Figure 5. Neck ulcer appearance after 30th day of treatment**

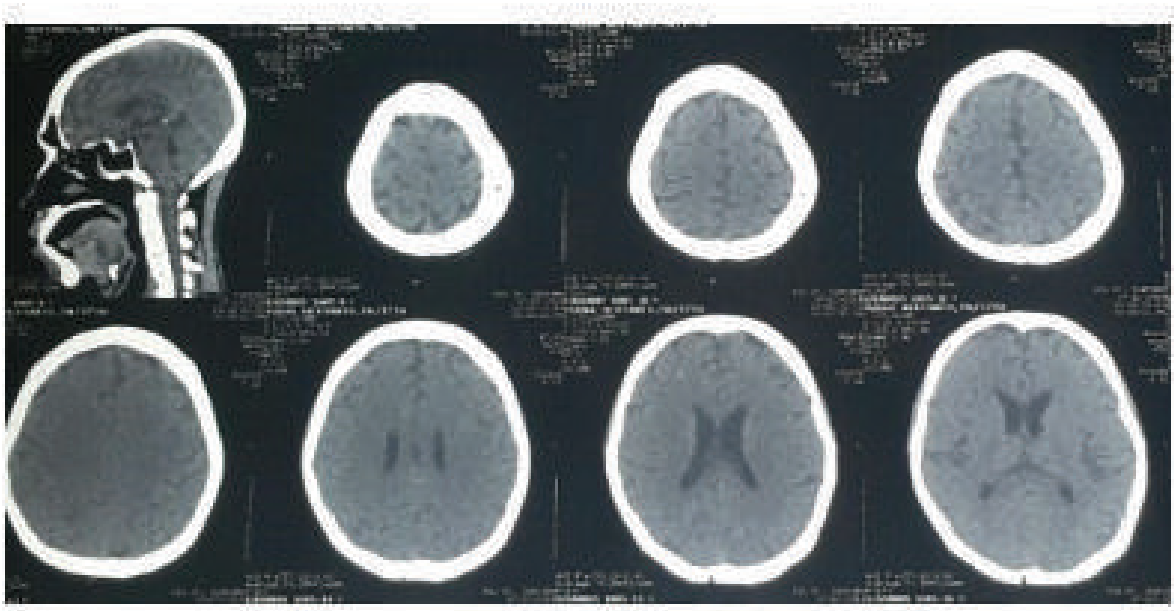

**Figure 6. Head CT-scan with contrast after treatment, no rim contrast enhancement was detected**

## Discussion

HIV-TB co-infection leads to challenges in both diagnosis and treatment. WHO recommendation regarding HIV patient was doing TB screening at the time that HIV infection is diagnosed.[5] Extra-pulmonary TB (EPTB) is associated with HIV infection. The clinical presentations may vary depending on the affected organs such as neck stiffness in TB meningitis, chest pain in pleural TB (pleurisy), enlarged superficial lymph nodes in TB lymphadenitis, and spinal deformity in TB spondylitis. Diagnosis method of extra-pulmonary TB is based on bacteriological and/or histopathological examination on affected body tissue.[6-9]

Tuberculous lymphadenitis may occur during primary tuberculous infection or as a result of reactivation of dormant foci or direct extension from a contiguous focus. Primary infection occurs on inhaled droplet nuclei which contain tubercle bacilli to pass mucociliary defenses of bronchi and lodge in terminal alveoli of the lungs. The Mycobacterium tuberculosis multiplies in the lung which is called ghon focus. The lymphatic drains the bacilli to the hilum lymph nodes, and it turns to be the primary complex. The infection may spread from primary focus to regional lymph nodes. The bacilli continue to spread via the lymphatic system or may pass through the nodes to reach the bloodstream, subsequently, it disperses to other organs in the body.[10]

This patient had performed the histopathological examination, as the result showed chronic inflammatory granulomatous tuberculous with acid-fast bacilli detected in Ziehl Nielsen staining, which indicates this lymphadenitis caused by tuberculosis infection.[2]

Another manifestation of EPTB is central nervous system involvement. The clinical spectrum of CNS tuberculosis includes meningitis, abscess, and tuberculoma. CNS involvement occurs in 10–20% of patients with AIDS-related tuberculosis, and mortality in these patients is high.[11] CNS tuberculosis are obtained by performing a CT scan examination to identify the location, rim contrast enhancement, perifocal edema, or midline shift. In case of the intracranial mass lesion within the posterior fossa of the brain, MRI should be done.[12, 13]

Appearances of the focal lesion with rim contrast enhancement, mass effect, and perifocal edema which are obtained from head CT scan of HIV/AIDS patient could be suspected either toxoplasmosis infection, cerebral tuberculoma, primary CNS lymphoma, or pyogenic brain abscess.[12-15] Rapid detection of HIV/AIDS-associated opportunistic infection in CNS is crucial to determine causative organism of CNS lesion. This patient has a history of tuberculosis, which is confirmed by examining a lymphadenitis sample. Thus, clinical presentations tend to cerebral tuberculoma.[15]

Treatment for EPTB does not differ significantly between patients with or without HIV/AIDS infection. However, the ATD regimen should be given 2-4 weeks before ART.[13] In this case, we determine this type of patient is the default (lost to follow-up) case because this patient had been given tuberculosis standard treatment for about 1-2 months yet later on lost to follow up.[2, 7-9]

ATD regimen is prescribed to this patient. It began with 1 gram of Streptomycin injection via intramuscular daily for a month, 4 Fixed Drugs Combination (150 mg of Rifampicin, 75 mg of INH, 400 mg of Pyrazinamide, 275 mg of Ethambutol) 4 tablets every 24 hours. This regimen should be completed for 12 months.[2]

First-line ART followed the ATD regimen after 2 weeks of EPTB treatment. Efavirenz is one of the NNRTI (Non-Nucleoside Reverse-Transcriptase Inhibitor) groups which is recommended since it has mild interaction with Rifampicin compared to Nevirapine. Fixed Drugs Combination ART which available in Indonesia are 300 mg of Tenofovir, 300 mg of Lamivudine, 600 mg of Efavirenz, these FDCs are commonly used as first-line ART in Indonesia.[7-9]

The outcome of combination treatment between ART and ATD in this patient has remarkably favorable. Another case report of HIV-TB coinfection who received this combination also shows good response to ART and ATD.[16] Cerebral tuberculoma of this patient has completely vanished during treatment.

## Conclusion

Concurrent infection of HIV/AIDS and TB is a notable consideration for HIV patient management. This case report demonstrates a good outcome of ART and ATD combination therapy for cerebral tuberculoma and tuberculous lymphadenitis in HIV/AIDS patients. The diagnostic procedure also plays an important role to be performed in HIV/AIDS patients. Overall, the clinician should understand the knowledge of opportunistic infection in HIV/AIDS patients properly, so that mortality and morbidity could be declined substantially.

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**Funding**: None.

**Ethical Clearance**: Not required for a case report.

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# The Balanced Score Card in Improving Performance in the Health Care Sector: A Literature Review

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## Abstract

This paper is a literature review that discusses the use of the Blanced Score Card (BSC) to improve organizational performance, especially in the field of health services. The purpose of this study is to explore and connect theories from various studies. This paper identifies 21 articles with related topics published in the last decade. The results in this study indicate that BSC has been used in the health sector but is mostly used in the hospital sector, amounting to 87.5% (measuring performance against national standards and guidelines in the essential package of hospital services, assessing the quality of health services to strengthen health services in developing a country, integrated health care in hospitals, measuring service quality with the cost of patient care in the hospital, evaluate hospital performance, evaluation of the main performance of the hospital as an effort to achieve the hospital's strategic goals efficiently) and in part small bada in the field of pharmaceutical services by 12.5% (measuring and evaluating the performance of pharmacies and stressing patient-centered services). While in other health service sectors there has not been any research that causes this cause based on this research it can be used to develop other research to examine the effectiveness of the use of BSC in the broader health service sector such as national health insurance, basic health services, or at the ministry or agency health.

***Keywords:*** *BSC, health care sectore, performance.*

## Introduction

Management strategy is very important in decision making to determine the life span of the health service sector. Health services are currently experiencing many complaints, especially related to the behavior of patients who are increasingly demanding and dissatisfied with the services provided. Therefore, the role of strategic management, especially related to strategic instruments that can improve the performance of hospital services, is important to be studied [1]. The health care sector is a complex organization that has a lot of professional staff and resources in various medical therapy services, so a systematic measurement of performance indicators is needed. For this reason, the Balanced Scorecard (BSC) tool is used to measure performance management or performance performance effectively and efficiently in the hospital [2]. Balanced scorecard (BSC) makes a company's strategic target into a balanced performance evaluation index, in this way a reliable performance evaluation system is built to achieve strategic targets. Evaluation of company performance is divided into four indexes, including finance, customer satisfaction,

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internal processes, innovation and improvement [3–5]. So it is interesting to do a review whether the BSC is really able to improve performance, especially in the health service sector.

## Literature Review

The management strategy model of a company describes the way the company conducts its business, it describes the interdependent activities carried out by the company and by its partners [6]. The company will not be able to compete for a long time if it does not have a management strategy [7]. The health care sector is a complex organization that has a lot of professional staff and resources in various medical therapy services, so a systematic measurement of performance indicators is needed. For this reason, the Balanced Scorecard (BSC) tool is used to measure performance management or performance performance effectively and efficiently in the hospital [2]. Balanced scorecard (BSC) makes a company's strategic target into a balanced performance evaluation index, in this way a reliable performance evaluation system is built to achieve strategic targets. Evaluation of company performance is divided into four indexes, including finance, customer satisfaction, internal processes, innovation and improvement [3–5].

The BSC is an instrument developed by the Harvard Professor in management, namely Kaplan and Renaissance [8]. This tool is applied by analyzing four main aspects, namely finance, customers, internal business processes, learning and growing to measure organizational and individual performance and then evaluated to improve organizational and individual performance. So that the BSC is a management tool that is able to create a multi-perspective organizational strategy and is a system for measuring organizational performance [8,9]. If viewed from the Fred David Strategic Model, the management strategy is divided into 3 stages which include strategy formulation, strategy implementation and strategy evaluation, strategy formulation consists of developing a vision and mission, auditing. Internal and external environment, long-term goals for formulating strategies. The second stage is strategy implementation, setting annual goals, formulating policies for each business function and allocating resources for organizational achievement. The third stage is a strategy evaluation. This stage is carried out by conducting work evaluations and taking corrective action at each stage of the strategy. BSC is a strategy tool that can be used from the strategy formulation stage to strategy evaluation [10].

Several previous studies conducted at health facilities in the Lubin area showed that 71.4% of health facilities had implemented a management strategy and 28.6% had not implemented a management strategy. Health facilities that have implemented management strategies use methods such as SWOT analysis 64.2%, break-even point analysis 42.9%, PEST analysis 14.3% and none of the health facilities strategy research uses analysis with a Blanced Scoredcard which indicates a lack of knowledge of related managers. Tools of strategic management [1]. Based on the research above (Jaworzynska, 2017) it is known that the BSC is one of the tools that has been used in strategic management in the field of health services. BSC measures management performance from financial and non-financial dimensions [1]. So in addition to providing financial performance indicators as usual given by the accounting function, the BSC also provides performance indicators other important dimensions are not financial such as customer satisfaction, customer retention, acquisition of new customers, service time (delivery time), quality, job satisfaction, skill mastery level, market segment [11].

BSC will be able to work well if its implementation is supported by an organizational culture that is willing to accept new approaches so that the organization will be able to find new things to measure and new goals in various fields to achieve organizational growth [12]. The advantage of this method is to consistently guide managers, departments, human resources, technology and financial resources towards organizational strategy. The disadvantages of this method are that

it creates high costs and the lack of expertise in the use of this instrument causes obstacles to appear in its implementation. Balanced scorecard (BSC) is proven to be able to improve aspects of performance, strategy, alignment, communication, resource allocation, decision making and competitiveness [13]. Research related to BSC analysis in health facilities that has been conducted includes measuring performance against national standards and guidelines in the essential package of hospital services [14]. BSC is also used to assess the quality of health services to strengthen health services in developing a country [15].

## Method

This paper is a literature review that discusses the use of the Balanced Score Card (BSC) in improving performance, especially in the health sector. The articles are collected by google database, google scholar and mendeley. Reviews are carried out on articles by topic about Balanced Score Card (BSC) in improving performance, especially in the health sector. We identified about 21 articles published in the last decade and analyzed them and drew conclusions.

## Results and Discussion

Based on several studies, it shows that the use of BSC in the health service sector has been widely used in measuring and improving the performance of health services. The use of BSC in health includes measuring performance against national standards and guidelines in the essential package of hospital services, assessing the quality of health services to strengthen health services in developing a country, integrated health care in hospitals, measuring service quality with the cost of patient care in the hospital, evaluate hospital performance**,** evaluation of the main performance of the hospital as an effort to achieve the hospital's strategic goals efficiently, measuring and evaluating the performance of pharmacies and stressing patient-centered services. So that if a resume is carried out based on the research, it can be shown in the table below

**Tabel 1: Summary of the use of the BSC in the health service sector**

| Sector | Percentage (%) | The role of the BSC |
|---|---|---|
| Hospital Service | 87,5% | measuring performance against national standards and guidelines in the essential package of hospital services |
| | | assessing the quality of health services to strengthen health services in developing a country |
| | | assessing the effectiveness and efficiency of the hospital strategic plan combined with the AHP method |
| | | measuring service quality with the cost of patient care in the hospital |
| | | integrated health care in hospitals evaluation |
| | | evaluation of the main performance of the hospital as an effort to achieve the hospital's strategic goals efficiently |
| | | tools for strategic planning in hospitals |
| Pharmacy service | 12,5% | measuring and evaluating the performance of pharmacies and stressing patient-centered services |

Source : [14–21].

Based on the summary of the various studies above, it is known that the BSC is a health service performance evaluation tool but can also be used as a strategic management tool to achieve organizational goals. Most of the BCS implementation in the health sector was applied to the hospital sector and a small part in the pharmacy service sector. So based on this research it is still possible to carry out various other studies to use BSC, for example in the field of national health insurance, basic health services, the ministry of health, the pharmaceutical industry and other health sectors.

## Conclusion

The BSC is a health service performance evaluation tool but can also be used as a strategic management tool to achieve organizational goals. Based on this review, it is known that most of the use of BSC in the health service sector is in the hospital sector amounting to 87.5% (measuring performance against national standards and guidelines in the essential package of hospital services, assessing the quality of health services to strengthen health services in developing a country, integrated health care in hospitals, measuring service quality with the cost of patient care in the hospital, evaluate hospital performance**,** evaluation of the main performance of the hospital as an effort to achieve the hospital's strategic goals efficiently) and a small portion in the pharmacy service sector by 12.5% (measuring and evaluating the performance of pharmacies and stressing patient-centered services). This is a research opportunity to develop the use of BSC in other health fields.

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# A Patient with AIDS and Embolic Stroke: A Case Report

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## Abstract

Human immunodeficiency virus (HIV) infection is a global concern. Globally, 36.7 million people are living with HIV and 1 million people died at the end of 2016. There is an association between human immunodeficiency virus (HIV) infection with neurological manifestations. Between 1% and 5% of patients with HIV develop stroke. The pathogenesis of AIDS with stroke is still controversial. A 37-year-old male patient with AIDS had an embolic stroke. The diagnosis of the patient was based on clinical condition and the CT scan results of the head with contrast. The stroke-causing mechanism in patients is HIV-associated vasculopathy that results from accelerated atherosclerosis. After administration of antiplatelet and statin, the patient's condition improves.

***Keywords:*** *Embolic Stroke, Vasculopathy, Atherosclerosis, HIV*

## Introduction

Human immunodeficiency virus (HIV) infection is a global issue. There are approximately 36.7 million people in the world infected with HIV in 2016 and 1 million people who die each year[(1)]. People with HIV / AIDS have a higher risk of developing neurological disorders and the most common neurological disorder is ischemic stroke. [(2),(3)]

HIV patients who had a stroke were recorded at 1-5%, and 4-34% of HIV patients had ischemic stroke lesions at the time of autopsy. In the USA, the incidence of ischemic stroke in HIV patients reaches 5.27 per 1000 population compared to 3.75 in patients without HIV. This number has increased by 43% in the last 9 years. [(4),(5),(6)]

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Stroke has a high mortality rate and reduces the quality of life of the sufferer. WHO in 2002 recorded that 15 million people in the world suffer from strokes each year, with 5 million of them causing death, while the other 5 million have permanent disabilities. The pathogenesis and management of AIDS sufferers who experience a stroke are still being debated. [(4),(7)]

Seeing the high incidence of stroke in HIV / AIDS patients and the magnitude of the problems that occur, the authors would like to discuss more about stroke in HIV / AIDS patients.

## Case Description

Mr. M.N., age 37 y.o., came to the emergency department of Dr. Soetomo Hospital complained of weakness of right extremities. The patient presented with acute right hemiplegia with the difficulty of speaking 3 days before admission after woke up in the morning. He denied seizures, nausea, vomiting nor decreased of consciousness. He hadn't complained of diarrhea, headache, or cough. He complained difficulty of eating and drinking. He also felt pain when swallowing. Weight decreased from 55 kg to 48

kg in the last 1 year.

Patients were diagnosed with HIV / AIDS since 10 years ago. History of stroke 2 months before admission and treated in Sidoarjo hospital. No history of diabetes mellitus, hypertension, heart disease, and blood disorders. There is a history of injecting drug use. Freesex is denied. The patient has married with 1 child. Neither his wife nor his child is infected.

On physical examination, body-weight 48 kg, body-height 161 cm, BMI 18,5 kg/m2. Blood pressure 110/80 mmHg, heart rate 92 bpm, breathing 20x/minutes, axillary temperature 36.5$^0$C, oxygen saturation 99% room air. No enlargement of a lymph node, anemia, icterus, and cyanosis. Examinations of heart, lung, abdomen were normal. There was right hemiplegia with 0 motoric scores for the right extremities.

From the laboratory examination, hemoglobin 13 g/dL, leucocyte 8.97x10^3/uL, thrombocyte 255x10^3/uL, HbsAg and Anti-HCV non-reactive, total cholesterol 156 mg/dl, triglyceride 164 mg/dl, HDL 22 mg/dl, LDL 108 mg/dl, AST 27 u/l, ALT 31 u/l, BUN/creatinine 17/0.72 mg/dl, CD4 9 cell/uL. Head CT scan with contrast: subacute-chronic tromboembolic cerebral infarction in left fronto-temporo-parietocipital lobe, left putamen and left nucleus caudatus (left MCA and LSA). Echocardiogram and echocardiography within normal limit.

The patient was diagnosed with AIDS with a second attack of embolic stroke. As treatment, patient received high calories and high proteins diet 2100 kcal/day, Cytidine 5'-diphosphocholine 500 mg every 12 hours (iv), acetylsalicylic acid loading dose 300 mg continued 100 mg daily (PO), Simvastatin 20 mg per day at night (PO) and fixed-dose combination (FDC) of antiretroviral therapy (Tenofovir/TDF 300mg, Lamivudine/3TC 150mg efavirenz/EFV 600mg) 1tab/day (PO). After 1 week, the patient could move his extremities with 3 motoric scores for the right extremities.

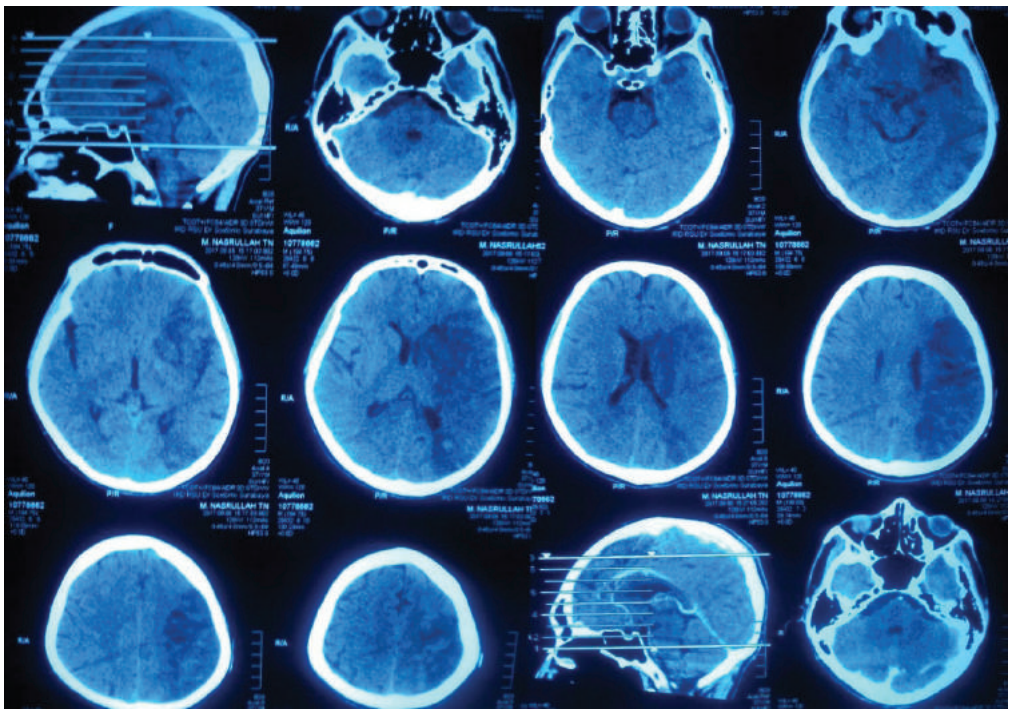

**Figure 1: Head CT scan with contrast. Subacute-chronic thromboembolic cerebral infarction in left fronto-temporo-parietocipital lobe, left putamen, and left nucleus caudatus (left MCA and LSA).**

## Discussion

AIDS is a collection of symptoms or diseases caused by decreased immunity due to infection by the HIV which belongs to the retroviridae family where AIDS is the final stage of HIV infection [8],[9]. The classification of HIV / AIDS stages based on WHO can be seen in table 1.

Our patient had a history of HIV / AIDS with difficulty swallowing and wasting syndrome so we diagnosed him with AIDS.

Stroke is a brain injury caused by a sudden disruption of blood supply to the brain. The diagnosis of stroke is based on clinical, focal neurological disorders and based on imaging studies. The stroke itself is classified into ischemic, hemorrhagic stroke, and transient ischemic stroke (if the symptoms are less than 24 hours). [10],[11]

Our patient had a history of stroke and focal neurological disorders in the form of weakness of the right extremities with a motor score = 0 and positive pathological reflexes. Head CT scan revealed subacute-chronic thromboembolic cerebral infarction in the left fronto-temporo-parietoocipital lobe, putamen, and left caudate nucleus (according to MCA and left LSA territories). So our patient was diagnosed with AIDS with a second attack embolic stroke.

The mechanism of stroke in HIV infection includes opportunistic infection, vasculopathy, cardioembolism, and coagulopathy[4]. The mechanism by which stroke occurs in HIV infection determines the necessary therapy. This mechanism can be seen in table 2.

The patient had an embolic stroke. There are no signs of infection or neoplasm that can cause a stroke. Cardiovascular examinations were within normal limits so that the cause of a stroke is suspected associated with vasculopathy which is accelerated atherosclerosis.

The pathogenesis of HIV-related vasculopathy occurs due to the role of inflammation and remodeling of blood vessel walls. Direct exposure to HIV and viral particles to the endothelium of blood vessels cause endothelial dysfunction. Indirect damage to the endothelium occurs due to the migration of infected monocytes to the endothelium of blood vessels. The CCL2 chemokine released by infected monocytes attracts leukocytes even more. This condition causes inflammation of the blood vessels resulting in upregulation of adhesion molecules that increase the adhesion of both infected and uninfected leukocytes, as well as the release of HIV virions into the smooth muscle of blood vessels and active replication of the virus in the blood vessel walls. The resulting inflammatory cytokines will also increase the production of reactive oxygen species (ROS), disruption of the coagulation system, and occur remodeling blood vessel walls. Remodeling of blood vessels in the form of hyperplasia of the tunica intima and elastic lamina, formation of atherosclerosis, and triggering thrombotic occlusion. Administration of antiviral drugs (ARVs) can also cause endothelial dysfunction. This process is thought to occur due to autoreactive self-destruction due to an increase in the autoimmune system.[12],[13]

The management of HIV patients with embolic stroke is the same as that of stroke patients in general. Based on the 2014 AHA / ASA guidelines, the management of stroke caused by stenosis of the intracranial large arteries is by administering aspirin 325 mg/day. Aspirin is recommended over warfarin. If the blockage is very severe (70-90%), clopidogrel 75 mg can be added for 90 days although currently there are no studies regarding dual antiplatelet administration. In addition, the systolic blood pressure was kept below 140 mmHg. Statin is recommended as a preventative for recurrent stroke events. Invasive measures such as stenting, angioplasty, and thrombolysis drugs are controversial and are not currently recommended. ARV drugs are still controversial because they can also lead to endothelial dysfunction. But given the

enormous benefits it has for HIV therapy and the many other mechanisms that can trigger stroke in HIV patients, it is currently recommended to keep it.[4),(11)]

Patients received therapy: head-up position $30^0$, high calories and high protein diet, NaCl infusion of 0.9% 1000 ml per 24 hours, injection of 500 mg Cytidine 5'-diphosphocholine every 12 hours. Patients have also given an oral loading dose of 300 mg acetylsalicylic acid followed by maintenance of 100 mg acetylsalicylic acid per day, paracetamol 500 mg per 8 hours, and simvastatin 20 mg per day at night. In addition, we provide ARV FDC once per day.

Therapy for patients is following the 2014 AHA / ASA guideline, where we provide antiplatelet in the form of acetylsalicylic acid with a loading dose of 300 mg followed by maintenance of 100 mg per day with a statin. We don't give anti-hypertensive drugs because the patient's systolic blood pressure is below 140 mmHg. ARV drugs are also given to patients. In the course of the patient's condition improved.

## Conclusion

A 37-year-old male patient with AIDS accompanied by an embolic stroke was reported. The patient's diagnosis was made based on clinical and head-contrast CT scan results. The mechanism that causes stroke in patients is HIV-associated vasculopathy which occurs due to accelerated atherosclerosis. After administering antiplatelet and statins, the patient's condition improved.

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*Article type: Research article*

# Association between Cortisol and Infection Risk of Children with Acute Lymphoblastic Receiving Induction and Consolidation Chemotherapy in Dr. Soetomo General Hospital Surabaya

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## Abstract

**Background:** Mortality due to Infection associated therapy in acute lymphoblastic leukemia (ALL) children remains high, although therapeutic success and survival rate are substantially improving.

**Methods:** This study used a pre and post-test group design for children aged less than 18 years with ALL newly diagnosed and receiving chemotherapy in pediatric patient of Dr. Soetomo hospital Surabaya.

**Results:** The study involved 25 subjects, 10 subjects were male, average age ranged from 3 to 9 years. The high risk-ALL subjects were 15 (60%), hyperleukocytosis 4 (16%) subjects and 7 (28%) subjects with leukopenia. Statistical analysis showed a significant difference in decreasing cortisol levels in week-4 compared to week-0 ($p < 0.001$) and an increased in cortisol levels between week-12 compared to week-10. The incidence of infection during induction phase is higher than the consolidation phase (40vs15). The relationship between the mean cortisol levels and the incidence of infection in general showed a significant difference in the induction phase of week-0 ($p=0.029$), week-4 ($p=0.041$), and week-6 ($p=0.005$).

**Conclusion:** In the induction phase, there is an association between mean cortisol levels and the risk of infection, but there is no such association in the consolidation phase.

***Keywords:*** *Cortisol, Infection, Chemotherapy, Mortality, ALL children.*

## Introduction

Acute lymphoblastic leukemia (ALL) is a type of cancer that is found in most of the children's cancer cases[1]. Treatment success and survival rates in ALL children continuously improved over time. Despite advancements in therapeutic outcomes, however, treatment-related toxicity remains high (21.4%)[2]. The survival rate for ALL patients has increased since 1980. Patients diagnosed with ALL since 2005-2009 are estimated to have a 10-year survival rate of 88% -93%[3,4]. However, the mortality rate from cancer, as well as toxicity from side effects and infections associated with therapy, is still quite high[5,6]. According to the data obtained from the medical records of Dr. Soetomo hospital in June 2006-December 2010, it was found that 23.9% of child ALL patients died while completing induction phase chemotherapy.

Glucocorticoids have an important role in the therapy of ALL in children by inhibiting growth and inducing lymphoblast apoptosis[7,8,9]. Genomic and non-genomic effects will increase with increasing doses given. Glucocorticoid therapy is given during the induction phase at high doses (Prednisone 40-60mg / m2 / day or Dexamethasone 6mg / m2 / day). Long-term, high-dose administration of glucocorticoids can suppress the hypothalamus-pituitary-adrenal axis (HPA). Reduced cortisol levels in the body can reduce anti-inflammatory effects and increase pro-inflammatory cytokines, increasing infection risk. Adrenal suppression triggered by corticosteroid induction may be a major factor in this infection[10,11].

While studies on cortisol levels in Indonesia as a result of high-dose glucocorticoid therapy are still contradictory, few studies have related high-dose glucocorticoid administration to adrenal suppression, infection, and the time required for adrenal suppression to recover during the induction and consolidation phases. Based on these data, research was conducted in order to examine the relationship between cortisol levels and the incidence of standard and high-risk child ALL patients infection during the induction and consolidation phases, as well as to evaluate the effect of pediatric therapy management and outcome for ALL children in Dr. Soetomo hospital in Surabaya.

## Materials and Methods

This research examines the time periods of the induction and consolidation phases in a prospective observational analytic study with a pre and post-test group design. The study population was new patients diagnosed as high-risk and standard -risk ALL who received chemotherapy from the induction phase to the consolidation phase. Meanwhile, the sample of the study were children with new high-risk and standard-risk ALL who were treated in the inpatient room of the Hematology at Pediatric ward of Dr. Soetomo hospital who received chemotherapy until the consolidation phase at the time of the study and met the inclusion criteria.

Furthermore, the results of blood tests (cortisol) were reported in ALL patients without intervention or treatment of the chemotherapy protocol used at SMF Children's Health Sciences, Dr. Soetomo Surabaya's Hematology Oncology Division. The patient's progress will be monitored while they are undergoing chemotherapy at Pediatric ward of Dr. Soetomo hospital in Surabaya.

## Results and Discussion

A total of 39 pediatric patients diagnosed with ALL and receiving care at the IRJ Pediatric Hematology-Oncology Department of Pediatrics, Dr. Sutomo Surabaya from June 1, 2019 to August 31, 2019. There were 25 patients who met the eligibility criterion for this study, while the remaining patients were not considered due to exclusion and drop-out criteria. There were 8 patients who stopped taking their medications after being diagnosed with ALL, and 6 patients died during the observation phase.

Following the diagnosis of ALL, the patient's cortisol level was tested for the basic cortisol level at week-0 (W0) before the patient received prednisone or dexamethasone, which formed the basis for the patient's cortisol level. The patient's blood was drawn seven times, week-0 (W0), at week-4 (W4), week-6 (W6), week-7 (W7) induction phase chemotherapy and at week-8 (W8), week-10 (W10) and week-12 (W12) consolidation phase chemotherapy. Throughout the course of the report, the occurrence of infection was tracked and registered. The study results were analyzed using patient characteristics, cortisol level profiles, and the rate of infection during the observation period. Prednisone and dexamethasone are two corticosteroids used in ALL treatment[12].

Cortisol levels were measured three times during the induction phase: before prednisone or dexamethasone treatment was administered at week-0 (W0), at week-4 (W4), and at week-6 (W6). The results revealed a cross reaction with prednisone treatment, which patients regularly took at 07.00 in the morning, obscuring the significance of cortisol levels at the

time of review. Cortisol levels started to rise by 38% in the seventh week of healing. Adrenal suppression is described as a reduction in the patient's cortisol levels as a result of prednisone and dexamethasone exposure to the HPA axis, as shown by cortisol levels falling below the normal range of cortisol levels according to age.

Reduced cortisol levels in the body can reduce anti-inflammatory effects while activating pro-inflammatory cytokines, raising the risk of infection. Adrenal suppression caused by corticosteroid induction may be a factor in this infection[10,11]. According to Table 1, the prevalence of adrenal suppression was observed in four patients prior to undergoing chemotherapy, with the majority of cases occurring between weeks four and six of the induction period. There were no patients who suffered adrenal suppression during the consolidation process, but hypercortisol was observed in some patients.

**Table 1. Incidence of induction and consolidation phase of induction and consolidation phase of adrenal suppression in ALL-HR and ALL-SR patients.**

| Cortisol level profile | Number of patients (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | W0 | W4 | W6 | W7 | W8 | W10 | W12 |
| ∑ Suppression | 5 (20) | 13(52) | 14 (56) | 5 (20) | 0 (0) | 0 (0) | 0 (0) |
| ∑ Normal | 16(64) | 12(48) | 10 (40) | 15(60) | 23(92) | 2 (8) | 19(76) |
| ∑ Hyper | 4 (16) | 0 (0) | 1 (4) | 2 (8) | 2(8) | 4(16) | 6 (24) |
| Percentage of suppression (%) | 20 | 52 | 56 | 20 | 0 | 0 | 0 |

Many patients in the induction process were found to have infections during the follow-up time. The percentage of high-risk ALL patients who get infected is higher than the number of standard-risk ALL patients. Fever, conjunctivitis, parotitis, mucositis, acute otitis media, measles, diarrhea, bacterial exanthema, abscess cellulitis, candidiasis, urinary tract infections, and sepsis are also possible clinical complaints. Positive cultures were obtained in four ALL patients, two of whom had Acinetobacter baumannii (+) culture results and two of whom had ESBL (+) E. coli culture results. There was no adrenal crisis syndrome in the patient during the observation period, which included frequent clinical testing and laboratory examinations for each point of chemotherapy.

This research found statistically and clinically significant variations in cortisol levels between patients with infection and those who did not during the induction period of week 0 ($p = 0.029$) and week 6 ($p = 0.031$). This is also supported by the fact that the trust index range is narrow. The data also revealed that there was no statistically relevant relationship between cortisol levels and the rate of infection at the week-4, week-7, and week-10 stages. The following tables provide a rationale for the preceding research:

**Table 2. Statistical analysis of ALL-SR cortisol levels with the incidence of induction and consolidation phase infections**

| Data | Infection | n | Meana ± SD | p-valueb | CI 95% |
|---|---|---|---|---|---|
| Cortisol W-0 | Yes | 9 | 16,13 ± 3,85 | 0,029 | 10,76 (1,39-20,12) |
| | No | 1 | 5,37 ± 0,0 | | |
| Cortisol W-4 | Yes | 4 | 8,92 ± 3,70 | 0,056 | -5,43 (-11,09-0,24) |
| | No | 6 | 14,35 ± 1,28 | | |
| Cortisol W-6 | Yes | 2 | 3,51 ± 2,16 | 0,031 | -11,65((-21,95-(-1,34)) |
| | No | 8 | 15,16 ± 5,99 | | |
| Cortisol W-7 | Yes | 1 | 9,74 ± 0,0 | 0,428 | -9,82 (-36,95-17,31) |
| | No | 9 | 19,56 ± 11,16 | | |
| Cortisol W-8 | Yes | 0 | - | -* | -* |
| | No | 10 | 17,83 ± 8,21 | | |
| Cortisol W-10 | Yes | 1 | 12,55 ± 0,0 | 0,533 | -4,38 (-19,88-11,13) |
| | No | 9 | 16,93 ± 6,38 | | |
| Cortisol W-12 | Yes | 0 | - | - | - |
| | No | 10 | 22,40 ± 4,90 | | |

a. Cortisol level (μg/dL).

b. Significant if $p < 0.05$ with paired t-test statistical test.

* The data cannot be analyzed because one of the variables with a value of 0.

The table below shows the outcomes of statistical analyses comparing ALL-HR cortisol levels to the rate of infection during the induction and consolidation processes. Except at the week-4 cortisol stage, the findings revealed that there was no substantial change in cortisol levels in patients with the occurrence of infection in ALL-HR during the induction and consolidation processes. The Mann-Whitney research study on the cortisol week-4 hypothesis test for the prevalence of infection reported p value = 0.009, indicating that there is a substantial differential between those who have an infection and those that do not.

**Table 3. Statistical analysis of ALL-HR cortisol levels with the incidence of infection in the induction and consolidation phase**

| Data | Infection | n | Meana ± SD | p-valueb | CI 95% |
|---|---|---|---|---|---|
| Cortisol W-0 | Yes | 13 | 5,60 ± 5,97 | 0,991 | 0,05 (-9,42-9,52) |
| | No | 2 | 5,55 ± 2,34 | | |
| Cortisol W-4 | Yes | 8 | - | 0,009c | - |
| | No | 7 | - | | |
| Cortisol W-6 | Yes | 4 | 0,38 ± 0,51 | 0,226 | -1,73 ((-4,68-,1,21)) |
| | No | 11 | 2,11 ± 2,65 | | |

***Cont...*** **Table 3. Statistical analysis of ALL-HR cortisol levels with the incidence of infection in the induction and consolidation phase**

| | | | | | |
|---|---|---|---|---|---|
| Cortisol W-7 | Yes | 4 | 1,27 ± 2,18 | 0,224 | -3,85 (-10,37-2,67) |
| | No | 11 | 5,12 ± 5,77 | | |
| Cortisol W-8 | Yes | 3 | 6,55 ± 1,53 | 0,064 | -4,62 (-9,57- 0,32) |
| | No | 12 | 11,17 ± 3,80 | | |
| Cortisol W-10 | Yes | 3 | 11,59 ± 1,86 | 0,312 | -2,26 (-6,88-2,37) |
| | No | 12 | 13,84 ± 3,52 | | |
| Cortisol W-12 | Yes | 3 | 13,89 ± 2,03 | 0,425 | -1,85 (-6,71- 3,01) |
| | No | 12 | 15,74 ± 3,68 | | |

a. Cortisol level (μg/dL)

b. Significant if $p < 0.05$ with paired t-test statistical test

c. Mann-Whitney test

During the induction process of chemotherapy, there were patients with adrenal repression, which was identified by cortisol levels that were lower than the range limit value for cortisol levels according to age. The amount of time it takes for the adrenal glands of each condition to return to natural cortisol levels following prednisone or dexamethasone treatment is different. ALL patients are given elevated doses of glucocorticoids for an extended period of time during chemotherapy. During the induction process, about half of the patients will be readmitted to the hospital due to febrile neutropenia or sepsis. If the HPA axis is suppressed at the moment, this will have a negative impact on the patient's health[13]. In this study there were 10 out of 11 patients (91%) who experienced an incidence of infection during the induction phase and 8 patients out of 10 (80%) during the consolidation phase (table 3).

In induction phase ALL chemotherapy, there was an increased incidence of mortality due to infection by 10% when prednisone therapy (40 mg/m$^2$/day) was replaced with dexamethasone (6 mg/m$^2$/day)[14]. Dexamethasone induces a sluggish and delayed rise in proinflammatory cytokine levels, resulting in an insufficient inflammatory response and a masking effect on the signs of infection that occur. Long-term dexamethasone treatment is accumulative and reduces the inflammatory reflex; additionally, it may induce an insufficient stress response and raise the likelihood of deadly infections[14].

Infection in high-risk ALL cases is not only the result of long-term utilization of high doses of dexamethasone, but may also be the result of the patient's disease path. The ALL patients have clinical symptoms such as anemia, thrombocytopenia, leukopenia/leukocytosis, and/or neutropenia as a result of bone marrow invasion. Infection in high-risk ALL cases is caused not only by long-term utilization of high doses of dexamethasone, but also by the patient's disease path. The ALL patients have clinical symptoms such as anemia, thrombocytopenia, leukopenia / leukocytosis, and/or neutropenia as a result of bone marrow invasion. Fever without a clear etiology is a manifestation of cytokine release or can be caused by neutropenia and immunosuppression[15].

In this study, four patients recovered from adrenal gland function at week eight, while the remaining

patients recovered normally at week ten. According to the findings of Salem et al., serum cortisol and ACTH levels returned to normal in the majority of ALL-HR patients around week 4 and around 2 weeks in ALL-SR patients. Serum DHEAS levels returned to normal in 45 percent of ALL-HR patients and 85-90 percent of LLA-SR patients two weeks before full adrenal recovery[16].

## Conclusion

The following conclusion can be drawn from the analysis and discussion:

1. The decrease in children's cortisol levels during induction phase chemotherapy differs between ALL-SR and ALL-HR.

2. During the consolidation process of ALL-HR, there are differences in cortisol levels in ALL pediatric patients.

3. Infection occurs more frequently in pediatric ALL patients during the induction phase.

4. During the consolidation phase, the incidence of infection in pediatric ALL patients is lower.

5. During the induction phase, there is a relationship between the incidence of infection and cortisol levels in ALL children.

6. During the consolidation phase, there was no association between the incidence of infection and cortisol levels in ALL children.

**Conflict of Interest:** There was no report for potential conflicts of interest in this study.

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**Ethical Clearance**: We obtained an approval of whole project from Ethical Committee Review Board of Dr. Soetomo General Hospital Surabaya. The Ethical Clearance has issued by the Clinical Research Unit of Dr. Soetomo General Hospital Surabaya, Indonesia number 1207/KEPK/2019.

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# Mastoid Canals and Grooves in Human Skulls: A Dry Bone Study

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## Abstract

**Background:** The mastoid canals and mastoid grooves are formed within the mastoid part of temporal bones of skull, these are present on the external surface of mastoid region, anterior to the occipitomastoid suture, behind and parallel to the petrosquamous suture and anteroinferior to the asterion. The study was conducted to observe the incidence of mastoid canals and grooves in dry adult human skulls.

**Methods:** Total of 120 dry adult human skulls consisting of 80 male & 40 female were examined to determine the incidence of mastoid canals and grooves. The mastoid parts of both sides were examined for the presence of mastoid canals and grooves. Then length of mastoid canals was measured.

**Conclusion:** Out of the total of 120 skulls, mastoid canals were observed in 8.75% of skulls. The incidence of mastoid canals was 10% in females and 8.12% in males. The incidence of mastoid grooves was 10.42% of total skulls. It was 11.88 % in male skulls and in female skulls, it was 7.55%. The knowledge of mastoid canals and grooves of the temporal bones is very useful to the neurosurgeons and ENT surgeons while doing surgery to avoid severe bleeding.

***Key words:*** *Mastoid grooves, Mastoid canals, Skull*

## Introduction

The mastoid canals and mastoid grooves are formed within the mastoid part of temporal bones of skull, these are present on the external surface of mastoid region, anterior to the occipitomastoid suture, behind and parallel to the petrosquamous suture and anteroinferior to the asterion. The mastoid canals are of variable diameter and length, and the knowledge of them is very useful for neurosurgeons and ENT surgeons. As they have an arterial branch of occipital artery with its associated vein, which can be injured and can cause severe bleeding. It is probable that during development of the mastoid process, the squamous part of temporal bone ossifies over the vessels, giving rise to the mastoid canals(Schaeefer JP,1953 and Hollinshead WH, 1982). Therefore, the study was conducted to observe the incidence of mastoid canals and grooves in dry adult human skulls.

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## Materials and Methods

Total of 120 dry adult human skulls consisting of 80 male & 40 female skulls were examined to determine the incidence of mastoid canals and grooves. The skulls were retrieved from the department of Anatomy, PT. B.D. Sharma PGIMS, Rohtak. The study was conducted in the year 2019-20. The study was conducted after ethical clearance from the Institutional Ethics Committee. The mastoid parts of both sides were examined for the presence of the mastoid canals and grooves. A thin metallic wire was passed through the canal for its confirmation. Then, the length of mastoid canals was measured with the help of the digital vernier caliper.

## Results and Discussion

Out of the total 120 skulls, the mastoid canals were observed in 8.75% (21) of total skulls (Table-1, Fig.-1). The mastoid canals were observed 10% (13) in female skulls and in male skulls, it was 8.12% (8). In 3 male skulls and in 1 female skull mastoid canals were present bilaterally.

The lengthwise measurement of mastoid canal in male skulls on the right side was 2.01 -12 mm and in female skulls it was 5.6 -14 mm. In male skulls on the left side it was 3.85 -13.51 mm and in female skulls it was 1.1 -11.8 mm (Table-2).

In this study, the incidence of mastoid grooves was 10.42% (25) of total skulls. It was 11.88 % (19) in male skulls and in female skulls, it was 7.55% (6) (Table-3, Fig.-2).

Both the mastoid canals and grooves were found bilaterally in one male skull.

In the present study, the incidence of the mastoid canal was 8.75 % which is in consensus with the study performed by Choudhry et al. 1996. In the Japanese skulls, Singh et al. 2004 reported the incidence of the mastoid canal as 52.4% while in the South Indian skulls the incidence of mastoid canal was reported as 59.2% by Hussain et al. 2012. Hadimani and Bagoji, 2013 on North Karnataka skulls reported it as 53% (Table-4).

The incidence of the mastoid groove was less in this study (10.42%) when compared to the results on others. Singh et al. 2004 reported that the incidence of the mastoid groove was 13.56% in the Japanese skulls. And it was 18% in the North Karnataka skulls reported by Hadimani and Bagoji, 2013 while it was 20% in the South Indian skulls reported by Hussain et al. 2012 and according to Saadia et al. 2016 it was 24% in the Egyptian skulls (Table-4).

In this study both mastoid canal and groove was observed on the same side in 1 male skull while the incidence of both mastoid canal and groove on the same side was 2% as reported by Hadimani and Bagoji, 2013 on the North Karnataka skulls.

**Table No. 1 Incidence of Mastoid Canals in Males and Females**

| Gender | Present (% ) | Absent (% ) | Total |
|---|---|---|---|
| Male (n =160) | 13 (8.12 % ) | 147 ( 91.88 %) | 160 (100%) |
| Female (n = 80) | 8 (10 % ) | 72 (90 % ) | 80 (100% ) |
| Total | 21 (8.75 % ) | 219 (91.25%) | 240 (100% ) |

**Table No. 2 Incidence of Length of Mastoid Canals in Males and Females**

| Gender | Right Side | | Left Side | | Total |
|---|---|---|---|---|---|
| | 1-10 mm | 11-20 mm | 1-10 mm | 11-20 mm | |
| Male (n=160) | 4 (2.5 %) | 2 (1.5 %) | 4 (2.5% ) | 3 (1.9%) | 13 (8.12%) |
| Female (n=80) | 2 (2.5 %) | 2 (2.5% ) | 3 (3.8 %) | 1 (1.25%) | 8 (10 %) |
| Total (n=240) | 6 (2.5 %) | 4 (1.7%) | 7 (2.9 %) | 4 (1.7 %) | 21 (8.7%) |

**Table No. 3 Incidence of Mastoid Grooves in Males and Females**

| Gender | Present (% ) | Absent (% ) | Total |
|---|---|---|---|
| Male (n =160) | 19 (11.88 % ) | 141 ( 88.12 %) | 160 (100%) |
| Female (n = 80) | 6 (7.5 % ) | 74 (92.5% ) | 80 (100% ) |
| Total | 25 (10.42 % ) | 215 (89.58%) | 240 (100% ) |

**Table No. 4 Incidence of Mastoid Canals and Grooves as observed in the Present Study and Compared the Same with Earlier Studies**

| Author | Choudhry et al[3] | Singh et al[4] | Hussain et al[5] | Hadimani et al[6] | Saadia et al[7] | Present study |
|---|---|---|---|---|---|---|
| Population | Indian | Japanese | South -Indian | North Karnataka | Egyptian | North Indian |
| No. of Skulls | 265 | 435 | 125 | 100 | 100 | 120 |
| Mastoid Canals Present | 7.7% | 52.4% | 59.2% | 53% | 28% | 8.75% |
| Mastoid Grooves Present | - | 13.56% | 20% | 18% | 24% | 10.42% |



**Figure 1: Wire passing through mastoid canal**

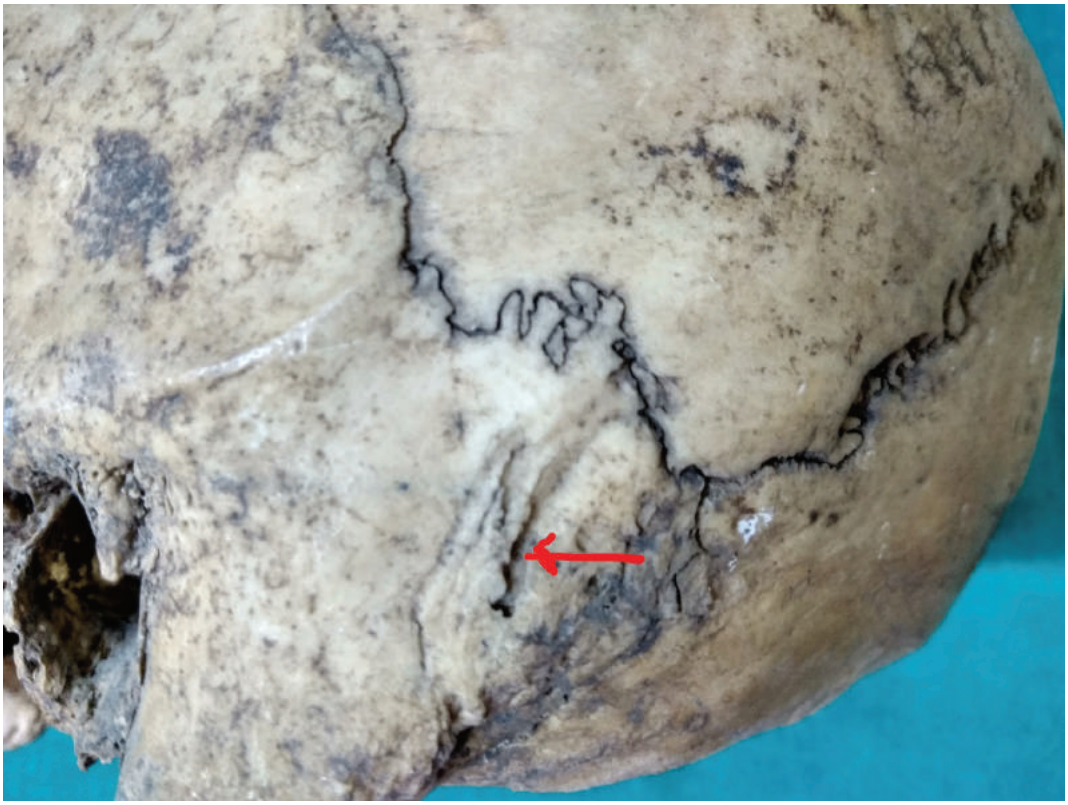

**Figure 2: Red arrow indicating on mastoid groove**

## Conclusion

The knowledge of the mastoid canals and grooves of temporal bones is very useful to the neurosurgeons and ENT surgeons due to increasing use of the transtemporal route for surgeries relating to structures in the posterior cranial fossa and the mastoid air system. As the mastoid canals and grooves have an arterial branch of occipital artery with its associated vein, it can be injured and can cause severe bleeding when they are doing surgery.

**Ethical Clearance –** Taken from the Institutional Ethics Committee vide letter No. SGTU/FMHS/D/795 Dated 26. Nov. 2018.

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**Conflict of Interest-** NIL

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# Predictor of Mortality COVID-19 in Two Referral Hospital in Surabaya, Indonesia

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## Abstract

**Introduction**: World Health Organization had identified COVID-19 in January 2019. This disease is caused by SARS-CoV-2, which spread throughout the world and became a pandemic on March 20, 2020. COVID-19 is still a health problem because it has not clear whether the patients will be cured and survive from the disease or not. This study aims to determine the predictors of mortality from COVID-19 at Dr. Soetomo General Academic Hospital and Universitas Airlangga Hospital in Surabaya, Indonesia.

**Method**: This study was conducted in Dr. Soetomo General Academic Hospital (referral hospital for COVID-19, 1500 beds) and Universitas Airlangga Hospital (Referal Hospital for COVID-19, 600 beds). The study used data on patients with confirmed COVID-19 who were hospitalized at these two referral hospitals. Predictors of mortality were analyzed using logistic regressions.

**Result**: There were 247 COVID-19 patients enrolled in this study, all patients were tested positive PCR SARS-CoV-2. The main complaints were cough, nasal congestion, dyspnea, and fever. Significant predictor mortality in this study were age >60 years old (OR: 3.24, 95% CI, 1.36 - 7.70), chronic kidney disease (OR: 5.71, 95% CI, 2.05 - 15.89), obesity (OR: 8.22, 95% CI,1.5 - 54.17), malignancy (OR: 6.025, 95% CI, 1.1- 33.00), coronary heart disease (OR: 5.31, 95% CI, 1.28 - 21.98) , and C-reactive protein >10 mg/L (OR 4.603, 95% CI, 2.03 - 10.44).

**Conclusions**: Obesity and the presence of malignancy, chronic kidney disease, heart disease and age >60 years old are the strongest predictors of mortality in people with COVID-19, despite high CRP results.

***Keywords***: *Predictors mortality, COVID-19, Indonesia, good health and well-being*

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## Background

Coronavirus disease-19 (COVID-19) was identified in December 2019 in the city of Wuhan, China. This disease is caused by Severe Acute Respiratory Coronavirus-2 (SARS-CoV-2). This virus is very contagious, spreading all over the world, and

become pandemic on March 2020.[1] Until now, the problem of morbidity and mortality from COVID-19 has not been resolved properly. The mortality rate for this disease in Indonesia is still high. Based on world meters info on April 21, 2021, there are 43,777 (2.7%) cases of the total cases of 1,614,849. This disease mainly attacked the respiratory system; although it can attack other organ systems, mostly around 80% of symptoms resolve without treatment. However, about 20% of patients will develop serious disease, most notably pneumonia, acute respiratory distress syndrome, sepsis or septic shock, thrombotic stroke and myocardial infarction, and mortality around the world was about 2.1%. [1-4]

The predictor of mortality had not clear yet whether the patient would suffer mild, moderate, or severe disease. Meanwhile, there has not been established proper treatment to overcome the problem of mortality for this disease. Several studies have shown this mortality predictor, including old age and the presence of various comorbid factors such as diabetes mellitus, chronic renal failure, and malignancy. [5,6]

This study aims to determine the predictor of mortality of COVID-19 at Dr. Soetomo General Academic Hospital Surabaya and Airlangga University Hospital Surabaya to improve the case management of COVID-19 as well as predict the prognostic condition of the patient based on existing comorbidities.

## Material and Methods

### Population and health care setting

The study was conducted in Dr. Soetomo General Academic Hospital (referral hospital for COVID-19, 1500 beds), and Universitas Airlangga Hospital (referral hospital of COVID-19, 600 beds), in Surabaya from April 2020 to June 2020. The study used data on patients with confirmed COVID-19 who were hospitalized at the two referral hospitals.

### Study design and inclusion procedure

COVID-19 patients confirmed by PCR who were hospitalized at the Dr. Soetomo General Academic Hospital and Universitas Airlangga Hospital in Surabaya from April 2020 to June 2020 were included in this study. Patients with incomplete data and records were excluded. The study was conducted retrospectively by looking back at the medical records of COVID-19 patients who were hospitalized in the isolation room. Subject characteristic, underlying comorbidities, symptoms, and signs at presentation, laboratory results, the outcome at discharge were collected and evaluated.

## Results

There were 247 COVID-19 patients enrolled in this study, age more than 60 years old were 50 (20.2%) patients, female 18(47.7%) patients. The most frequent symptoms were cough 175(70.9%), dyspnea 161 (65.2%), and fever 155 (62.8%), characteristic of the subject can be seen in (Table 1). The most common comorbidities found in these patients were diabetes mellitus, hypertension, and chronic kidney disease.

**Table 1 Characteristic of subject study: symptoms of patients on admission**

| Characteristic Subject | N (%) |
|---|---|
| Age > 60 y | 50 (20.2) |
| Female | 118 (47.7) |
| Cough | 175 (70.9) |
| Nasal congestion | 90 (36.4) |
| Dyspnea | 161 (65.2) |
| Fever | 155 (62.8) |
| Anosmia | 1 (0.29) |
| Diarrhea | 45 (18.2) |
| Nausea or vomiting | 69 (27.9) |
| Abdominal pain | 30 (12.1) |

**Table 2. Comorbidities associated with the subject**

| Comorbid conditions | N(%) |
|---|---|
| Diabetes mellitus | 49 (19.8) |
| Hypertension | 41 (16.6) |
| Chronic kidney disease | 25 (10.1) |
| Malignancy | 8 (3.2) |
| Heart disease | 11 (4.5) |
| Autoimmune diseases | 5 (2.0) |
| Obesity | 6 (2.4) |
| HIV | 3(1.2) |
| Pregnancy | 2(0.8) |

**Table 3. Univariate analysis predictor mortality of COVID-19**

| | Alive | Deceased | P | Odds Ratio | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Age > 60 y | 36 | 14 | 0.006 | 2.94 | 1.38 | 6.26 |
| Female | 103 | 15 | 0.219 | 0.71 | 0.35 | 1.44 |
| DM | 34 | 15 | 0.001 | 3.53 | 1.66 | 7.49 |
| Hypertension | 28 | 13 | 0.002 | 3.52 | 1.61 | 7.71 |
| CKD | 14 | 11 | 0.000 | 5.92 | 2.43 | 14.41 |
| Obesity | 4 | 2 | 0.222 | 2.94 | 0.52 | 16.68 |
| Malignancy | 5 | 3 | 0.102 | 3.62 | 0.82 | 15.839 |
| Autoimmune disease | 3 | 2 | 0.163 | 3.94 | 0.64 | 24.45 |
| Heart disease | 6 | 5 | 0.014 | 5.31 | 1.53 | 18.43 |
| Pregnancy | 2 | 0 | 0.722 | 0.99 | 0.99 | 1.004 |
| HIV | 3 | 0 | 0.613 | 0.99 | 0.97 | 1.002 |
| CRP >10 mg/L | 56 | 24 | 0.000 | 5.08 | 2.42 | 10.65 |
| N/L ratio >5.5 | 82 | 24 | 0.003 | 2.88 | 1.39 | 5.98 |

**Table 4. Predictor of Mortality by Binary Logistic Regression analysis**

| | **P** | **Odds Ratio** | **95% Confidence Interval** | |
|---|---|---|---|---|
| | | | **Lower** | **Upper** |
| Age > 60 y | 0.008 | 3.24 | 1.36 | 7.70 |
| CKD | 0.001 | 5.71 | 2.05 | 15.89 |
| Obesity | 0.028 | 8.22 | 1.25 | 54.17 |
| Malignancy | 0.038 | 6.025 | 1.10 | 33.00 |
| Heart disease | 0.021 | 5.31 | 1.28 | 21.98 |
| CRP >10 mg/L | 0.000 | 4.603 | 2.03 | 10.44 |

## Discussion

In this article, we summarized the symptoms and signs most commonly found in COVID-19 cases, namely cough, dyspnea, and fever. In univariate analysis, data was obtained that factors such as age >60 years, female, DM, HT, CKD, obesity, malignancy, autoimmune disease, heart disease, pregnancy, HIV, CRP >10 mg/L, and N/L ratio >5.5 were related to mortality. However, from these factors, age >60 years (p = 0.008), CKD (p = 0.001), obesity (p = 0.028), malignancy (p = 0.038), heart disease (p = 0.02), and CRP >10mg/L (p = 0.000) was statistically significant after being analyzed using logistic regression.

COVID-19 remains the disease-causing world pandemic with a high mortality rate. It is important to know the prognostic factors that play a role in patients with COVID-19.[7] Predicting mortality using pre-existing clinical and laboratory data provides an advantage because it can help stratify the next patient in treatment which then has implications for therapy.[8] The findings of Yang et al.[7] indicated that fever and dyspnea are signs of disease severity.[7] Although our study did not link clinical symptoms with mortality, the findings of this study also found that the majority of these symptoms were found in patients with COVID-19.

Age is an essential factor in the course of the COVID-19 disease. Advanced age is a significant factor in the occurrence of mortality. In a cohort study by Jain et al. of 425 patients, patients aged >65 years had a high risk of mortality, the OR (4.034; 95% CI 1.68–9.71; p=0.002) of in-hospital mortality, but this finding was not that far off in comparison with patients aged >47 years.[6] Research by Estiri et al.[9] also suggests that death occurs in two age groups, namely 45-65 and 65-85. The 45-65 years age group has a high risk of death due to underlying diseases such as DM and mean cancer, while 65-85 years is due to the pulmonary system, including interstitial lung disease, chronic obstructive pulmonary disease, lung cancer, and smoking history.[9] Therefore, age is a prognostic factor for mortality because it is associated with comorbidities in the study population.[6]

A decrease in glomerular filtration rate indicated by an increase in serum urea-creatinine was statistically associated as a predictor of death. Acute kidney injury (AKI) itself is a predictor of mortality in critically ill patients.[10] In the case of COVID-19, data from autopsy shows that the kidney is one of the targets of the SARS-CoV2 virus invasion.[11] The kidney is involved in the pathophysiology of AKI-COVID-19 because the kidneys express angiotensin-converting enzyme 2 (ACE-2) receptors as the entrance to SARS-CoV2 100 times greater than the lung. This mechanism can be a direct cytopathic effect. In addition, immune deposition of viral or viral antigen complexes induced specific immunological effectors also causes kidney damage.[10]

Several cohort-based population studies suggest that obesity is associated with high comorbidities such as DM, HT, and heart disease. The mortality rate is also increased with BMI. Obesity makes patients more susceptible to infection due to a decreased inflammatory cascade. Chronic inflammation in obesity reduces levels of cytokines, adiponectin, leptin and also disturbs the micro-macrovascular response. Lung function is also impaired due to mechanical problems and airway resistance.[12,13]

A single retrospective study by Erdal[14] found the mortality prevalence of cancer-COVID-19 patients to be 23.9%.[14] This figure is relatively high when compared to the general population. This figure is relatively the same as the research in Wuhan, which is around 28.6%. Analysis of the factors associated with the high mortality of the population was an abnormality of inflammatory biomarkers such as low lymphocytes, high CRP, procalcitonin, and D-dimer. The inflammatory response plays an important role in COVID-19, and cytokine storms will cause disease severity [14]. This can occur due to cancer itself or due to chemotherapy.[14]

The latest meta-analysis states that CVD increases the incidence of death in COVID-19 four time-folds.[15] The overactivity of the ACE network contributes to HT and cardiovascular. ACE-2 is associated with cardiac contractility and apoptotic processes due to hypoxia, affecting the outcome of cardiac capacity. In the case of COVID-19 with heart disease, there was an increase in cardiac troponin and an abnormality of electrocardiography by 7.2% and other cardiac biomarkers of 20%.[16]

Markers of inflammation such as lymphopenia have been reported to play a role in the mortality of COVID-19.[8] Although our study did not find a significant association, we found other inflammatory markers that have been widely used in monitoring the progression of COVID-19. C-reactive protein is a valuable marker and gauge of inflammation; it plays an important role in host defense against invading pathogens as well as in inflammation. Early rise in the C-reactive protein also had the strongest association with mechanical ventilation or mortality.[5]

This study has several limitations that need to be studied. First, this study was a retrospective design. Second, we did not perform a subgroup analysis for analysis of prognostic factors for mortality. We also did not analyze the effects of the medication or treatment given to the patients because the standards of care in managing COVID-19 are changing rapidly.

## Conclusion

Obesity and the presence of malignancy, chronic kidney disease, heart disease, and age >60 years old are the strongest predictors of mortality in people with COVID-19, besides high CRP results.

**Conflict of Interest:** All authors state that there are no conflicts of interest.

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*Original Article*

# Assessment of Patients' Satisfaction with Fixed Partial Denture and its Correlation with Patients' Evaluation of Clinicians

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## Abstract

I**ntroduction**: Patient satisfaction affects clinical outcomes, patient retention, and patient-centered delivery of quality health care. It is a very effective indicator to measure the success of doctors and healthcare organizations. Hence, the present study was designed to investigate the relationship between patients' satisfaction with Fixed Partial Denture and their perception regarding their clinicians' in a dental school in India.

**Methods:** A cross-sectional survey was carried out among 250 patients attending the OPD of the Department of Prosthodontics of a dental college in Faridabad, India. Patients having atleast3 unit fixed prosthesis were interviewed regarding the demographic details, oral hygiene aids used, and dental care utilization. Patient satisfaction questionnaire and patients' evaluation of dentist questionnaire was also administered. Data were statistically analysed using SPSS version 21.0 and significance was set at $p<0.05$

**Results**: The overall PSQ score for the present study was 6.58+0.59. It was higher in males and increased with social class. Most of the patients agreed or strongly agreed with positive dentist conduct. There was a strong positive correlation between patient satisfaction scores and patient agreement with the dentist.

**Conclusion:** The majority of patients were satisfied with the services received at the facility. Social class was associated with a difference in the level of patient satisfaction

***Key words-*** *Dental patients, fixed prosthesis, oral health, satisfaction*

## Introduction

Patient satisfaction is an important and commonly used indicator for measuring quality in health care. Patient satisfaction affects clinical outcomes, patient retention, and malpractice claims. It affects the timely, efficient, and patient-centered delivery of quality health care. Patient satisfaction is thus a proxy but a very effective indicator to measure the success of doctors and healthcare organisations.[1]

Patient satisfaction leads to customer (patient) loyalty and improved patient retention.[2] Patient satisfaction leads to customer (patient) loyalty.It also leads to Improvedpatient retention – according to the

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Technical Assistant Research Programs (TARPs), if one customer is satisfied, the information reaches four others. If there is one unsatisfied customer, it spreads to 10, or even more if the problem is serious.[3]

There is sufficient evidence to prove that organizations with high customer loyalty can command a higher price without losing their profit or market share. It is now universally accepted that various accreditation agencies like the International Organization for Standardization (ISO), National Accreditation Board for Hospitals (NABH), Joint Commission on Accreditation of Healthcare Organizations (JCAHO), etc., all focus on quality service issues.[4]Satisfying patients should be a key task for all dental providers.

As with general healthcare, patient satisfaction has also been shown to influence compliance and, in turn, treatment quality. This is relevant to all aspects of dentistry. When planning treatment, dentists should take into consideration objectives such as function, structure, esthetics and biology. This requires the clinician to rely on several disciplines in dentistry to deliver the highest level of dental care, which should lead to a higher level of patient satisfaction.[5]

Edentulism and dental disease have been shown to affect patients adversely. Patients with dental disease suffer from an altered self-image.[6]Dento-facial problems have known effects on patient's satisfaction with their dentition as they affect esthetics, performance, and function.[6]

Fixed partial dentures (FPDs) have been the treatment of choice for the replacement of missing teeth for some years. The dental literature has more than 10,000 articles on the topic of FPDs. However, only a few numbers of them deal with patients' perceptions of clinical outcomes and level of satisfaction with FPD treatment.FPD is indicated in short span edentulous arches, presence of sound teeth that can offer sufficient support adjacent to the edentulous space.

Early prosthodontic patient satisfaction studies focused on removable prostheses, while most current studies have focused on implant treatment for the edentulous patient there are few studies for the fixed partial denture. Verbal, numeric, Likert (combined verbal/numeric), and visual analog scales (VASs) are employed routinely to record responses in questionnaire-based studies. No one scale is accepted universally as the gold standard, and all four have been used to assess prosthodontic patient satisfaction.[7]

Patient-evaluated prosthesis satisfaction questionnaires should be simple to understand and short enough to facilitate compliance but broad enough to evaluate treatment objectives. They should gather information without causing coercion (limiting invasive questions, ensuring nonidentity), be easy to use in other study centers, and provide data that is simple to collate. They provide a practical basis for patient evaluated dentistry, which is increasingly being recognized as a necessary consideration of overall prosthodontic success.[8]

An English questionnaire to explore patients' satisfaction with fixed prosthodontic treatment was developed, with reference to guidelines published by the International Epidemiology Association European Questionnaire Group. The questionnaire was formulated from literature-based evidence, nominal group expertise.[9]The general conclusion is that patient satisfaction is a complex and multidimensional phenomenon, much of which remains unclear. Studies to investigate patients' satisfaction were carried out in different countries, including Sweden, Finland, The Netherlands, Croatia, and Singapore, and all concluded that patients' satisfaction with FPD was very high; however such studies have not been carried out in India.[10-13]

Nowadays health care is being transformed from a provider-centered approach to a patient-centered approach in which satisfaction of the patients' needs is part of the definition of quality. Hence, a commitment to providing high-quality service and to

achieving patient satisfaction is important for the oral health care provider. Dental school clinics must also constantly strive to find a balance between meeting the needs of the patient and meeting the needs of the student, all the while knowing that "patients and their satisfaction are critical to the education of the students as well.

Therefore, the present study aimed to investigate the relationship between patients' satisfaction with Fixed Partial Denture and their perception about their clinicians' in a dental college, Faridabad, India.

## Methodology

A cross-sectional survey was carried out in the patients at a dental college in Faridabad, India. Faridabad is the most populous city and also known as the industrial hub in Haryana, India. It has a geographical area of 742.90 square kilometers. Faridabad District and Division is located on the South-Eastern part of the State. Its dense shape located in the south of Delhi.

The study was conducted after ethical approval from the Institutional Ethical Review Board (IERB), Manav Rachna Dental College, Faridabad.

The sample size was calculated using prevalence data from previous literature, by taking an estimated proportion of (83.3%) from a previous study.[14]Using the given prevalence, 95% confidence interval, absolute precision of 5%, and a design effect of 1, the sample size was calculated to be 223which was further rounded off to 250.

Non Probability (Convenience) sampling technique was used as patients with recently delivered FPD therapy could be approached easily at the tertiary care hospital.The study was scheduled over five months (September 2019 to January 2020). Patient reporting to the Out Patient Department (OPD) at the Department of Prosthodontics, Manav Rachna Dental College, Faridabad for Fixed Partial therapy were checked for the eligibility criteria. Those found eligible were recruited in the study. Informed consent was administered to the participants in the Hindi/ English language. The aims and objectives were explained to the patient before taking consent. The study included patients above 18 years of age**,** **p**artially having at least 3 units fixed prosthesis and giving written informed consent for participation in the study. The exclusion criteria included patients with Implant prosthesis, patients not able to understand the content of the questionnaire, and patients who were mentally and visually challenged and completely edentulous patients.

The investigator (UK) was trained for interviewing the patients in the department on ten patients. The eligible participants were interviewed by a single investigator (UK) and questionnaires were administered using face to face interview method. Baseline data including the demographic details (name, age, sex, occupation, level of education, and annual income), oral hygiene aids (brushing frequency, duration of brushing, oral hygiene aids used), and dental care utilisation (number of missing teeth replaced by FPD, time span of prosthesis) was collected for each subject. The participants were interviewed one week after the treatment.

### The Patient Satisfaction Questionnaire

The Patient Satisfaction Questionnaire (Layton D & Watson TR, 2011)[9] and Patient evaluation of Dentist (adapted from Siqueira GP et al. 2012)[14] were used in the current study.

*The Patient Satisfaction Questionnaire (PSQ)* was used for the study which assessed the patients' satisfaction with appearance (2 questions), mastication (1 question), and cleansibility (1 question) of their prostheses. Apart from the above, two questions further assessed the patients' perceived satisfaction and cost when their prostheses were inserted initially. One question was asked whether they would recommend their friends for treatment. Patients answered the questions using a Visual Analog Scale (VAS). They were directed to cross a 10-cm line at the point representing the appropriate response between

the worst possible satisfaction/discontent and the best possible satisfaction. A single question (yes/no response) sought whether the patients would undergo the same treatment again.

Patient evaluation of dentist questionnaire consisting of 9 close-ended questions, using a 5 point Likert scale (Strongly Disagree to Strongly Agree), was also used to note patients' evaluation of the dentists' conduct.

The data was entered into the computer (MS-Office, Excel 2010) and subjected to statistical analysis SPSS (Statistical Package for Social Sciences) Version 24.0. The values were represented in numbers, percentages, and Mean±S.D. The association between patient satisfaction/ Patient evaluation of dentist and various demographic factors, utilization of dental services, oral hygiene practices was subjected to the statistical test of significance by using Independent Students unpaired t-test, One-way Analysis of Variance (ANOVA). Correlation Analysis (Pearson rho) was also performed. The statistical significance was set at a 5% level of significance. ($p<0.05$)

## Results

The study population comprised of 250 subjects in the age range of 20 to 64 years. A modified Kuppuswamy scale was used to classify the subjects according to their socioeconomic status (SES). The majority of the subjects i.e. 45.2% (n=113) belonged to the upper lower class. The majority of the study population (62.4%; n=156) brushed only once in the day. A total of 73.6% (n=184) brushed atleast for two minutes. Most of the study subjects (64.8%; n= 162) wanted replacement of either two or more teeth.

The overall PSQ score for the study participants was 6.58+0.59. The mean PSQ score regarding esthetics was 8.50+1.05 on the day of treatment and 8.73+1.12 on the day of the interview. Regarding chewing capacity, the mean PSQ was 8.25+0.96. (Table 1)

Concerning the questionnaire regarding patients' evaluation of the dentists' conduct, it was seen that there was a positive response from the patient regarding the dentists' conduct. Most of the patients agreed or strongly agreed with positive dentist conduct. A significant positive correlation was found between various PSQ scores such as esthetics and comfort with phonetics and esthetics on the day of the interview. (Table 2) The mean PSQ score (6.62+0.51) was highest in the age group of 20-34 years and was least in the 35-44 years age group but the differences were not statistically significant ($p= 0.60$). The mean PSQ score was higher in males (6.57+0.63) as compared to females (6.60+0.54) but again the difference was not statistically significant($p=0.74$). The mean PSQ score increased with raise in social class but this difference was also not significant ($p=0.76$).

On the questionnaire items that concerned the patients' evaluation of the dentists' conduct, statistically significant differences were found for patients' scores for satisfaction with for different answers to question 1 (i.e. 'The dentist I saw thoroughly explained the recommended treatment before it commenced') on comfort in cleaning ($p= 0.026$), cost ($p=0.044$), overall experience ($p=0.0001$) and desire to get treated again ($p = 0.012$). Also, question 2 "I am confident that I received good dental care", question 8 "The dentists I saw were impersonal or indifferent towards me " and question 9 "The dentists I saw answered my questions" were also significantly correlated with patients satisfaction with their denture with regards to chewing capacity and esthetics.

When the patients' evaluation of the dentists' conduct was assessed based on age and gender separately, there was no significant difference among the age groups and gender for any of the nine questions asked. When the patients' perception of dentist conduct was assessed based on SES, it was seen that study subjects from almost all the SES had a positive evaluation of the dentist. However, regarding the dentists' behaviour in answering the questions($p=0.012$) and carefulness in examining the

patient (p=0.012) was found to be more satisfactory by the upper lower class as compared to other SES classes.

**Table 1: Patient's satisfaction with FPD based on PSQ**

| PSQ | PSQ score (Mean) | S.D. |
|---|---|---|
| Esthetics after treatment | 8.50 | 1.05 |
| Esthetics on the day of interview | 8.73 | 1.12 |
| Chewing capacity | 8.25 | 0.96 |
| Comfort in cleaning teeth | 8.54 | 1.03 |
| Cost | 8.44 | 1.04 |
| Overall experience | 8.25 | 1.25 |
| Patient desire to get treated again | 0.98 | 0.13 |
| Recommendation to friends | 1.02 | 0.18 |
| Overall PSQ | 6.58 | 0.59 |

**Table 2: Correlation between patient's expectations and satisfaction with FPD**

| Patient satisfaction | | PSQ2. Esthetic s on the day of interview | PSQ3. Chewing capacity | PSQ4. Comfort in cleaning teeth | PSQ5. Cost | PSQ6. Overall experience | PSQ7. Patient desire to get treated again | PSQ8. Recommendation to friend s |
|---|---|---|---|---|---|---|---|---|
| PSQ 1 | R2 | 0.725 | 0.477 | 0.371 | 0.338 | 0.453 | 0.183 | 0.000 |
| | P | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 0.004* | 1.00 |
| PSQ 2 | R2 | 1 | 0.459 | 0.472 | 0.452 | 0.404 | 0.197 | -0.058 |
| | P | | 0.001* | 0.001* | 0.001* | 0.001* | 0.002* | 0.358 |
| PSQ 3 | R2 | | 1 | 0.555 | 0.528 | 0.427 | 0.165 | -0.023 |
| | P | | | 0.001* | 0.001* | 0.001* | 0.009* | 0.716 |
| PSQ 4 | R2 | | | 1 | 0.424 | 0.365 | 0.160 | -0.135 |
| | P | | | | 0.001* | 0.001* | 0.011* | 0.033* |
| PSQ 5 | R2 | | | | 1 | 0.413 | 0.084 | -0.124 |
| | P | | | | | 0.001* | 0.183 | 0.050 |
| **PSQ 6** | **$R^2$** | | | | | 1 | 0.128 | -0.522 |
| | **P** | | | | | | 0.043* | 0.001* |
| **PSQ 7** | **$R^2$** | | | | | | 1 | 0.011 |
| | **P** | | | | | | | 0.857 |

## Discussion

In health care, considerable efforts are being made to ensure services are effective, beneficial, evidence-based, and acceptable to the patient. In this context, patient satisfaction is considered an essential component of quality improvement plans. Patients' perceptions of their oral health status are important outcomes in prosthodontics. The present study found that 80.9% were satisfied with their prosthesis. This similar level of patient satisfaction with their fixed prosthesis was described in some studies. Kashbur et al[15]reported that 80.9% patients and Zavanelli et al.[16]reported that 72.58% of the patients were satisfied with their fixed prosthesis. Tan et al.[17]and Kola et al.[18]and an 18-year retrospective study by Napankangas et al[19]observed very high levels of satisfaction in relation to functional aspects of fixed prosthesis like aesthetics, mastication, speech, and comfort levels. This high satisfaction level could be attributed to the fact that fixed restorative treatment might have restored the feeling of "normality" to the patient, as he/she feltthe prosthesis more like a natural tooth as observed by Al-Quranet al.[20]

Geiballaet al.[21] described that almost half of their patients had noticed an improvement in their masticatory function while in the present study, around one-fifth of the group were not happy with their masticatory function. Thus, patients have to be aware that having regular checkups after receiving their prosthesis is important to avoid additional impairment to their masticatory ability. Geiballaet al.[21]also reported that 20% of their patients were not satisfied with the esthetic aspect of their prosthesis. In the present study, only 14.7% found their fixed restorations esthetically unpleasant. Their dissatisfaction was related to the mismatch in color with the natural teeth (9.1%), mismatch of shape and size, or improper artificial tooth position in the fixed prosthesis (4.4%) whereas 1.2% of the participants were told by other people that their prosthesis looks not good. Therefore, the clinician needs to pay great attention to select the proper shade of the prosthetic teeth, particularly where anterior teeth are involved. Another important aspect is matching the position and angulation of the prosthetic teeth and natural teeth.In the present study, a significant positive correlation was found between various PSQ scores such as esthetics and comfort with phonetics which was similar to the study done by Banerjee et al which reported that more than 90% of the individuals had no phonetic alteration with their FPD.[22]

The high percentage of males among the investigated sample suggests that males were more concerned about getting a replacement to their missing teeth may be due to financial independence deciding to get the treatments done as most females were financially dependent on their male relatives or spouses to bear the cost of the treatments which is not as per the several other studies done abroad where females were more concerned about replacement of missing teeth.

Considering the importance of the patient/professional relationship to the success of prosthodontic therapy a questionnaire was used to assess an important component of this relationship (i.e. the patients' evaluation of the dentists' conduct), which showed a clear predominance of positive ratings for all questions. The most satisfactory attribute of dental care in this study was the communication skills of treating students. Patients were also highly satisfied with the quality of care, particularly the interpersonal aspects of care. Karydis A et al.[23] concluded that the largest quality gap was also observed in characteristics regarding responsiveness which was found positive in this study. It enables dentists to determine a proper diagnosis and treatment plan empowering patients to take responsibility for their health leading to better patient education, adherence with treatment, and better treatment outcomes.

Dewi FD et al.[24] concluded that priority should be given to the dentist's communication and dental assistant's knowledge of patient's needs to enhance the service quality. A proper explanation of treatment

procedures is an important aspect of the patient-dentist relationship and in this study was reported to be very high. Aldosari MA et al.[25] also reported that patient satisfaction is increased with friendly and understanding staff. Moreover, meeting patient expectations by taking time to understand the needs and giving the right instructions is associated with higher satisfaction, and the fact that patients were highly satisfied with the clinician's communication abilities indicates that clinician's in this facility pay proper attention to explaining treatment options/ procedures to patients.

In the current study, patients are capable of differentiating between technical (quality, interpersonal aspects) and non-technical (aesthetics) aspects of care and improving satisfaction is more about improving quality and interaction and communication with providers rather than amenities. Based on these findings, patient experience measures provide important information, are the best judges to evaluate some aspects of quality, and their viewpoint is the keystone of patient-centered care Therefore, healthcare quality cannot be defined as seen from healthcare professional perspectives . It is a distinct, complementary aspect of quality that can be used along with conventional clinical measures. Thus, the null hypothesis was defeated in the present study

However, few limitations of this study included that since the sample was a convenience sample, its external and generalizability can be affected, and also the satisfaction of current patients was assessed rather than former patients, responses could have been varied if the former patients were also assessed.

## Conclusion

Overall, the results of this study indicate that the majority of the patients were satisfied with the services received and the patient's assessment of care may be influenced by several other factors that extend beyond the actual care received. In the present study, social class was associated with a difference in the level of satisfaction with some aspects. Dental students must be aware of socio-demographic and health status disparities in satisfaction identified in the population receiving care at this dental faculty. Such information may help them to target improvement efforts.

The information obtained from this study could be useful in establishing new strategies to improve patient experiences with the dental services delivered. One effective means for addressing patients' concerns is to focus on how they perceive care. Moreover, the objective of patient-centered care could not be achieved without gathering data on patient satisfaction and responding to it appropriately. The study provides important information on important aspects of quality form patients' perspectives and identifies areas that may need improvement.

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# Production, Purification and Characterization of Bacteriocin Produced by Novel *L. Pentosus*MW857478 for Enhancement of Food Safety and Shelf-Life of Paneer

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## Abstract

This research paper is based upon the production, purification and characterization of bacteriocin by *L.pentosus*MW857478 followed by its enhancement of food safety and shelf life of paneer. In recent year LAB (Lactic acid bacteria) produced bacteriocinattract a great attention of researcher due to their many potential applications. This paper focused on isolation, identification, evaluation of broad spectrum inhibitory activity, production and purification, characterization and evaluation of food safety and shelf life of paneer. Bcateriocin produced by*L. pentosus*showedantagonistic activity against food spoiling pathogens in broad range ofBacteriocin production parameter was optimized with pH 5.5 incubated at 35°C for*L. pentosus*. Bacteriocin was purified by ammonium sulphate precipitation and purified bacteriocin with single band on SDS-PAGE for molecular weight. The purified bacteriocin stableat 2-10 pH and 30-75°C temperatures, suggesting *L. pentosus*a potent candidates for safety and extending shelf life of paneer for 15 days.

***Keywords**: - Antagonistic activity, Bacteriocin, purification, raw milk, pathogens, lactic acid bacteria*

## Introduction

Lactobacilli are important microorganism as they recognised as their many potent abilities in food quality as preservation and also have suitability to produce drastic changes in the taste, flavour and texture, also they show broad range of spectrum against pathogenic and spoilage microorganisms. Therefore they found naturally in fermented foods, assumed to be safe without create any health risk of consumers, and designated as GRAS (Generally Recognized as Safe) organisms because of producing various compounds such as organic acids, hydrogen peroxide and bacteriocin.

Bacteriocins are extracellular peptides or proteins that exhibiting bactericidal activity against species and closely related species [11].Although they may be found in many Gram positive and Gram negative bacteria, those produced by LAB have received particular attention of consumer in recent year due to their potential application in the food industry as natural preservatives. They have been shown very important role in improving microbiological quality and shelf life of many fermented food products and set very excellent examples of bio-preservation.They are mostly heat stable and responsive forprotelolytic

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inactivation so there role as novel food preservatives have received great attentiontowards the bacteriocin producing lactic acid bacteriaspecies*Lactococcus, Lactobacillus, Streptomyces, Staphylococcus, Bacillus, Pediococcus* and *Carnobacterium*as reported till now [12].

## Materials and Methods

### Isolation, phenotypic and molecular characterization of *L.pentosus*

*L.pentosus*was isolated from raw milk samples from cows and goats were collected from different localities of Roorkee, Uttrakhand state of India. Milk sample was utilized for making initial dilution ($10^{-1}$). Further serial dilution up to $10^{-6}$, 1ml of first dilution was transferred into 9 ml of sterile peptone water. 1ml of this dilution was transferred in sterilized Petri-plates and poured then 15 ml melted and cooled de Man Rogosa (MRS) agar and mixed properly and allowed to cool. Plates were incubated at 37°C/48 hours. After incubation, different types of colonies appeared on plate with different morphology such as colour, shape and size were picked with the help of sterilized tooth picks into sterile MRS agar plates. This process was repeated 2-3 times on fresh MRS agar media by incubating at 37°C/24 hours to have pure cultures[16].The purified cultures were preliminary screened for catalase test. The pure culture were further characterized by Gram's staining and, and other biochemical tests. The cultures were characterized based on cell morphology and biochemical tests[13]. The selected strains were identified formolecular characterization of lactobacilli isolates was performed by 16SrRNA gene sequencing[4].

### Screening of antagonistic activity of bacteriocin producing*L.pentosus*

*L.pentosus* exert antagonistic activity against indicator microorganisms*viz.,E. coli*(ATCC 25922), *B. cereus*(ATCC 14579), *S. aureus*(ATCC 25923) was performed by disc diffusion method. The isolated *L.pentosus*was inoculated in 5ml MRS broth and incubated 37°C/18-24hrs. Cell-free supernatant (CFS) by centrifugation of this culture at 10000×*g* for 10 min at 4°C. To rule out anypossibility of antimicrobial activity due to organic acid ($H_2O_2$), CFS was adjusted to pH 7.0 by adding 1N NaOH.The CFS also treated with catalase to eliminate the inhibitory effect of $H_2O_2$ produced by lactobacilli isolate. The discs were prepared from Whattman filter paper and autoclaved at 121°C/15 min. Culture free MRS broth disc were used as negative control. The discs were placed on Muller-Hinton agar (MHA) seeded with 18hrs active culture of indicator microorganism. Plates were incubated at 37°C/ 24hrs forclear zone of inhibition around the discs, used to determine bacteriocin activity according to[3].

### Production and purification of bacteriocin

24hr old culture of *L.pentosus*was propagated by10% of inoculum on MRS broth and incubated for 48h at 120 rpm at 37°C. The whole broth centrifuged for 1180g for 15 min and CFSused as crude bacteriocin[11]. The bacteriocin sample protein concentration determined by[8].and bovine serum albumin (BSA) used as standard. For purification of CFS of bacteriocinwas saturated with 60-70% ammonium sulphate and stored at 4°C to precipitate out the proteins, pellets were collected after centrifugation at 1180g at 4°C for 30 minutes.

### Molecular weight Determination

The molecular weight of purified bacteriocin was detected by SDS-PAGE(sodium dodecyl sulphate polyacrylamide gel electrophoresis [6].

### Characterization of bacteriocinactivity on the basis of effect of enzymes, pH, temperature

The bacteriocin of isolate *L.pentosus* showing clear zone of inhibition was characterized for temperature, pH and enzymes[2].

Enzyme(proteinase K, trpsin or pepsin) was tested on the antagonistic activity of crude bacteriocin preparation at a final concentration of 1mg/ml and

incubated for 2hr/30ºC,wheras effect of temperature (heat resistance) was tested at different temperatures 30ºC, 45ºC, 60ºC, 75ºC and 100ºC, bacteriocin activity was detected against selected pathogenic bacteria for 30, 45 minutes, Effect of pH on bacteriocin activity was tested at various pH 2.0 to 10.0 adjusting through sterile 1mol/lNaOH or 1 mol/lHCl.

**Shelf Life studies of paneer**

Preparation of samples

Freshly prepared paneer sample that was prepared in KanyaGurukul campus, department of microbiology lab the pieces 5gm 3cm×3cm and 0.5mm thickness both the samples was sprayed equally by partially purified bacteriocin 5µg/g on the surface of the piece with the help of hand operated sterile spray bottles and kept and stored at under refrigerated conditions (4°C) and observe at every 3 days interval until spoilage for the parameter via microbiological analysis test like total plate count (TPC), coliform count [1].

**Microbiological analysis**

The paneer samples were taken out at different time intervals until spoilage. For plate count of total aerobic bacteria, serial dilutions were prepared with normal saline (0.85% NaCl). 1 g of sample was taken and added into 9ml of normal saline solution and termed as $10^{-1}$ dilution. After mixing it homogenously, 1 ml of sample was taken from $10^{-1}$ dilution and added in 9 mL of normal saline solution ($10^{-2}$ dilution). Similarly, further dilutions were prepared. From appropriate dilution, 0.1 mL sample was taken and plated on solidified and dried (one day at 37°C) standard plate count agar.Plates were incubated at 37°/48hr and colonies were counted through Darkfield Quebec Colony counter.

## Results

Identification of bacteriocin producing LAB

Out of 56 isolates,a total of 18 isolates were analysed for the potential of bacteriocin producing lactobacilli from different milk samples whereas on thebasis of morphological, and biochemical characterization of Cm12were confirmed to be lactobacilli. The data has been summarising in Table 1 and figures 1 and 2.

**Table 1: Morphological and biochemical characterization of *L.pentosus*Cm12**

| S No | Test performed | L. pentosus |
|---|---|---|
| 1 | Gram's Reaction | + |
| 2 | Shape | Rods |
| 3 | Catalase | + |
| 4 | Glucose | + |
| 5 | Arbinose | + |
| 6 | Lactose | + |
| 7 | Galactose | + |
| 8 | Maltose | + |
| 9 | Ribose | + |
| 10 | Manitol | - |
| 11 | Gas formation | + |

(+) Positive test, (-) Negative test

Molecular characterization and submission of sequence to NCBI

*Lactobacillus* isolates Cm12 was identified by 16S rRNA sequence homology as a strain of *Lactobacilluspentosus*.The 16S rRNA sequence of the isolate Cm12 showed 99.64% identity to *Lactobacillus pentosus*strain LMEM1001. The phylogenetic tree for the isolate was constructed and has been depicted in figure 3. The 16S rRNA gene of isolate Cm12 was successfully sequenced and deposited to gene bank with accession number MW857478 was obtained. BLAST homology search showed 100% sequence similarity with *Lactobacillus pentosus*strain JCM 1149.

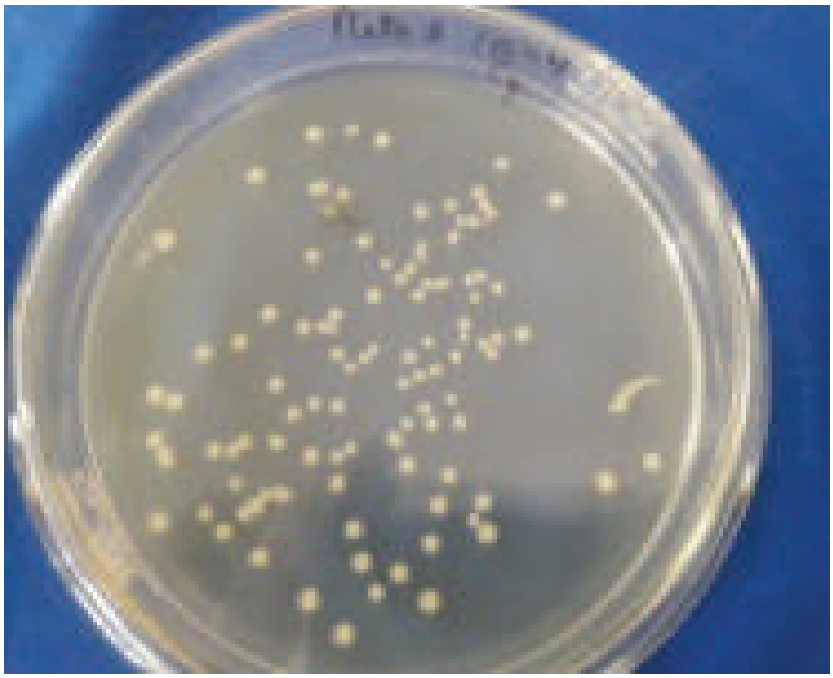

**Fig 1: Colony morphology of *L. pentosus* Cm12 on MRS agar.**

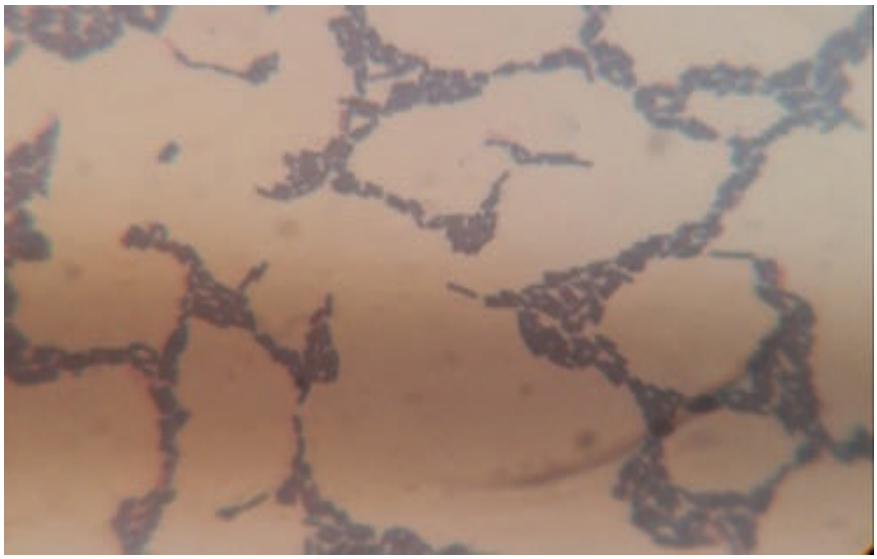

**Fig 2: Microscopic observation (1000x)of *Lactobacillus pentosus***

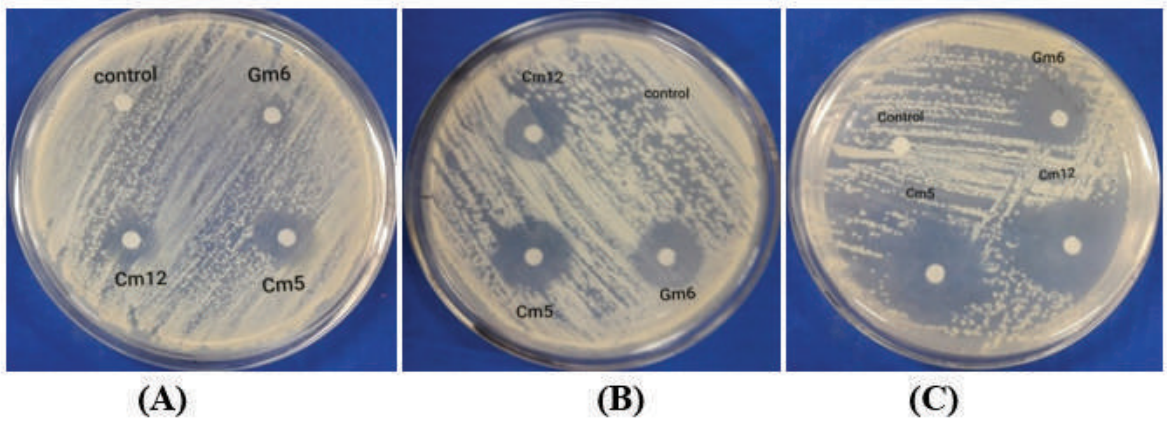


**Fig 3: Antagonistic activity against selected food borne pathogens (A) *B. cereus*ATCC14579 (B) *S. aureus*ATCC 25923 (C) *E. coli* ATCC 25922**

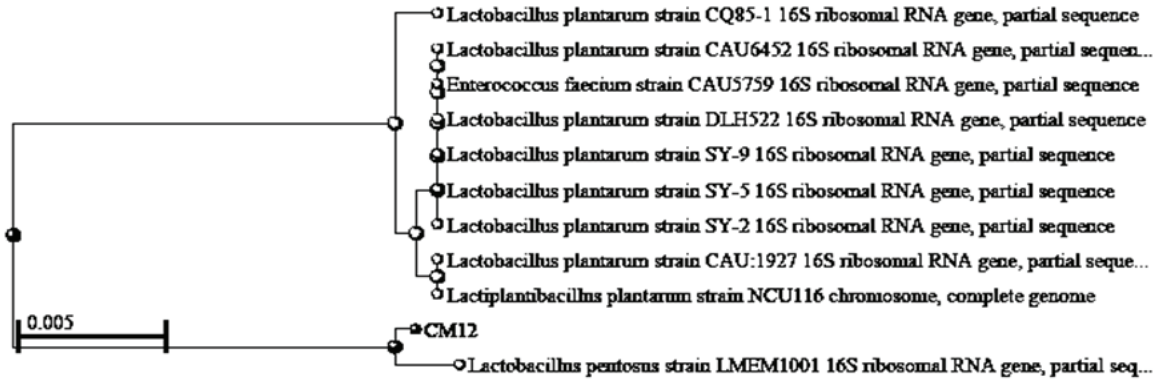


**Fig 4: Phylogenetic tree of L. pentosus Cm12**

**Antagonistic activity of bacteriocin producing LAB**

Observations on antagonistic activity of identified lactobacilli isolates obtained from cow and goat milk have been presented in table 2 and figure 3. Antagonistic activity of lactobacilli strains was determined by disc diffusion method. The cell free extract was neutralized with 1M NaOH to eliminate acid effect that could inhibit growth of indicator bacteria. *L. pentosus* Cm12 out of these demonstrated highest antagonistic activity i.e. >15 mm zone of inhibition against three tested indicator *S.aureus*ATCC25923,*B. cereus*ATCC14579 and *E. coli* ATCC25922 indicative of broad spectrum inhibition. Our observations are in corroboration also showing inhibitory effect of LAB against*S.aureus*and*B.cereus*to the tune of8 and 9 mm zone of inhibition[16]. Reported that inhibitory activity of *Lactobacillus* can be enhanced selectively and confirmed that *L.cornyimis* XN8 exhibited broad spectrum antimicrobial effect against *S.aureus*.

**Table 2:Antimicrobial activity of bacteriocin producing lactobacilli isolates by disc diffusion method on Muller-Hinton agar.**

| Isolates | Zone of inhibition (mm) | | |
|---|---|---|---|
| | E. coli ATCC25922 | B.cereusATCC14579 | S.aureusATCC25923 |
| L.pentosus | 20.66±0.33 | 15.33±0.33 | 18±0.33 |

*Value of mean of triplicate assays ± standard error, - No zone of inhibition.

**Growth and bacteriocin production of *L.pentosus***

The result of the study of growth and bacteriocin production revealed that there was a positive correlation existed between the growth and bacteriocinproduction.The growth increase was up to 24hour of incubation but bacteriocin production attained maximum at 18hour of incubation and thereafter no increase was noticed maximum bacteriocin production of *L.pentosus*is 6.14 mg/ml.results are summarised in table 3.

**Table3: Total protein concentration of bacteriocin produced by *L. pentosus***

| Strain | Purification stage | Volume (ml) | Protein concentration (mg/ml) |
|---|---|---|---|
| L. pentosus | Culture supernatant (Crude) | 500 | 21.42 |
| | Ammonium sulphate precipitation (Partial purification) | 20 | 6.14 |

**Determination of molecular weight by SDS PAGE**

Molecular weight of *L.pentosus*bacteriocin was carried out by SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis).Single protein band havingmolecular weight ±11kDa after stained with coomasie brilliant blue that clearly indicated the purity of protein.

**Characterization of bacteriocin**

*L.pentosus*was stable over at 30-75°C and more at 60°C for 30 minutes and declined afterwards against different food borne pathogens (*S.aureus ,E.coli*and *B.cerus*) results were showing in figure 5,However pH on bacteriocin activity was tested by incubating at various pH at 2.0-10.0 and the stability of *L.pentosus* on bacteriocin activity is stable at 2.0-10.0 pH showing in Figure 4 and more at 6.0 pH,Whereas *L.pentosus* exhibited complete in inactivation of antimicrobial activity. After the treatment of bacteriocin with proteinase K, trypsin and pepsin which confirms its proteinaceous nature showing in Table 4.

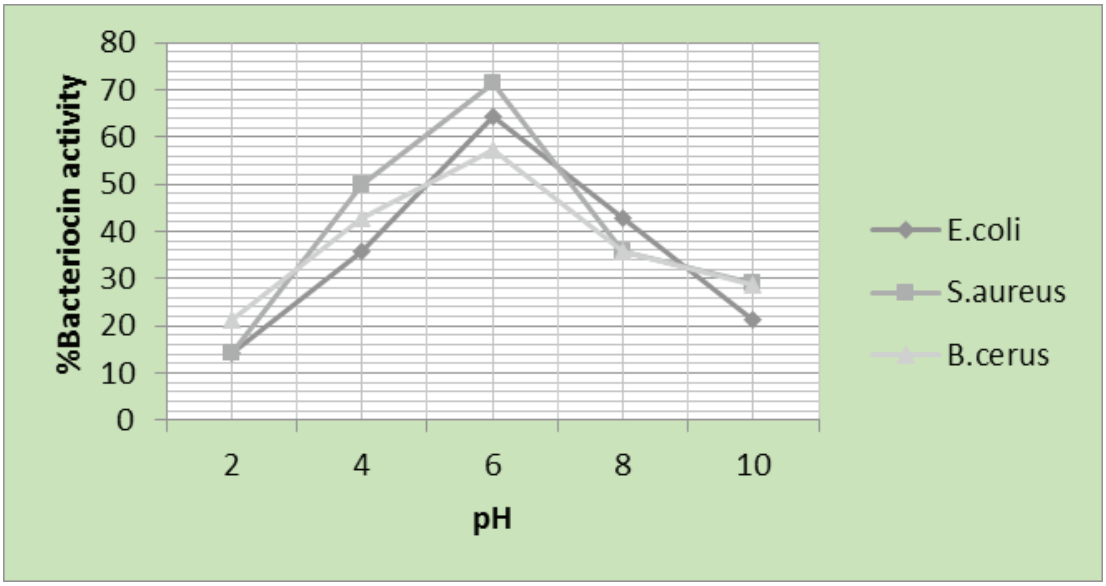


**Fig4: Effect of pH on characterization of bacteriocin of *L.pentosus***

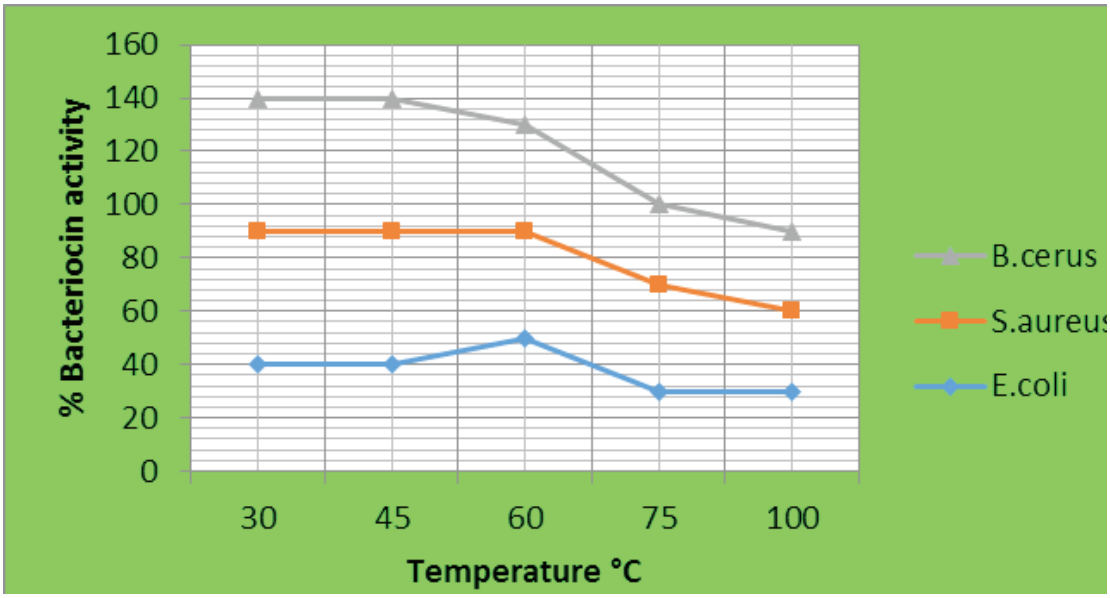


**Fig5: Effect of temperature on characterization of bacteriocin of *L.pentosus***

**Table4: Showing effect of enzymes on *L.pentosus***

| Enzymes | Bacteriocin Activity |
|---|---|
| | L.pentosus |
| Protinase K | - |
| Trypsin | - |
| Pepsin | - |
| Catalase | + |

**Evaluation of Shelf Life Extension Potential of Bacteriocin**

Freshly panner sample was prepared in KanyaGurukul campus, department of microbiology lab the pieces 5gm, 3cm×3cm and 0.5mm thickness both the samples was sprayed equally by partially purified bacteriocin 5µg/g on the surface of the piece with the help of hand operated sterile spray bottles and kept and stored at under refrigerated conditions (4 °C) and observe at every 3 days interval until spoilage for the parameter via microbiological analysis test like total plate count (TPC), coliform count[1].through microbiological analysis the plates for standard plate count were incubated at 37 °C for 48 h and colonies were counted with the help of Quebec Colony counter. Showing the microbial analysis of paneer for control and pentocin during refrigerator storage (4±1°C) in which there is no coliform detected until day 15 in pentocin while TPC is 4.74±0.08cfu/g at day 15 ofpentocinwhile control TPC at day 3 is 5.82±0.08 cfu/g andnot performed (NP), As per the Bureau of Indian standards (IS:1983), the TPC should not exceed $5\times10^5$ at day 6,9,12 and 15 because numbers are too high for count incontrol,Hence pentocinbacteriocin could be easily used as biopresevative for extending the shelf life of paneer after incorporatedmaximum 15days in refrigerator condition (4±1°C).

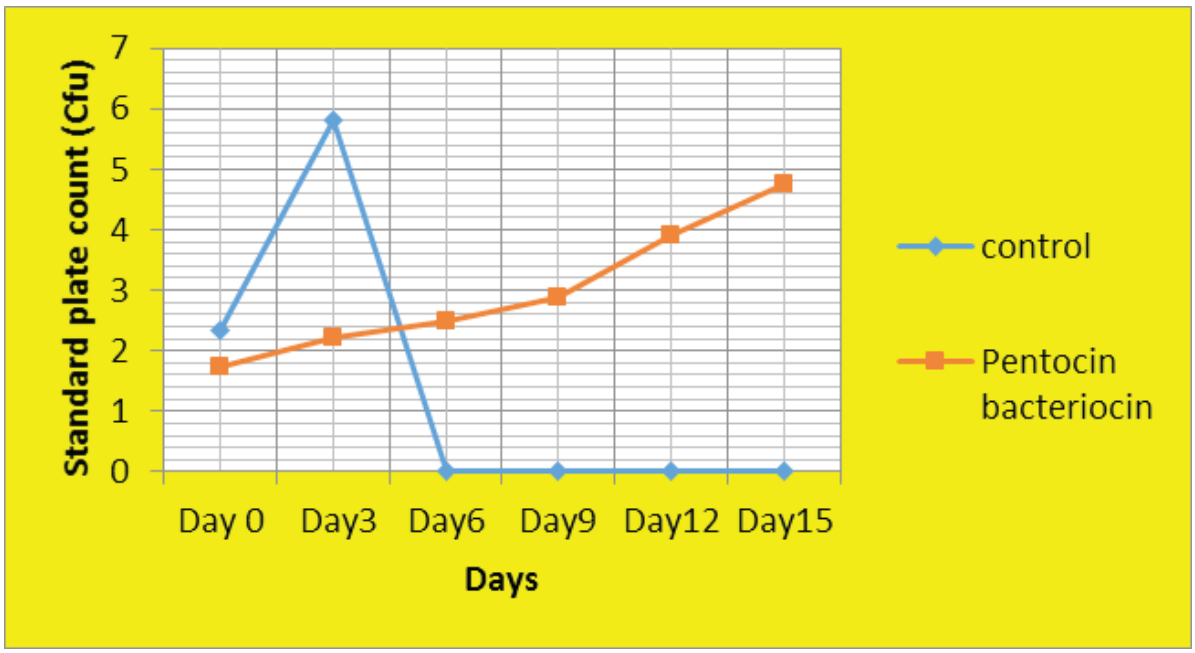


**Fig 6: Microbiological analysis of paneer with different treatments during refrigerated storage (4±1°C) until spoilage**

## Discussion

This study aimed to evaluate the ability of *L.pentosus*to produce bacteriocin and enhance the shelf life of paneer. The bacteriocin production and detection was formed in-vitro accompanied by the production of other metabolites like hydrogen peroxide and lactic acid. Thus neutralize the effect of other metabolites and assure that protein extract was not related to these metabolites. The results suggest that a protineceousnature of bacteriocin produced by *L.pentosus*. Based on the described results we conclude that *L.pentosus*produce bacteriocinwith an expected size ±11kDa. This molecular weight is within the range of the most frequently reported in[5]. Further studied may include the enhancement of

shelf-life of paneer.

In parallel we conducted antimicrobial tests of crude and partially purified bacteriocin against different food borne pathogens showing the effectiveness of bacteriocin against gram negative and gram positive bacteria, then pentocinbacteriocin produced by *L.pentosus* showing the effectiveness in enhancing the shelf life of paneer by incorporating of pentocin.

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# Body Mass Index as an Indicator for Endometrial Biopsy in Premenopausal Women with Heavy Menstrual Bleeding

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## Abstract

**Background:** Although Obesity is a risk factor of endometrial hyperplasia/ carcinoma, there is no consensus on using the BMI as a predictor for endometrial biopsy screening in patients with heavy menstrual bleeding in pre-menopausal women. Therefore, it's clinically important to identify BMI cutoff values as a predictor for endometrial hyperplasia/ carcinoma in premenopausal women with heavy menstrual bleeding.

**Aim of the Study:** To determine the feasibility of using Body Mass Index (BMI) as an indicator to perform endometrial biopsy in premenopausal women presented with heavy menstrual bleeding.

**Patients & Methods:** This is a cross-sectional study that was conducted in Obstetrics and Gynecology Clinic /Al-Emamain Al-Kadhemein teaching medical city in Baghdad where women 150 women who are 40-50 years old with heavy menstrual bleeding where sent for endometrial biopsy. 100 women had abnormal endometrial biopsy results, histopathological results of the abnormal endometrial samples were classified into following groups: hyperplasia without atypia, hyperplasia with atypia, and hyperplasia with atypia and cancer. Statistical analysis conducted to correlate The Body Mass Index (BMI) with the status of endometrial biopsy.

**Results:** 42% of the women with BMI≥30 kg/m$^2$ have hyperplasia with atypia or carcinoma compared to 10% of women who BMI<30 kg/m$^2$ ($p<0.001$). from 54 women who had hyperplasia with atypia in endometrial sample results; 77.7% of such patients were BMI≥30 kg/m$^2$. Receiver operating characteristic analysis shows that using BMI ≥30 kg/m$^2$ as a predictor of hyperplasia with atypia or carcinoma carries a sensitivity of 80.77 % and specify of 86.00% with area under the curve (AUC) value of 0.83 (CI. 0.75-0.92) and ($p<0.001$). women with BMI≥30 kg/m$^2$ has a relative risk (RR) of 3.98 of developing endometrial hyperplasia (without atypia or with atypia+carcinoma) and a relative risk (RR) of 4.11 of developing hyperplasia with atypia or carcinoma ($p<0.001$).

**Conclusion:** Our results indicate that BMI regardless of the age is the highest risk factor for abnormal endometrial hyperplasia premenopausal women. Also, BMI≥30 kg/m$^2$ has highest risk for developing endometrial hyperplasia with atypia or carcinoma. BMI≥30 kg/m$^2$ can be used as predictor of endometrial hyperplasia in premenopausal women with heavy uterine bleeding.

***Keywords:*** *Body mass index, endometrial biopsy, menstrual bleeding,*

## Background

Obesity and overweight estimated to account for up to 45% of endometrial cancer incidence in Europe[1] and 57% in the United States[2]. Endometrial cancer (EC) is the second most common gynecological malignancy worldwide. The incidence of EC is steadily increasing, primarily due to an aging population and escalating rates of obesity[3]. The unopposed exposure of endometrium to the effect of estrogen is a risk factor in endometrial hyperplasia & carcinoma. Other factors that are influencing estrogen exposure like obesity, polycystic ovarian syndrome, anovulation, nulliparity, and type II diabetes mellitus also increase the risk of endometrial cancer[4].

Although, different parts of the world started to research the use of Body Mass Index (BMI) as an indicator for endometrial biopsy, the data obtained are inconstant from country to another, this might be attributed to the racial and demographic variabilities associated with women in different part of the world[5]. It may also be that most of the researchers examined the role of BMI in undedicated studies but in retrospective manner using the non-objective reported BMI for the patients[2,5] The mechanism underlying BMI measurement is done by dividing the individual's weight (in kilograms) by height (in meters squared). The resulting unit is $kg/m^2$ [6].

Epidemiologically speaking; endometrial hyperplasia is common in women aged 50-54 years with body mass index (BMI) over 30, the average age for EH is 52 years, which is nine years lower than the average age for EC[7]. The increased risk of endometrial cancer among overweight (BMI > 25) and obese persons have been observed recently. Endometrial hyperplasia is one of the most common causes of abnormal uterine bleeding, which leads to endometrial carcinoma if left untreated. ***Therefore; in our study, we will examine the role of BMI in premenopausal women***. ***We hypothesize*** that Body Mass Index (BMI) correlates positively with histopathological features of endometrial biopsy in premenopausal women with abnormal uterine bleeding, thus BMI can be used an indicator for endometrial biopsy.

## Patients and Methods

This is a cross-sectional study that was conducted from 1st of August 2018 to the end of May 2019 in Obstetrics and Gynecology Clinic /Al-Emamain Al-Kadhemein teaching medical city in Baghdad where women 150 women who are 40-50 years old with heavy menstrual bleeding where sent for endometrial biopsy. 100 women had abnormal endometrial biopsy results, histopathological results of the abnormal endometrial samples were grouped into following groups: hyperplasia without atypia, hyperplasia with atypia, and hyperplasia with atypia and cancer. Statistical analysis conducted to correlate The Body Mass Index (BMI) with the status of endometrial biopsy.

## Results

Our results showed that 42% of the women with BMI≥30 $kg/m^2$ have hyperplasia with atypia or carcinoma compared to 10% of women who BMI<30 $kg/m^2$ ($p<0.001$). Out of 54 women who had hyperplasia with atypia in endometrial sample results; 77.7% of such patients were BMI≥30 $kg/m^2$. Receiver operating characteristic analysis shows that using BMI ≥30 $kg/m^2$ as a predictor of hyperplasia with atypia or carcinoma carries a sensitivity of 80.77 % and specify of 86.00% with area under the curve (AUC) value of 0.83 (CI. 0.75-0.92) and ($p<0.001$). It was observed that women with BMI≥30 $kg/m^2$ has a relative risk (RR) of 3.98 of developing endometrial hyperplasia (without atypia or with atypia+carcinoma) and a relative risk (RR) of 4.11 of developing hyperplasia with atypia or carcinoma ($p<0.001$).

**Table 1 shows the patients with abnormal endometrial biopsy characteristics.**

**Table 1: Patients Characteristics**

| Variable | No. | % |
|---|---|---|
| **Age (years) Mean ± SD (Range)** | 46.1±3.67 | (40-50) |
| **BMI Mean ± SD (Range)** | 29.88±2.71 | (20.81-37.64) |
| **Parity Mean ± SD (Range)** | 4.62±2.09 | (0-8) |
| **Nulliparity** | 13 | (8.6%) |
| **Diabetes** | 56 | (37.22%) |
| **Hypertension** | 60 | (40%) |
| **Family History** | 23 | (15.33%) |

**Table 2: Distribution of the cases based on Age and BMI cutoff values**

| Variable | | Benign | Hyperplasia without atypia | Hyperplasia with atypia | Carcinoma | Total |
|---|---|---|---|---|---|---|
| **Age (years)** | **40-44** | 19 | 16 (55.17%) | 11 (37.93%) | 2 (6.90%) | 29 (29%) |
| | **≥45** | 31 | 32 (45.07%) | 34 (47.89%) | 5 (7.04) | 71 (71%) |
| | | | | | | |
| **BMI** | **<25** | 1 | 7 (87.5%) | 1 (12.5%) | 0 | 8 (8%) |
| | **25-29.9** | 42 | 29 (76.32%) | 9 (23.68%) | 0 (0%) | 38 (38%) |

***Cont...*** **Table 2: Distribution of the cases based on Age and BMI cutoff values**

| | | | | | | |
|---|---|---|---|---|---|---|
| (kg/ $m^2$) | 30-34.9 | 7 | 12 (22.64 %) | 35 (66.04% ) | 6 (11.3 2%) | 53 (53%) |
| | ≥35 | 0 | 0 (0%) | 0 (0%) | 1 (100 %) | 1 (1%) |

Table 2 shows the distribution of the cases stratified according to age and BMI cutoff values.

**Table 3 shows the prevalence analysis of hyperplasia with atypia or carcinoma with different age and BMI**

**Table 4: Prevalence of hyperplasia and carcinoma according to different age and BMI groups.**

| Variable | | Hyperplasia with Atypia + Carcinoma prevalence (%) |
|---|---|---|
| **All patients** | | 52/100 (52%) |
| **Age ≥45 years** | | 39/71 (55%) |
| **BMI ≥30** | | 42/54 (77.7%) |
| **Age <45** | BMI ≥30 | 12/15 (80%) |
| | BMI <30 | 1/14 (7.14%) |
| **Age ≥45** | BMI ≥30 | 30/39 (76.9%) |
| | BMI <30 | 9/32 (28.13%) |

groups.

**Table 4: Risk factors for endometrial hyperplasia and carcinoma in premenopausal women with Abnormal menstrual bleeding**

| Risk Factor | All Abnormal Findings | | | Hyperplasia with atypia + Carcinoma | | |
|---|---|---|---|---|---|---|
| | *RR (95% CI)* | *p* | *Odds Ratio* | *RR (95% CI)* | *p* | *Odds Ratio* |
| Age≥45 (years) | 1.13 (0.60-2.32) | 0.73 | 1.17 | 1.27 (0.85-1.92) | 0.316 | 1.61 |
| BMI ≥30(kg/m$^2$) | 3.98 (1.92-8.25) | <0.001 | 6.65 | 4.11 (2.32-7.32) | <0.001 | 15.96 |

Table 4 shows multivariate analysis of the risk factors Age (≥45 years old) and BMI (BMI≥30 kg/m$^2$)

**Table 5: show ROC analysis which demonstrated a significant relationship between all abnormal endometrial histological findings and BMI.**

| Cut-off BMI (Kg/m2) | SENSITIVITY | SPECIFICTY | ROC (95% CI) | P |
|---|---|---|---|---|
| | (%) | (%) | | |
| All Abnormal findings | | | | |
| ≥25 | 91 | 2 | | |
| ≥30 | 52 | 86 | 0.68 (0.60-0.77) | 0.00026 |
| ≥35 | 3 | 100 | | |
| Hyperplasia with atypia + carcinoma | | | | |
| ≥25 | 98.08 | 2 | | |
| ≥30 | 80.77 | 86 | 0.83 (0.75-0.92) | < 0.0001 |
| ≥35 | 5.769 | 100 | | |

## Discussion

The findings of this study revealed abnormal endometrial histology in 66.6% of cases (100 out of 150) of premenopausal women with abnormal uterine bleeding. The major risk factors for endometrial hyperplasia and carcinoma in this study were BMI ≥ 30 kg/m$^2$ and diabetes mellitus. Even that the hyperplasia without atypia was reported with age of 40-44 years and the hyperplasia with and without atypia were reported with BMI index of 25.9-29.9 kg/m$^2$ but the association did not reach the significant level.

The mean age and BMI of the patients in this study was higher than that those who has been reported by Sun et al were they found that the mean age of included women was 35.9±9.3 years and the mean BMI was 23.5±3.9 kg/m2[8]. The difference in age average is because Sun Y et al inclusion criteria for age was 15-55 years old while our study design used age selection of 40-50 years old. Our data shows higher BMI average for our samples in comparison

to Sun et al, which might be attributed to the older women in our study (40-50 years old) as BMI tends to increase with age.[9]

The authors Wise et al found that the mean age of included women with abnormal uterine bleeding was 43.7 ± 6.4 in the non-obese group and 42.0 ± 7.2 in the obese group.[10] The diabetes mellitus and hypertension were reported in 37.22% and 40% respectively and nulliparity in 8.6%. The significant association of abnormal uterine histological findings was confirmed with diabetes mellitus only. Nearly similar proportion of patient with such comorbid illness and nulliparity were reported by Epplein et al who were found an increased risk of endometrial hyperplasia with increasing body mass index and nulliparity but no association with diabetes or hypertension was found.[11].

With cases of age> 45 years old, our results revealed that the hyperplasia without atypia also represent the commonest abnormal finding (45.07%) followed by hyperplasia with atypia (47.8%) but the prevalence of carcinoma was higher than younger patients (7.04% vs.6.9%) as well 39 out of 71 cases of age ≥45 years were showed hyperplasia with Atypia & Carcinoma. These finding in accordance with the results of Guraslan et al that found the commonest pathology was hyperplasia without atypia followed by hyperplasia with atypia and carcinoma with both age groups and higher prevalence of carcinoma also reported with a women of ≥ 45 years old.[12]

Our findings were partially in discordant with authors Nicholls-Dempsey et al who has been found 13% of cases had endometrial hyperplasia or neoplasia but 42% of patients with Abnormal uterine bleeding between 41 and 45 years old did not showed hyperplasia or neoplasia.[15] Our results showed the hyperplasia without atypia, hyperplasia with atypia or endometrial carcinoma represented were reported with BMI >25-29 and >35 kg/m$^2$. These findings agreed with Guraslan et al as they also found that all the abnormal findings including hyperplasia without atypia, hyperplasia with atypia, and carcinoma were observed among patients with BMI ≥30 and the difference was statistically significant[12].

Patients with BMI>24 kg/m$^2$ are obese, since in patients with higher BMI, the fat constituent of the body is higher, this will lead to overproduction of estrogen, as excessive adipose tissue increases the peripheral aromatization of Androstenedione to estrone in premenopausal women. This abnormal feedback loop associated with premenopausal women will cause anovulation, which cause unopposed effect of estrogen on the endometrium leading to thicker endometrium and higher risk for abnormal uterine bleeding[14].

These results were in consistent with findings of retrospective cohort study that carried out to evaluate the effect of BMI on endometrial hyperplasia and cancer done in Auckland from 2008 to 2014 (916 women met the inclusion and exclusion criteria) were they found that the obese women had higher odds of having complex hyperplasia or cancer compared to non-obese women[14], also in line with Giannella et al that found the odd ratio for association with endometrial hyperplasia(EH)/endometrial carcinoma(EC)by univariate analysis for different factors as following; for BMI ≥ 30 was 8.13( 95% CI 2.34 - 28.21)[15].

Our findings also revealed that BMI is risk factor for hyperplasia with atypia and carcinoma ($p<0.001$) were in disagreement with Parslov M et al who found that the BMI was not demonstrated to be an independent risk factor in their study for endometrial cancer but the family history (OR; 2.1), completion of 1 term pregnancy (OR; 0.6), receiving hormone replacement therapy for 1-5 years (OR; 1.4-7) are the indicator of predicting endometrial carcinoma.[16]

Our results also showed that diabetes mellitus and BMI≥30 kg/m$^2$ are the factors that significantly associated with increasing the incidence of hyperplasia with atypia and carcinoma (P=0.001 for both) and this findings are in agreement with

Guraslan et al concerning the association of diabetes mellitus disease with increasing the incidence of these pathologies but in discordance with regard to the nulliparity status, however they identified the PCOS as a risk factor associated with these disorders but they found when hyperplasia without atypia was excluded, age of 45 years and older determined as a risk factor but PCOS was not a risk factor (p = 0.02 and 0.12, respectively)[12]. The findings were in disagreement with Wise MR et al concerning the associated risk factors for abnormal uterine histological findings as they found nulliparity (OR, 2.51; 95% CI, 1.25-5.05), anemia (OR, 2.38; 95% CI, 1.25-4.56), and thickened endometrium on ultrasound (OR, 4.04; 95% CI, 1.69-9.65) as other variables associated with the outcome[10]. It was also against the findings of Soliman et al who found the nulliparity is a risk factor for abnormal uterine finding[17].

Our study revealed that abnormal findings were higher with BMI >30 kg/m2 regardless the age group of the patients. These results were in agreement with Xu et al were also concluded that high BMI at all adult ages significantly predicted complex endometrial findings.[18] But our results were in discordance with findings of Thomas CC et al that found the increased risk of abnormal uterine findings with women who had BMIs of at least 35 who are younger than 45 years at the time of last menstrual period[19].

At the best cut-off value, sensitivity and specificity were 75.0% and 90.79%, respectively; the PPV and NPV were 30.0% and 98.6%, respectively.[10] Our findings showed that the BMI not the age of the patient is risk factor for endometrial hyperplasia and carcinoma as the results revealed significant high odd ratio with BMI ≥30 (kg/m2), OR; 4.11,P<0.001. These findings in agreement with Yamazawa et al who were found the odd ratio (OR) of obesity (BMI≥30) was 6.66 (p = 0.001; CI 2.40–18.48) for endometrial cancer and this ratio significantly considered as good indicator or predictor of endometrial carcinoma.[20] It has been concluded in their study that the body mass index showed a positive trend for risk of endometrial carcinoma as the BMI of the woman increases the risk of endometrial carcinoma was also increases.

## Conclusions

The results of the current study confirm that obesity is the most regardless the age of the women is a significant risk factor for endometrial hyperplasia with atypia and carcinoma in premenopausal patients with abnormal uterine bleeding as we found the patients with BMI 30 whether their age is 45-years old or <45-year-old showed the highest risk for endometrial hyperplasia with atypia or carcinoma while the patients with BMI <30 whether aged <45-years old or 45-years old had the lowest risk for endometrial hyperplasia with atypia or carcinoma. Diabetes mellitus also identified as a risk factor associated with increasing the incidence of endometrial hyperplasia with atypia and carcinoma. BMI is independent indicator for prediction of serious medical condition such as endometrial carcinoma in patient with abnormal uterine bleeding

**Ethical Clearance**- Taken from Al-Emamein Al-Kadhemein Medical City, Baghdad, Iraq

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**Conflict of Interest** - None

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# The Experience of Health Services in Handling Covid-19 Pandemic in Nine Provinces of Indonesia

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## Abstract

**Background:** Indonesia's experience in handling the Covid-19 pandemic is essential to prepare for future pandemic.

**Aim:** This research aims to know the experience of health services in handling Covid-19 pandemic in nine provinces of Indonesia.

**Methods:** This research used qualitative and case study approach, with a total of 94 informants coming from all levels of health service agencies from nine provinces in Indonesia. The data collection process was carried out by in-depth interviews, and data analysis were done by means of triangulation of sources between informants.

**Results:** Each province has a variety of local policies with the addition of varying amouns of the budget for handling Covid-19. In addition, the workload of health workers increased after the pandemic, and the availability of facilities, medicines, and single-usage medical materials were deemed inadequate in dealing with the Covid-19 pandemic.

**Discussions:** Accounting for the use of funds for handling Covid-19 as well as increasing surveillance capacity needed to prepare for future pandemic in Indonesia.

***Keywords:*** *Covid-19, Single-usage medical materials, Pandemic, Indonesia*

## Introduction

Since declared on March 11 2020 by WHO that it is a pandemic, COVID-19 has spread rapidly across Indonesia[1]. If on March 11 2020 34 cases were recorded in several province, on June 2nd 2020, the total Covid-19 patients leaped to 27.549 people in all provinces in Indonesia[2]. Moreover, Indonesia is an archipelagic country which consists of 33 provinces that adopts a decentralized system. Furthermore, it is possible that each province has its own obstacles and solutions in handling the Covid-19 pandemic[3].

Compared with China and South Korea, they were able to handle the spread of Covid-19 quickly, which reached the peak of daily case within 2-3 weeks. Indonesia, however, experienced a sloping growth in cases[4]. Learning from various cases from other countries, it seems that the decline in the Covid-19

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cases has been slow, thus the end of the epidemic in Indonesia is hard to predict[5]. There are, however, other determinants of factors, especially public's discipline in obeying the government policies and high proportion of elderly people associated with comorbidities[6].

In Indonesia, pandemic control is carried out by means of large-scale social engineering, which affects economic activity. The experience of the Indonesian health system in handling the Covid-19 pandemic is very much needed to prepare for any future pandemic for Indonesia and other developing countries[7]. Furthermore, the capacity of each province is different in terms of the level of economic progress and health. Therefore, the objective of this research is to assess the **experience of Covid-19 pandemic management handling in nine provinces in Indonesia**. Thus, it is hoped that the experience of handing Covid-19 can be seen from various provinces with good and inadequate fiscal capacity in Indonesia.

## Methods

This research is a qualitative research with a case study approach. Research informants came from nine provinces in Indonesia, namely Sumatera Utara, DKI Jakarta, banten, Jogyakarta, Jawa Tengah, Kalimantan Selatan, Sulawesi Selatan, dan Papua. These provinces are selected based on high and low fiscal capacity in Indonesia. This study had a total of 94 informants (IF1-IF94) from nine provinces in Indonesia. The informants came from the Provincial Health Office, Dictrict/City Health Offices, Public Health Centers, and Government Hospitals as well as Private Hospitals. These agencies represent all levels of basic and referral health services in Indonesia.

The aspects seen in the handling of Covid-19 in the nine provinces include policies and regulations regarding Covid-19, financing of Covid-19 handlement, the human resources, quality of health services for Covid-19 handlement, and availability of drugs and medical tools for handling Covid-19 as what have been done in previous work[8–11].

The study procedure was initiated by licensing all research insititutions in nine provicnes in Indonesia. The data collection process was carried out by in-depth interviews. The data is then transcribed and summarized in the form of a matrix. Data analysis was carried out by means of triangulation of sources between informants. This research has received ethics approval from the Health Research and Development Agency of the Republic of Indonesia on June 17, 2020.

## Results

### Policies and Regulations

Informants from the nine provinces revealed that there were various rules or regulations, starting from the district or city level to the provincial level. It is known that the provincial level regulations are more fcocused on the formation of task force, the appointment of referral hospitals, and policies regarding health protocols which are follow-ups to the Covid-19 prevention guidelines made based on regulations from central government. One of the provinces that has regulations on this focus is East Java Province, where regulations were found implementing health protocols, providing local tax incentives for Covid-19 handlement, implementing sanitation in worship places, tracing (to break the Covid-19 spread), waste management in the context of preventing Covid-19, and regulations regarding working from home. Likewise, Central Java Province made regulations about Covid-19 as an outbreak status.

In addition to the formation of regulations from the provincial and district/city levels, various other efforts have also been made in handling the Covid-19 pandemic. One of the efforts is the creation of Covid-19 prevention guidelines. The guidelines for preventing Covid-19 have changed five times. Despite those changes, informants from various provinces said that the guidelines were still easy to understand and apply. It only tookt he informants a few days to get used to and implement the changes to the guidelines.

Guidelines for the prevention and control of Covid-19 Revision 5 and Guidelines for Covid-19 management, August 2, 2020 edition (IF15 from DKI Jakarta)

The protocol is easy to understand and so far, there have been no difficulties in implementing the Covid-19 handling protocol (IF56 from Jawa Tengah)

In various provinces, there were obstacles in disseminating health protocols. The Jawa Timur province had difficulty sozializing health protocols online related to signal problems. Whereas in Papua, it was found that not all people belive about the existance of Covid-19, thus some people refused to be examined. The following are detailed explanations of the health protocol socialization obstacles in various provinces.

**Table 1. Obstacles in the Socialization of Health Protocols in Nine Provinces**

| North Sumatera | Yogyakarta | East Java | Papua | South Sulawesi |
|---|---|---|---|---|
| There are no definite laws, thus there are still many health protocol violations (IF3) | The most difficult thing is social distancing due to the opening of tourist attractions, and in those places, it starts to get out of control (IF33) | The difficulty in disseminating regulations and guidelines without directly dealing with them (through online media) is deemed not optimal, especially if there are internet network problems and voices are not clear (IF57) | Difficulty in directing the public to conduct checks, tracing, and the disbelief in the existence of Covid-19, (IF92) | Yes, the difficulty is just how we distribute it to the public because the guideline's fourth revision is very different from the fifth revision in terms of hadling Covid-19 patients (IF80) |

**Financing**

In this study, it was found that the budget for handling Covid-19 was different for each province and agency. At RSUP H. Adam Malik in North Sumatera, the budget for handling Covid-19 was IDR 2.502.205.616. Unlike the hospitals in North Sumatera, DKI Jakarta's total budget is only IDR 376.369.001 for Covid-19 handlement. Meanwhile in Jogjakarta Province, the Kulon Progo Health Office has a budget of IDR 2.520.874.028. At Semarang City in Central Java Province, the budget for handling Covid-19 is IDR 3.076.967.670 while Salatiga City Health Office reached IDR 28.734.8736.664.

According to an informant from North Sumatera, the total Covid-19 budget in several hospitals is actually still insufficient for the provision of BMHP, PPE, and others. The increase in PPE and BMHP prices is also the cause of insufficient Covid-19 budget funds. However, the Covid-19 budget funds were still sufficient because of the Unexpected Expenditure APBD funds.

If you it is enough, obviouslt it is not. Especiial for the provision of BMHP, PPE, monitoring operations, and incentives for health workers. However, with the existing budget, efficiency is still being implemented, such as unexpected fund diversions (IF3 from North

Sumatera)

It cannot be said that it is enough, but it also cannot be said that it is insufficient because the prices of PPE and BMHP all went up. Attempted by personnel who do not have direct contact with patients, occasionaly using a cloth (IF24 from Banten)

The budget for handling Covid-19 from the government and other institutions or agencies is used for various things. At a hospital in North Sumatera, there is a budget allocation for handling Covid-19 which is used for transportations costs for officers, procurement of BHMP, and procurement of PPE. Hospitals in Yogyakarta and Centra Java also use the budget for handling Covid-19 to procure PPE.

The transport for isolated, suspected, and close-contact patients are taken from BOK for routine transport (IF5 from North Sumatera)

Tracing implementation because it requires PPE and a low of manpower (IF21 from Yogyakarta)

The budget is mostly used for PPE (IF48 from Central Java)

**Service Quality**

According to the informant's explaination in this study, it was found that health workers at the time of the Covid-19 pandemic had inappropriate proportions in certain segments. The shortage of medical personnel is a major facor at this time due to the lack of human resources. Efforts made by health facilities include a rolling system. The following is the informant's explaination.

With the number of cases continuing to increase, there is a shortage of human resources. For this hospital, there should be shifts or rotation of officers every four month. However, the availability of human resources is very minimal (IF9 from North Sumatera)

The human resources for hospials in DKI Jakarta, Jogjakarta, and *Central Java* have more than enough, even though at certain times they are overwhelmed. This can be overcome by applying shifts or rotation to the officers on duty. The following is the informant's explaination.

Human resources are sufficient because some are still young and has the enthusiasm in handling Covid-19 (IF17 from DKI Jakarta)

It is enough and not lacking. It's just that the workload has indrased (IF24 from Banten)

Until now, human resources have been sufficient because we cannot recruit anymore. So, there is only shifts or rotation, especially for the paramedics because they work 24 hours. So, there is a rotation happening (IF38 from Yogyakarta)

Human resources handling Covid-19 patients at the beginning of the pandemic were sufficient, but overtime a little overwhelmed (IF56 from Central Java)

Hospitals in East Java and South Kalimantan often experience a shortage of health workers. This happened due to the increasing number of patients who were positively infected with Covid-19. In South Kalimantan, a shortage of human resources also occurred because officers were found to be infected with Covid-19. The lack of human resources at hospitals in East Java has been carried out by several solutions, such as adding health personel from internships and volunteers. The following is the informant's explaination.

The need for human resources at Arosbaya Public Health Center is still limited when compared to the number of Covid patients being served. The solution includes: 1) Assistance of staff from interns, 2) Transfer of duties and functions, as well as additional job descriptions for personnel whoa re active in public health efforts to the Covid-19 ploy clinic for screening, recording, and tracing (IF61 from East Java)

There is a shortage of doctors because there are only three people in the Health Centre, then there are doctors who have been confirmed positive for Covid.

So, only two are active effectively. And one of the two doctors is also a doctor who on maternity leave (IF72 from South Kalimantan)

So far, human resources are sufficient and we have received 400 volunteers (IF18 from DKI Jakarta)

Various trainings have been carried out by health workers to assist in handling Covid-19 patients. The training that health workers received in handling Covid-19 is training for swabber, Covid-19 coutnermeasures, Infection Prevention Guidelines (PPI), and many other trainings. However, further training is needed for the health workers.

**Table 2. Training Needed for Health Workers**

| Training Needed for Health Workers | Informant Code |
|---|---|
| How to do tracing correctly. Maybe there are more detailed interview techniques that can be used to do tracing effectively. | IF58, IF80 |
| The desired training is data processing and analysis of Covid-19 because data is constantly changing. | IF46, IF58 |
| Need to train non-health workers to be able to help in the filed in order to be able to shift as well as maintain the health and safety of staff. | IF12 |
| Provided with the latest education related to Covid hadnlers and providing excellent service. | IF19 |
| Tracing training is included for volunteer tracing implementation. | IF57 |
| Technical guidance for Health promoters. | IF57 |
| ICU room attendant training for ventilator installation. | IF57 |

The risk of being exposed to Covid-19 is very high for health workers. However, in Sumatera Utara, most of the officers infected with Covid-19 are officers who are not on duty in isolation rooms and do not directly handle Covid-19 patients. This is different from what happened in Jogjakarta, where 40 health workers were positively infected by Covid-19 because the implementation of health protocols was not optimal in several rooms.

From 20% of the total health center staff who participated in the mass swab, the results were two people confirmed positive, namely 1 nurse and 1 part of management (IF6 fro North Sumatera)

Approximately five doctors were exposed. One person is a physician Non-Covid-19 polyclinic, and the other doctor in management is most likely exposed outside the hospital (IF26 from Banten)

According to an informant from Jogjakarta, several hospitals have provided a special place for isolation of health workers who are positive for Covid-19. The hospital has also disseminated K3

guidelines to all employees. Not only that, the hospital also carried out tracing according to the guidelines for preventing Covid-19 at the Hospital.

Provide a place of isolation, socialize guidelines from K3 and tell all employees to wear PPE, adjust it to their workplace, social distancing and wash their hands frequently. Provide special wards for them if they are infected with Covid. It's on the 3rd floor. It is dedicated to our employees if they are positive for Covid. So, they isolated themselves in this hospital (IF44 from Yogyakarta)

Based on the results of this study at a hospital in North Sumatera, incentives given to health workers were carried out as it should be. Incentive is only given to officers who handle Covid-19 patients directly. Incentive is considered as a form of appreciation or concern for the loyalty of officers in handling Covid-19.

Regarding the calculation of health workers' incentives not to be limited by the number of recipients, due to the fact that in the field, the tasks were carried out by a team (IF19 from Banten)

Incentives that do not differentiate between Public Health Centers and Hospitals (IF49 from Central Java)

Incentives for Public Health Centers are less than hospitals. Whereas in the field that carry out a lot of tracking, tracing, and other activities, most of them are Public Health Center people, so the workload of Public Health Center worker is a lot (IF71 from South Kalimantan)

**Health Services**

Health services during the Covid-19 pandemic certainly experienced many changes, such as health workers who had to use PPE and the application of health protocols in hospitals. It is known that in North Sumatera in providing health services during the Covid-19 pandemic, health protocols were implemented such as obtaining a place to wash hands and the officers on duty had already used PPE properly. The flow of services at the hospital is also in accordance with applicable regulations and procedures. The following is the narrative.

Waiting rooms are not yet ideal for patients. Patients with suspected Covid-19 wait outside the building (IF34 from Yogyakarta)

If we get a positive Rapid Test result, we are confused. What is the status of this patient? Fit into what kind of criteria? It means that something is out of sync. So, the guidelines cannot be executed, do not have the ability to force the situation, and the existing circumstances have never been seen in the gudelines (IF44 from Yogyakarta)

Various problems and obstacles are of course experienced by health facilities in handling and minimizing the spread of Covid-19. In North Sumatera, it was found that hospitals only have rooms for screening health personnel. Several Public Health Centers in North Sumatera have also found difficulties in early detection of Covid-19 in the community. This is because so far the observation has only been carried out independently by the Public Health Centers. The following is the story of the informant regarding the screening flow in North Sumatera.

Currently, RSUP only has room for screening health personnel only. Temporary house is needed to provide a sense of security and comfort when health workers finish their activities at the hospital (IF7 from North Sumatera)

People Without Symptoms (OTG) is one of the things that is troubling during the current pandemic, so it is necessary to make efforts to minimize the spread of Covid-19 through OTG. One of the efforts that are often carried out in various regions is to provide a shelter for OTGs. However, some areas in Indonesia, such as in Serang, Banten, do not yet have a shelter for OTG. So, that at this time almost all Banten people carried out the screening process and accessed health facilities to carry out a swab test.

The incidence of patients who have died and tested positive for Covid-19 with undetermined swab test results has occurred evenly in all regions in Indonesia. This is due to the length of the test results. Some health facilities have been accused by the local community with the term Covid of probable patients who died before the results of the swab test were published. In Jogjakarta, this happened because it had to be done according to applicable guidelines. Not only in Jogjakarta, fixing of that matter is also opposed in South Kalimantan, along with his narrative.

Probable: Suspect person dies before the swab comes out, is it probable? What is the basis? (IF43 from Yogyakarta)

Some patients who wish to enter the hospital must undergo a rapid test. But the results of the rapid test have not yet come out, it turns out that the patient has died first, thus some people do not accept it, etc. Therefore, there is a lot of news circulating that the patient was deliberately made covid-19 " (IF69 from South Kalimantan)

**Medicine Availability**

This study found that the stock of medicines available at the hospital had constraints such as expiration and stocks of certain types of drugs were not available. However, Public Health Centers and hospitals have other drug alternatives to replace empty stock of drugs with a fast restocking process, so that the drugs available at the hospital are still sufficient.

So far there have been no significant obstacles. Even at the hospital, there were only drug stocks that had expired. That was immediately reported to the Provincial Health Office and the restock process was fast (IF3 from North Sumatera)

It is enough because the drugs given are more on symptomatic drugs (IF12) (IF13 from DKI Jakarta)

The results of this study also revealed that treatment services for Covid-19 patients in health facilities were not sufficient to treat patients. Most of the informants complained about this due to the lack of much needed antiviral drugs. The available antiviral drugs are also unclear on the instructions and criteria for their use. Another complaint expressed by several informants was that the availability of drugs for serious and critical illnesses were still lacking. The following is the narrative.

The drugs most needed for the treatment of Covid patients are antivirals and antibiotics (oseltamivir is hard to get) (IF54 from Central Java)

Availability of Covid drugs at dr. Soetomo for standard light medicine and medium is sufficient. However, for heavy and serious drug is still lacking due to inadequate distribution such as Resedimvir, Levofloxacin, and Metoclopramide (IF63 from East Java)

Several efforts by health facilities to avoid empty drug stocks have been made. Several informants revealed that the hospital immediately bought drug stocks, which did not use the funds to procure drugs. However, there are often problems in fulfilling drug stocks such as goods needed are not available in the market and need to submit to the Health Office and order from certain distributors. Most of the informants in this study also revealed that in order to supply new drugs, it is necessary to submit a proposal for assistance to the company and the Health Office. Several informants also revealed that another obstacle in drug procurement was due to unclear drug procurement regulations.

In the procurement of BMHP, there are no problems. Only problem is PPE, which is not available in the E-Catalog (IF39 from Yogyakarta)

The availability of goods needed in the market that does not exist, the solution is to contact the distributor directly who has been our partner (IF45 from Central Java)

There are no clear rules that the Health Office can procure drugs outside PKD (Basic Health Services) /

generic (IF57 from East Java)

The results of this study indicate that the tools for detection of Covid-19 are still lacking. Several informants revealed that Covid-19 detection tools such as PCR and TCM were only available for 1 unit in one hospital. This is an obstacle due to the lack of optimization and effectiveness of health workers in detecting Covid-19.

However, the problem is the PCR device and the TCM machine, only 1 unit is available at H. Adam Malik Hospital. For TCM even the cartridge is very hard to come by. the more tools are used, the faster the early detection of Covid-19 will be (IF7 from North Sumatera)

This study revealed that hospitals and Public Health Centers were experiencing a shortage of BMHP, such as masks and hand sanitizers. Most of the informants stated that the limited number of medical devices available caused the use that was not in accordance with standards. However, most informants also stated that the availability of medical devices was sufficient. This is supported by the fact that several health facilities purchase their own equipment and the large number of equipment grants for health workers.

**Table 3. Personal Protective Equipment (PPE) Problems**

| PPE | Problems |
|---|---|
| PPE in general | - There are also PPE deficiencies relying on donations (IF11)<br>- The PPE grant was not good, so the hospital decided to buy it (IF 18)<br>- When the tool is available, the room is not (IF11)<br>- According to the protocol, our rooms for installing PPE and removing PPE should be far apart. But for now, this cannot be done due to the limited space of the Public Health Centers, the term is called dopping and donning (IF70). |
| Hazmat Suit | - At the beginning of the pandemic, when PPE experienced a shortage of hazmat wearing raincoats. There were also donors who helped. Currently, PPE stocks are still safe (IF9)<br>- The hazmat suit, after wearing it, then wash it and dry it in the sun, then put it on again (IF11)<br>- Reuse, calculate monthly usage, for the next need (IF44)<br>- For hazmat use, change into a raincoat. To use the N95 in the sun after use. For medical masks it is still sufficient (IF61)<br>- Borrowing and creativity to make gloves by patching with tape and using raincoats as hazmat (IF51)<br>- At the beginning lacked PPE, so some health workers wore raincoats and plastics. (IF73) |
| Masks | - Masks until December are lacking, especially the N-95. Most surgical masks are needed, but the price issue is far. (IF33)<br>- The problem is the fulfillment of the N-95 mask, because the price is still expensive. So, I urge friends to wear a two-layer surgical mask. |
| Oxymeter | - Oximetry examination, the number is still limited. Only in the ER and those at the screening. Each unit does not have one. In FKTP, the initial detection is, at least the temperature is the same as oxygen saturation (IF35) |

The results of this study indicate that the suitability of the ICU room and medical devices is not sufficient. Most of the informants revealed that the need for a ventilator in the ICU room could not accommodate the large number of patients being treated in that room. This shortage of needs also includes medical devices such as stethoscopes and thermogun, which are not yet available. The following is the narrative of the informant.

Ventilator. Currently the hospital only has five ventilators, while more and more patients need ICU care (IF8 from North Sumatera)

A stethoscope that is used to keep a distance, but until now there is none (IF19 from Banten)

According to the informant's explanation, the lack of stock of certain tools for handling Covid-19 patients still occurs in the laboratory room. Most of the informants stated that the components of the laboratory needs came because of the various donations given. However, the various donations given did not meet laboratory standards. Several informants also revealed that they needed aerosols for dental services to prevent transmission. The following is the story of the informant.

Availability of supply providers is not always available, and there must be a distribution permit for certain equipment which is very much needed when in the laboratory. The aid reagents that come with different brands and the requirements for BMHP are not the same. Various sizes of aerosol barrier filter tips, 70% alcohol, nitrile gloves, micro tubes, parafilm, children's size dacron swabs, VTM which various brands often do not meet the needs in the lab, donation reagents (PCR and various extraction kinds of brands, with single gene or multiplex), etc. (IF63 from East Java)

VTM with various brands that often do not suit the needs in the lab, donation reagents (PCR and extraction of various brands, single gene or multiplex), etc. (IF63 from East Java)

For the Public Health Center, the most needed is aerosol, especially in dental services to prevent transmission (IF70 from South Kalimantan)

## Discussion

The results of this study reveal that every province in Indonesia has various regulations that focus on accelerating and preventing Covid-19. In the U.S., regulations prioritize high-risk communities for testing, treatment, and overcoming barriers to social and economic welfare [12]. The regulations in Taiwan since February 2020 have focused on the detection and isolation of local cases and limit the entry of foreigners from highly influential areas [8]. One of the policies has also been developed in various provinces in Indonesia, namely social restriction policies and implementation of health protocols. The implementation of this policy is expected to be able to reduce the rate of Covid-19, including in Indonesia.

The Covid-19 prevention guidelines made by the Indonesian Ministry of Health to reduce the rate of Covid-19 in Indonesia have undergone changes and added to the contents of the guidelines so that they are adjusted to the increasing situation and the development of findings regarding new things on Covid-19. One of the changes is in the chapters on clinical manifestations, infection prevention and control, specimen management and laboratory confirmation, and community empowerment risk communication [13]. Changes to the guidelines should also be developed in view of the situation and conditions in various provinces. The government should also ensure that the socialization of the revised guidelines runs smoothly.

The informants of this study complained that there were still people who did not believe in the Covid-19 pandemic due to a lack of socialization from the government. In fact, they are not worried about new clusters of transmission from public places or tourist attractions. Encouraging development and innovation from resilient villages to increase efforts to prevent Covid-19 have been carried out in Surabaya.

In Australia, the federal government implements a self-isolation policy by closing all services on March 23, 2020, including clubs, hotels, cafes and restaurants limited to takeaway services only [14]. This policy was also implemented in DKI Jakarta. However, fears of economic conditions have made these public places operational again by implementing health protocols.

Lack of public awareness in implementing health protocols in outdoor activities is very important to minimize transmission of Covid-19. A study conducted by Michael in developed countries shows that many adults are aware of the importance of preventing exposure to Covid-19, but this does not change the routines or plans they want to do [15]. According to informants in this study, people from economically disadvantaged circle find it more difficult to implement health protocols. The youth group hopes that the success of the socialization in implementing health protocols for the prevention of Covid-19 will run smoothly. Therefore, the socialization of Covid-19 prevention requires different approaches at different age groups and economic levels.

The socialization of the prevention of Covid-19 that has not been optimal has resulted in more and more people being affected by Covid-19. The high number of positive Covid-19 patients affected economic growth, so there were obstacles in the financing budget for Covid-19 patients. The financing budget for Covid-19 in Indonesia is sourced from the 2020 State Budget (APBN). In developed countries such as the UK, it provides £ 3.2 billion in emergency grants and more than £ 5 billion in cash flows to support local authorities in the month March and April 2020 [9]. The allocation of funds amounting to £ 1.6 billion is intended to meet the needs of health care expenditures during the pandemic. Not only that, the available funds are also allocated per person with most of them flowing to low-level districts for social safety net services [16]. In Indonesia, the government issued a financing regulation to handle Covid-19 in the form of (Perppu) No.1 of 2020 concerning State Financial Policy and Financial System Stability for Handling the 2019 Corona Virus Disease Pandemic (Covid-19).

In March 2020, the British government handled Covid-19 by mobilizing Covid-19 funds for five sectors [17]. These sectors are health services (service providers, equipment, vaccine testing and development), public services and emergency response, support for affected individuals, pandemic, and PPE support [9]. Based on the experience of developed countries, the Government of Indonesia should increase its budget, especially in terms of procurement of PPE and vaccine supply.

The Covid-19 pandemic does not only have an impact on the economy and funding and special Covid-19 budgeting. This also has an impact on health workers who experienced an increase in work shifting during the Covid-19 pandemic [18]. So, that additional officers are needed in handling Covid-19 such as volunteers.

The results of this study indicate that in handling the Covid-19 pandemic, the Indonesian government opens recruitment of medical and non-medical volunteers, and collects donations in handling Covid-19. Non-medical IT volunteers are needed to create systems and applications for accelerating information and policy making based on data [19]. In Indonesia, through the government, a Task Force was formed which has the aim of being a special volunteer in helping health workers handle Covid-19 [20]. This needs to be done evenly for fulfillment in health workers or volunteers in remote areas to handle Covid-19. Volunteers who have been recruited in handling the Covid-19 pandemic must have more abilities. One of the quick efforts to improve the capacity of officers is by providing training.

One of the capabilities expected to be improved by the informants of this study is surveillance capability. The availability and completeness of surveillance data that is real time, interoperable across units, and connected between regions is a navigation tool for policy-making that is urgently needed [21]. At times like this it will be better if human resource funding capacity

is increased and develop systems for surveillance and tracing for handling Covid- 19.

Covid-19 detection is carried out by tracing and several types of tests such as swab tests. The test results according to this study take a long time in several provinces. This research was also confirmed by the news that the results of the swab test tended to be accepted for a long time due to the lack of capacity for the PCR test [10]. In South Korea, the hospital formed a unit of officers that had the role of regulating a special hospital for Covid-19 referrals so that the results were faster [22]. The screening process for patients to detect Covid-19 are carried out by means of rapid tests and swab tests in primary health facilities [23]. Screening of medical personnel, namely by contacting the hospital via email by mentioning the symptoms they feel. Testing of medical personnel also through the Screening Pod using the RT-PCR method [24]. This requires strengthening the capacity of primary health facilities, including those at the Public Health Centers.

In Indonesia, health facilities for handling Covid-19 do not need to rely on hospitals but must also strengthen primary health services. This can be done by strictly implementing health protocols at health centers, improving technical or clinical skills in dealing with Covid-19, and conducting management and monitoring of isolation [7]. Strengthening primary health services can also be done by improving quality in the pharmaceutical sector. This is in accordance with research, which states that in the pharmaceutical sector or pharmacists have an important role in handling Covid-19 by monitoring the drugs used by Covid-19 patients [25].

The results of this study indicate that the stock of medicines available at the hospital has a problem in completeness of the drugs. This is also supported by the difficulty of finding a distributor of medical equipment providers at the start of the pandemic [26]. The need for oral drugs is also increasingly scarce and costly at the beginning of the pandemic [18]. According to the results of this study, ensuring sufficient drugs for the symptomatic handling of Covid-19 patients is a matter of the important one. According to the results of other studies showing that Vitamin C given to inpatients with Covid-19 has also decreased availability [27]. The procurement of these drugs does require funding and the right distributors.

In the Covid-19 pandemic, distributors are very much needed, especially to help in the shortage of personal protective equipment (PPE). The results of this study also indicate that it is difficult to find distributors and market prices are high. This shortage resulted in an increase in PPE production to meet demand, and as a result some substandard equipment began to enter the market [11]. The government should encourage an increase in the role of smalland medium enterprises in producing PPE domestically. So, that the community can divert the business sector during the Covid-19 pandemic, namely producing Personal Protective Equipment (PPE) and health masks / cloths that can be used repeatedly by the community [1]. In addition to producing PPE, the production of other tools such as tools for early detection of Covid-19 is also required.

Early detection of Covid-19 can be done with several tools, such as a body temperature measuring device. South China Morning Post (SCMP) reports that one of the efforts to detect and prevent the spread of the corona virus is to check the body temperature which is equipped with a handheld infrared thermometer, also known as a “thermogun”, because it looks like a gun that is fired in the forehead [3]. Other temperature sensor devices are also observed by Puput et al [28], also observed that the MLX 90614 Temperature Sensor for Early Detection of Covid-19 Symptoms before entering the building can be used with an average error of 0.6% and a standard deviation of 0.078. The results of this study indicate that the availability of thermogun is sufficient in health facilities.

Health facilities in several regions in Indonesia are known to have met various needs in handling

Covid-19 both in terms of facilities, human resources, and funding. The strength of this research is that it discusses variations in handling Covid-19 in nine provinces by presenting a fairly comprehensive policy for handling Covid-19. The budget for handling Covid-19 in this study is discussed in detail in each of these provinces. In addition, this study also found problems and proposed solutions in handling Covid-19 which were explored from the nine provinces in terms of policies, funding, human resources, and drugs and medical equipment. However, the weakness of this study is that it does not discuss too much about the social safety net that is needed by the community because this is not the essence of handling Covid-19. This research also does not discuss the technology used in handling Covid-19 because there are still not many developments in health technology in handling Covid-19 that have been implemented in health facilities. In addition, the handling of Covid-19 from the aspect of vaccine administration was not discussed in this study. For health technology and vaccine administration in the context of handling Covid-19 can be carried out in further research.

## Conclusion

This study concludes that every province in Indonesia has various regulations that focuses on accelerating and preventing Covid-19. The budget for handling Covid-19 also varies from province to province. Human resources in handling Covid-19 in certain provinces are sufficient but with an increased workload. Some health facilities still do not have the means to properly screen both medical personnel and patients. The stock of medicines available in hospitals has constraints such as limited stocks of certain types of drugs.

The government should make an accounting of the amount of the budget that has been given in handling Covid-19 to evaluate and prepare for the availability of funds in the following year. The Ministry of Health should increase funding and human resource capacity for surveillance and tracing handling of Covid-19. Local governments should encourage each agency to provide managerial and leadership training to the task force handling Covid-19 in various agencies. Further research on budget requirements and recording of expenditures in preparation for the next pandemic.

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# Study Effecting of Hetero Chitosan Mineralization on Structure of *Proteus spp*

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## Abstract

DE protection, demineralization, de colorization, and DE acetylation. Protein accesses were used to obtain chitosan from shrimp shell waste. Using FTIR, SEM and XRD. It was characterized. Also analyzed were the physiochemical parameter such as moisture content, hydrogen meter, viscosity, degree of DE acetylation and solubility The age of bacterial culture influenced its susceptibility to chitosan with cells being most prone to chitosan in the late exponential period. Hetero chitosan oligosaccharides were made up of partially DE acetylated chitosan's 90%, 75%, and 50% DE acetylated chitosan's. It was clear that in the presence of the hetero chitosan and their oligosaccharides, the growth of gram –negative bacteria is less inhibited than gram positive bacteria. These results revealed that hetero chitosan's and their oligosaccharide rely on the antibacterial effects degree of DE acetylation, and molecular weight.

***Keyword****: Chitosan, Proteus spp, mineral, antimicrobial medicines, Penicillin*

## Introduction

It has become great interest not only as an underused resource, but also as anew high potential function material in different fields, and recent progress in chitin chemistry is noteworthy. Antimicrobial medicines have brought about dramatic shift not just in the treatment of infectious disease, but also in the fate of kind. The medicines were synthetic substances in terms of protection and efficacy, andhadlimitations, Fleming had discovered penicillin in 1928. The antibiotic was named penicillin, and in the 1940 it reached clinical use. Penicillin, which is an excellent protection and efficacy agent, led by saving the lives of many wounded soldiers during world war II in the era of antimicrobial chemotherapy[1]. The antimicrobial activities of water soluble chitosan derivatives such as quaternary ammonium chitosan have been identified inseveral studies, hydroxyl propyl chitosan, N-car boxy butyl chitosan, car boxy methylated chitosan and sulfated chitosan. Chitosan copolymer consisting of of â-(1f4)- 2- acetate med-D-glucose, extracted from chitin in the presence of alkali through DE acetylation. This exhibits a broad range of biological activities such as antitumor activity. The possible uses of acetate solution as a food preservative as afresh, natural anti micro bio local agent for antimicrobial packaging films have been studied. The exact mechanism of chitosan's antimicrobial activity and itsderivatives is not yet completely understood but has been suggested to include cell lye sis breakdown of the cytoplasmic membrane barrier and chelation by the chitosan of trace metal cat ions may be required for the growth of the microorganism. A cationic chitosan must communicate with both membranes of the bacterial cell envelope in the killing of gram negative

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bacteria[3]. The genus *Proteus* contain gram negative, possible anaerobic, heterotrophic and proteolysis rods as opportunistic pathogens for human use. Some bacteria have changed the taxonomic classification many times. In the family these throw closely related genera formed the Protease tribe. The genus *Proteus* currently contains *P. mirabilis, P. vulgaris, P. pinner, P. Hauser* and three genome species. Based on molecular studies, *P. Hauser* as well as the genome species were isolated from P. *vulgaris and* from *P. vulgaris* group. Genome species and are labelled with numbers only because there were no metabolic properties indicated to allow their full differentiation[4].

## Materials and Methods

### Preparation of chitosan solution

A 1% chitosan solution used to be prepared by way of dispersing 150 g of chitosan in 1 l of water, dissolving it and stirring through including 400 ml of 1 M lactic acid and making up to 15 l with water. The pH used to be adjusted to be 5.5 with a saturated NaHCO3 solution.[5]

### Enzymatic hydrolysis of chitosan in the UF membrane reactor

The UF membrane reactor (Millipore Minitab" system, Millipore Co., USA). It consisted of a supplement tank, a reservoir tank, a water bath for control- ling reaction temperature, three peristaltic pumps, a membrane cartridge with molecular weight cut off (MWCO) 3000 Da and an enzyme reactor vessel. The quantity of reducing sugar produced from chitosan by means of the UF membran erea ctoratagiven on rate usedtobe incontrast withthe best conditions for the enzyme concentration, incubation time, and reaction temperature determined in the batchreactor. The oligosaccharide sproduced from chitosanat special permeation rates havebeen analyzed by HPL Con the TSK gel NH260 column.

The reactor system for semicontinuous production of oliosacharides was additionally operated beneath the choicest conditions. A new substrate used tobe brought to the reactor tankafter sufficient incubation and recycling time had been allowed to hydrolyze the chitosan solution[5].

### Assay for antibacterial activity

Antibacterial things to do of chitosan and chi to oligosaccharides were examined as the inhibitory effects against the increase of E.coli. A 0.5 ml of 1% sample solution in 0.05 M acetate buffer (pH 6.0) was introduced to the mixture of 0.5 ml of the cultured microorganism solution and 49 ml of try optic soy broth medium, and incubated with shaking at 37°C. The inhibitory results have been estimated periodically through measuring the turbidity of the cultured medium at 640 nm. In a manage group, 0.5 ml of 0.05 M acetate buffer in region of the oligosaccharide- ides used to be brought to the mixture[5].

## Results and Discussion

Binding of chitosan with mineral

The development of chitosan based materials as useful adsorbent polymeric matrices. In particular is a growing area in the area of adsorption science[6]. Chitosan composites have recently been developed to adsorb heavy metals and environmentally friendly dyes. The development of chitosan it has been proved that chitosan composites have better adsorption capacity and resistance to acidic conditions. Chitosan has a high affinity to the surface of silica based minerals due tothe interaction between a part of the polymer protonated amino groups and dissociated silica hydroxyl groups produced in aqueous solution. [7]. The ability of chitosan to organize heavy metal ions Zn(II), Cu(II), Cd(II) and Fe(III), is less than equal to the ability of mineral pores to hold ions of these metals without the formation of chemical bonds. Even though these minerals possess high capacity for adsorption, their structural modification will successfully booster their capabilities, indicates multilayer adsorption on composites for Cr(III) and Fe (III). Kinetic studies have shown that composites give fast kinetics to adsorb Cr(III) and Fe(III) . The

isomorphs substitutions of Al3+ for Si4+ in the tetrahedral layer and Mg2+ for Al3+ in the octahedral layer resulted in Mont morally nit having a negative charged surface. Generated Mont morale nit coated with chitosan for removal of Cr (VI) [8]

**Deficiency of chitosan effect on structure of bacteria**

Gram positive bacteria structure for Chitosan treated exhibited a biofilm –like structure formed on the surface and bottom of the tube. *B. cereus bears* more negative surface load than *Escherichia. Coli* despite having weak antimicrobial activity in the direction of gram positive, while *B. cereus* chitosan interaction was found to induce the formation of a biofilm like structure Antibiotic9. *B. cereus*produced polysaccharides secreted may some barriers and may increase the bacterial survival rate, that has been found to contain a higher exo polysaccharide content compared to *E. coli* in its colony, *B. cereus* developed red and blue color. By contrast E. coli *was* shown to be transparent and deemed not to produce a biofilm. The chitosan has been found to have lower gram positive B. cereus *inhibition*. Finally the widespread overuse of antibiotic in many natural habitats, such as waste water and river, lakes, drinking water and livestock has been reported to cause the existence of sub inhibitory concentration . [10]

**TABLE 1. Effect of various levels of chitosan on the outgrowth of E. coli at pH 6.5 or 5.5.[7] Wang, 1992.**

| Concentrations | | Incubation (d) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| E. coli cells/ml. pH of chitosan (%) | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 6.5 | I | 6.8 x 10'• | 4.5 x 106 | 5.1 x 10' | 9.8 x 10' | 6.4 x 10' | 4.7 x 10' | 3.6 x 10' | 2.7 x 10' | 1.3 x 10' |
| | 0.5 | 6.8 x 10' | 1.0 x 10' | 1.4 x 106 | 2.3 x 10' | 3.4 x 10' | 7.3 x 10' | 6.5 x 10' | 7.7 x 10' | 5.3 x 107 |
| | 1.0 | 6.8 x 10' | 4.4 x 10' | 3.9 x 10‘ | 8.7 x 106 | 9.3 x 10‘ | 1.2 x 10' | 6.7 x 10' | 4.2 x 10' | 8.3 x 10' |
| | 1.5 | 6.8 x 10" | 4.9 x 10' | 8.1 x 10‘ | 1.1 x 106 | 1.0 x l0^ | 4.2 x l0^ | 5.0 x l0^ | 6.1 x l0^ | 1.6 x 106 |
| | 2.0 | 6.8 x 10' | 2.3 x 10' | 4.1 x 10' | 1.3 x 10' | 9.4 x 10' | 9.8 x 10' | 7.8 x 106 | 3.0 x 10' | 3.2 x 10' |
| | 2.5 | 6.8 x 10' | 4.4 x 10' | 8.0 x 102 | 6.0 x 10° | 4.0 x 10° | 2.4 x 10' | 3.3 x l0• | 7.9 x 10" | 1.4 x 10' |
| 5.5 | 0 | 6.8 x 10' | 5.7 x l0• | 1.3 x 10' | 3.2 x 107 | 6.4 x 107 | 1.5 x 10' | 1.2 x 10' | 6.1 x 10' | 6.3 x 10' |
| | 0.5 | 6.8 x 10° | 3.6 x 10° | <102 | <102 | <10° | <102 | <102 | <10° | <102 |
| | 1.0 | 6.8 x 10' | 1.4 x 10' | <10° | <10° | <102 | <10° | <10° | <102 | <10° |
| | 1.5 | 6.8 x 10' | <10° | <10° | <10° | <10° | <10° | <10° | <10° | <102 |
| | 2.0 | 6.8 x 10° | <102 | <102 | <10° | <10° | <10° | <102 | <10° | <10° |
| | 2 5 | 6.8 x 10' | <10° | <10° | <10° | <10' | <10° | <102 | <10° | <10° |

**Mineralization with structure of bacteria.**

In the biological environment bio mineralization is common and is transmitted by bacteria, protests', fungi plants and animals. [11]. And other referred as biologically passive mineralization BPM. The difference between these modes is that the organism does not regulate and enforce morphology. Although three –mode classification is useful, in the context of non-cell driven mineralization or organic template driven mineralization. First quickly turn into a more stable calcite, calcite precipitation during metabolism has been well exemplified in more recent efforts by arrange of urease active bacteria. Where the breakdown of urea into ammonia and CO2 satisfies both the increase in hydrogen number and the production ofcarbonate The variety of metabolic processes leading mineralization of bacteria Carbonates represent the most common bacterial induced minerals that precipitate during the metabolism of organic matter in response to CO2. Bacterial metal sorption and subsequent precipitation can be necessary and useful for metal and radionuclide removal during metal and radionuclide contaminated waste bioremediation. Biogenic manganese oxide showed a greater metal binding ability than well crystallized synthetic manganese oxide a higher catalytic activity for organic compound degradation [12].

## Conclusion

Much work on chitosan and its derivatives has been done for tissue engineering drug delivery, wound healing, water treatment, antitumor and antimicrobial activity, as well as increasing the surface area for cell attachment, migration. The best antibacterial behavior was demonstrated by crab polymer chitosan, while squid polymer chitosan showed the best efficiency to inhibit *S. aurous, B. ceres, and B. subtitles*. The minimum inhibition concentration and minimum bactericidal concentration values of chitosan sources can be applied to different purpose in the food industry, such as natural food preservatives which extend food shelf life. Quarter noised chitosan ,which introduces the hydroxyl group or amino group of polymers to permanently positive charged quaternary groups enhances antimicrobial activity over aside hydrogen number range. Chitosan is extremely effective in extracting mercury from dilute solution. The poor diffusion properties of chitosan in the solid state, in terms of kinetic behavior ,cause a control of the absorption performance by sorbent particle size. However, the characteristic of chitosan makes it possible to use ultrafiltration in liquid form by coupling the chelation mechanism for mercury recovery and anionic dyes coagulation-flocculation. The use of liquid formed chitosan increases the accessibility and /or availability of reactive sites. During polymer dissolution ,the breaking of hydrogen bonds between amino groups and between hydroxyl groups (inter-chain or intra-chain bonds ) is far more interact with metal ions and anionic dyes. This can explain the much more efficient use of amino chitosan groups when the polymer is used for color removal in the dissolved –state This effect is less important when considering mercury, in this case the positive effect of using chitosan in the dissolved state (ultrafiltration coupled with chelation )is only important for binding because binding capacities at saturation tend to be values of the same magnitude order. *Escherichia coli* no viable cells were detected after 1 hour experimental groups (those with chitosan ) in the presence of 0-25 mm sodium chloride. However except for the 102 colony forming unit /ml inoculum ,the cell counts for chitosan supplemented cell suspensions with 100mM sodium chloride were similar to those of the control. Just 100 mM sodium chloride had an inhibitory effect on *E. coli*. we suspect that 100mM from sodium reversed the impact chitosan on *E. coli* is not dueto competition between chitosan and sodium for binding to the cell surface with negative residues, but because sodium and chitosan form a complex that reduces binding to the cell surface. The amount of free (uncompleted ) chitosan at low sodium concentration or with fewer cells ($10^2$ CFU/ml) would be sufficient to destroy all the cells.In the presence of 100mM

sodium, the efficacy of chitosan decreasedwith growing cell counts ($10^3$ to $10^5$ CFU /ml ). Better understanding is that chitosan was complexes with sodium. If the sodium and chitosan were varying, the bactericidal effect of chitosan would be independent of the cell counts. Chitosan binds strongly to various metal cat ions, such as Cu. The involvement of –OH and NH2 groups on glucosamine residues as ligands, since the NH2 groups are crucial sites for binding chitosan to cells, it will be predicted that the chitosan –sodium complex will not be able to bind cells, in the presence significant amounts of sodium and cells. The sum of free chitosan would not be adequate to bind all of the cells, so the number of *E. coli* cells will not be significantly reduced .Similarly, the divalent captions decreased the bactericidal effects of chitosan on *E .coli* at concentrations of 10-25 mm *E. coli* the barium, magnesium, sodium at concentrations of 10 and 25*mill micron* also decreased the activity of chitosan. Curiously, the reversal effect seen in 100mM sodium also occurs at 25mM sodium in this higher cell inoculum ($10^7$ cfu/ml). Chitosan found that enhanced adhesion of *E. coli*, by neutralizing the negative cell charges. The showed that inorganic cat ions (sodium, magnesium )inhibited chitosan –mediate adhesion of *E. coli*, revealed that chitosan induced calcium release the permeability of the membrane improved proved that leakage caused by chitosan was inhibited by divalent cat ions of the order of barium, calcium. Effects of 25 and100 mill micron sodium on activity of chitosan to *E. coli* only occurred athigh densities of the cells. The cat ions bind directly to the chitosan instead of binding on the cell surface, and that it is the formation of these complexes, with the consequent reduction of free chitosan, that leads to a reduction in the activity of chitosan. Magnesium indicated this occurred because inorganic cat ions could substitute the loss of calcium, from the cell surface to form new stabilizing complexes on the cell surfaces that prevent the leakage caused by chitosan. The possible contribution of *Proteus* spp. to intestinal diseases and infections has been somewhat neglected. Research into the virulence of *Proteus* spp. in the urinary tract using the bacteriology of ileac conduits and intestinal segments for bladder augmentation suggests that *Proteus* spp. Should be examined more closely for their potential as gastrointestinal pathogens. There is increasing evidence that *Proteus* species may play a role in inflammatory bowel disease through the direct action of the bacteria, compounded by host immune evasion and perturbation. As Gram- negativeorganisms, *Proteus* species areintrinsicallyproinflammatory result of the production of lipopolysaccharide (LPS) and immune stimulatory flagella proteins. There may be an association between *Proteus* species and inflammatory bowel disease, especially Crown's disease, mainly through population expansion and immune activation. The effect of treating the drug and the degree of mineralization of the chitosan yield load power. The degradation level observed in both chitosan with mineral and chitosan without affecting the absorption enhancing properties of chitosan is still not certain. This could be analyzed using degradation and bio adhesion measurements leakage of *E .coli* cause with chitosan. *E. coli cells* attracted to the medium by glucose. It appears that glucose leakagein the chitosan supplemented cell suspension increased inversely with the log of the viable cell count. We find the chitosan caused protein leakage and U.V absorbent content based on the 260 nm absorbance and protein concentration calculation.

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# Extraction of Mannanase from Bifidobacteria and its Effect On Starvation

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## Abstract

Any members of the human gut micro biota including members of the gut micro biota that promote health like bifid bacterium *bifidbacterium spp*. Catabolize manna's for food. Few informationis available in the gut ecological niche regarding the enzymology of man nan deconstruction, below the biochemical properties if the first 5 subfamily 8 glycoside hydrolase (GH5 8) manna n as e derived from the biochemical properties of probiotic. The relationship between gut micro biota and biochemical metabolism to explore the relationship between gut micro biota. In traycomponents and some substances which are produced by the host, the primary intestinal microbe :bacteria and microbial species associated with dietary carbohydrates metabolism, strictly anaerobic and gram positive strain of specified bifid bacteria known as, *B. Lon gum* (60 percent of the sample )was the most commonly identified species followed by *B. bifida* (60 percent of the samples ), while *B. Brief (*21 percent).

***Keyword****: Mannanase, Bifidbacterium,* Starvation, probiotic, digestive tracts

## Introduction

B-mannans are an excess of different structural plant and polysaccharide for storage. Some human man nans are abundant and complex polysaccharides of plant structure and storage. Some members of the human gut micro biota including health promoting members of the gut micro biota *bifid bacteriumspp*. catalyze man nans for food. Catabolize mannans for food, few information is available in the gut ecological niche regarding the enzymology of mannan deconstruction the is dependent on mannan deconstruction enzymology instomach. The biochemical features of probiotic biochemical mannanase of the first family 5 subfamily 8 glycoside hydrolase (GH5) mannanase *bifid bacterium animals bifid bacterium animals subs Lactic lactic*. Microbial manna n a se s are predominantly extracellular and can function in abroad range of industrial such as hydrogen number and temperature, pharmaceutical. Feed, oil and textile. Microbial mannanases have become biotechnologically essentialas they target the hydrolysis of complex plant tissue polysaccharides into pure molecules such as man no –oleo go saccharides and mannoses[1]. from plants and animals. Bacterial mannanases are often extracellular and can function in a wide range of hydrogen number and temperature, but more common are acidic and neutral mannanases. Complex manna structure and the complex of microbial enzymes involved in its complete breakdown, mannanase sources, conditions of growth. We characterized the β- GA lacto man Nan activity mechanism and the β-mannanase supplementation on the gut. The human colonic micro biota is a large and complex population of microbial[1,2].

This linked detaryportion metabolism, the human intestinal micro biota is a natural ecosystem that is now dynamic ecosystem that affects human death and wellbeing[1]. Morethan 400 species within the industrial tract canbe classified and can be classified within the intestinal micro flora as more than 400

species. In colon *Bifid bacterium bifid bacterium* were first discovered in infant feces by tissue who isolated a rare and tissue bacterium in infant feces, who isolated a unusual and distinctive Y-shaped bacterium and called it Y-shaped distinctive, and called it *Bacillus bifid us* [2]. This bacteria is a gram from 1900-1957 such bacteria are pleomorphic gram positive, non-motile, nonsporeform, pleomorphic. Speculate danaerobic bacteria with extremities[3] B*ifid bacterium bifid bacterium are* anaerobic, bacilli belonging to the dominant gut which belongs to the dominants gut micro biota. *Bifid bacterium bifid bacterium* have gained considerable attention in recent years because their association with various health promoting effects of *Bifid bacterium spp*. Isolation recently, bacteria play a crucial role in the digestive tract[4,5]. Probiotic are need to survive in the gastrointestinal microbial ecosystem adherenceto gut epithelium will enhance their ability to survive. Local isolation and detection of strains leads to specific growth potential. Specific *bifid bacterium* strains have a certain probiotic character in various biotopes[6, 7].

## Materials and Methods

### Isolation:

Bacteria were isolated from the digestive tracts of the bumblebee species Bombs Pascua rum, Bombs Pascua rum and Bombs lapidaries. Worker bumblebees were accumulated in central Bohemia at some point of summer 2006, and their intestinal tracts were weighed and transferred aseptically into tubes containing sterile MRS broth (Oxo id) supplemented with soybean peptone (5 g l−1) and cysteine hydrochloride (0.5 g l−1). The tubes have been flushed with O2-free CO2 and closed with rubber stoppers. The equal broth was used for serial dilutions of all samples. Aliquots (0.1 ml) were plated on TPY agar with mope Racine as described *Propionic bacterium acnes* and representatives of several novel species with incredibly low levels of 16S r R NA gene sequence similarity (92–95%) to the genus *Bifid bacterium* were present in the cultures[8].

### Chemical Analysis:

The gross energy contents of feed, excreta, and digest samples were determined on a 0.5-g sample the use of an adiabatic bomb calorimeter with benzoic acid as standard.

The nitrogen contents of feed and digest samples were decided on a 0.25-g sample in a combustion analyzer the use of EDTA as a calibration standard, with crude protein being calculated through multiplying proportion N by acorrection element (6.25).

Proximateanalyses of the CM and eatingregimen samples have been conducted in accordance to through strategies 920.39 (for crude fat), 982.30 E (for total lysine), 975.44 (for reactive lysine), 978.10 (for crude fiber), 973.18 (for impartial detergent fiber and acid detergent fiber and 942.05 (for ash). Hemicellulose content material was calculated as the difference between NDF and ADF.

The impartial detergent insoluble nitrogen used tobedeterminedthroughmeasuringthenitrogencontent material of the insoluble fraction received from the NDF assay. The Glico simulate content material of the meal was determined by way of colorimetric analyses the usage of a spectrophotometer according to the technique described via with the use of tetra chloral pall date as the quite particular regent for Glico simulates[9].

## Results and Discussion

### Mannanase and *bifid bacterium*

Mannans are known to be hydrolyzed by human digestive enzymes, and thus give manna no lytic gut bacteria a possible advantage. The fermentation of gum galactic man nan was demonstrated in the human gut and the ingestion of this polysaccharides partial hydro lysates induced the prolife ration of *bifid bacterium spp*[10]. but end β-1-4 mannanase which hydrolyze the internal β-1-4 linkages of backbone are central in man nan degradation. β- mannanase

are listed in the carbohydrate active enzymes (Casey) database of glycoside hydrolase (GH) families[11] many bacterial mannanase cluster according to a phylogeny based assignment in sub family 8 of GH5 8. GH5 mannanase use a double displacement mechanism with numeric structure retention. Many mannanase contain carbohydrate binding molecule (CBM) that have been assigned various molecules including targeting polysaccharides to enzymes, raising the concentration of local substrate or providing. *Bifid bacterium* animal is subs a probiotic bacterium B.lactis-*04*. In addition, the study proved that *Bifid bacterium* animal is subspp. is the GH5 mannanase retained within *bifid bacterium animal is subspplactic.* Displays optimum catalytic efficiency CBM members had been found to bind to insoluble microcrystalline cellulose. Insoluble mannan [11], the CBM 10 of the enzyme is the first low affinity man nan binding module described and to gather with close counterparts it forms a novel CBM 10 subfamily. The distinct differences in the biochemical properties of his enzyme compared to characterized gut micro biota β-mannanases illustrated the diversity of mannan utilization strategies which will be critical in adapting to a high competitive gut niche.. [12]

**Table (1) *bifid bacterium species*. In the intestine of infants and adults (according to Reuter, 1971.[13]**

| | biotypes Reuter/Mitsuoka | infant | adult |
|---|---|---|---|
| *B. bifidum* (Tissier, 1900, Orla-Jensen, 1924) | a | - | + |
| | b | +T | - |
| *B. adolescentis* (Reuter, 1963) | a x) | - | +T |
| | b xx) | — | + |
| | c xx) | - | + |
| | d xx) | — | (+) |
| *B. longum* (Reuter, 1963) | a | - | + |
| | b | +T | - |
| *B. infantis* (Reuter, 1963) | a | +T | — |
| | b | + | — |
| *B. breve* (Reuter, 1963) | a | (+)T | -* |
| | b | - | — |
| syn. *B. parvulorum* (Reuter, 1963) | c | - | — |

frequency:
+ : frequently
(+) : moderately
- : occasionally
T : including type strain T of species (Reuter, 1971)
* : also isolated from the human vagina
x) : *B. adolescentis* sensu stricto
xx) : *B. adolescentis* sensu lato, including *B. dentium* (b?), *B. catenulatum*, *pseudocatenulatum* (c), *B. angulatum* (d) (Scardovi, 1986; Gavini et al., 2001)

**Mannanase and carbohydrate metabolism**

Carbohydrate molecules are used as compounds in several different types, the outer structures on the cell surface of microorganism spreading the cellular inner and outer environment, the source of energy metabolism that is necessary for energy obtaining (TCA cycle), polymer structure for energy storage(glycogen), and inherited molecules as part of de oxy nucleic acids and ribonucleic acids. In addition , the polymer shape of the carbohydrate molecule modify the structure and stability of the protein molecule to maintain these biological processes, and many different types of modified sugar molecules are required. Nucleotide sugar molecules play one of the most important role for the construction of carbohydrate polymer structure among modified sugar molecules nucleotide sugar, an activated molecule for of sugar, is the sole substratum for polymer structure construction including a variety of sugar molecules. In

Achaea, the moiety GlcNAc is an essential component of the cell surface structure, while in eukaryote the activated molecule is necessary for the synthesis of chitin a component of fungal cell wall. The connector of glycosylate phosphatidylinositol a molecule that attaches a variety of cell surface proteins to the plasma membrane 14, 15.

**Carbohydrate metabolism and starvation**

Human diet is a complex mixture of cumulatively healthy, interacting components [16]. Mate holism macronutrient energies (carbohydrate, proteins, and lipids) are responsible for the bulk of the human dietary energy. Micronutrients (minerals and vitamins) play a central role in metabolism and tissue –function maintenance. Metabolism includes all the biochemical pathways that species use to synthesize and derive energy from structural and functional constituents. It is usually divide into anabolism, which involves macromolecules such as glycogen, proteins and lipids (triacylglycerol, TAG) and catabolism this involves macromolecular degradation to its simplest precursor: glucose, amino acids, glycerol and fatty acids. The free energy released via catabolic degradation through adenosine triphosphate and nicotine amide adenine diphosphate is used to drive anabolic biosynthesis endergonic processes. Anabolic hormones are pancreatic insulin and pituitary growth hormone (GH), other endocrine secretions (pituitary GH, adrenal cottice tropic hormone and prolactin) and weight gain. Androgens (testosterone) may have an anabolic synthesis of the proteins. Anabolism raises the requirements for all nutrients that (synthesis of glucose from non–glycosides substrates) and stimulation of adipose tissue to release free fatty acids (FFAs) and glycerol by lipolysis (TAG breakdown). Thyroid hormone (tri iodole thyroxin or T3) are catabolic and play a major in deciding the metabolism process over the long term. Acute injury catabolism (infection, surgery or trauma) leads to increased energy and protein breakdown, increasing vitamin and mineral requirements. The enzymes (and their substrates) of this bidirectional metabolism remain best studied in prokaryotic organisms (bacteria) such as *Coli*Importantly both gluconeogenesis and glycerol neo genesis are kata plea roti pathways as they convert cycle anions of citric acid into phosphor Enola pyruvate which is then used to produce either glucose or glucose 3 phosphate. Glucose and Fats are the most relevant energy substrates for most species (including humans) and the primacy of these fuels reflects the intermediate metabolism. Fasting metabolism also involves high levels of lipolysis and Fats through circulating to enable energy utilization. In fact, malnutrition is longer, potentially dangerous, and may result in a let hat outcome. Hunger is an adaptive response to food deprivation involving changes in the senses, cognitions and neuron endocrines. As carbohydrate reserves are rapidly depleted and protein supplies are low, the survival period of hungry people depends more on fat reserves than on muscle mass (obese people can live for many months without eating in clinically supervised weight loss programs). Breakfast, lunch and dinner corresponds to nocturnal fasting (approx. eight hours). Clearly adapted to fastening metabolism ensuring that endogenous substrate and resources are adequately utilized to sustain critical activity. It is characterized by low levels of insulin, high levels of glucagon, hepatic glycol gene lyeis, and gluconeogenesis to control serum glucose levels and cerebral function[17].

## Conclusion

Polysaccharide-degrading enzymes are very important in many industrial processes, the study of these enzymes is an important field of research. These enzymes include those which degrade cellulose and hemicellulose two of the main components in plant cell walls. Such enzymes are often composed of two or several separated modules which perform different functions. Carbohydrate- binding modules (CBMs) are frequently present and are known to be important for efficient hydrolysis of cellulose. The binding of the CBM is not directed to the mannan-substrate. However, these results may be a reflection of the tight and complex organization of cellulose

and hemicellulose in the plant cell wall. Such enzyme systems are not only of academic interest but also they have potential biotechnological applications in a wide range of industrial enzyme markets, including food and feed technology, coffee extraction, bioethanol production, slime control agents, pharmaceutical field, pulp and paper industry. Exploitation of biodiversity to provide microorganisms that produce mannanases well suited for their diverse applications is considered to be one of the most promising future alternatives. The presence atypical low affinity CBM, which increases binding to enzyme to soluble mannan while causing minimal decrease in catalytic efficiency as opposed to enzymes with canonical mannan binding modules. These features highlight of catalytic and binding properties to support man Nan binding modules. However, microbes are most potent producers of mannanases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and ready accessibility to genetic manipulation. Microbial man Nan as e s have been used recently in the food, feed and detergent industries. Due to the complex nature of the plant cell wall and man nan-based substrates, several issues need to be addressed in order to achieve a better understanding of man nan-degradation. Firstly, the enzyme-polysaccharide interaction of manno side- hydrolases in the degradation of the more complex hetero man nans, like O-acetyl-galactic glycol manan, should be studied in more detail. In particular, the influence of different substituents on the rate of hydrolysis needs to be investigated further. Secondly, a larger comparative study of man nan-degrading enzymes from different enzyme families would possibly reveal any differences or similarities in substrate specificity. In general, improved methods for the separation and detection of polysaccharides and oligosaccharides would be very useful in these types of investigations. A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The mannanases isolated from extremophiles organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. Exploitation of biodiversity to provide microorganisms that produce mannanases well suited for their diverse applications is considered to be one of the most promising future alternatives. The existing knowledge about the structure-function relationship of man nan as e s, in evolving mannanases that were never made in nature and that would meet the requirements of the multitude of mannanase applications.

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# Secondary Syphilis in the Second Trimester Pregnancy : Case Report

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## Abstract

**Background:** Syphilis is a sexually transmitted disease caused by *Treponema pallidum*, which is transmitted through sexual contact, blood transfusion and transplacental from an infected mother to the fetus. Syphilis in pregnancy can cause complications including abortion, low birth weight, premature birth, neonatal death or congenital syphilis infection.

**Case:** A 39-year-old woman, 16 weeks pregnant, presented with a complaint of a small lump on the genitals accompanied by red patches on the palms of the hands and feet. Serological tests showed a reactive VDRL of 1:512 and a reactive TPHA of 1:640. Based on the history, physical examination and serological tests the patient was diagnosed with secondary syphilis in pregnancy. The management of this patient was given a single dose of Benzathine Penicillin G injection of 2.4 million units intramuscularly.

**Conclusion:** Early screening for syphilis in pregnancy isvery important to prevent complications in the fetus. VDRL serological test examination 3 months after therapy was carried out to determine the success of therapy. In this case, there was a decrease in the VDRL titer to 1:4 in the absence of skin lesions 3 months after therapy with benzathine penicillin G 2.4 million units intramuscularly single dose.

***Key words:*** *Syphilis in Pregnancy, Serological tests, Treponema pallidum*

## Introduction

Syphilis is a sexually transmitted disease with varied clinical manifestations caused by *Treponema pallidum* thatonsisting of late latent syphilis and tertiary syphilis. Syphilis is transmitted through direct sexual contact in individuals who have active primary or secondary syphilis lesions, blood transfusion and vertical transmission from the infected mother to the fetus transplacental.[1,2] The most common complications of syphilis are neurosyphilis, involvement of the aortic valve or cardiosyphilis and if it affects pregnant women it can cause congenital syphilis in the fetus[3]

In 1956, penicillin was first introduced for the treatment of syphilis and reduced the incidence of primary and secondary syphilis from 66.4 cases to 3.9

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cases per 100,000 people.[3] According to the World Health Organization (WHO) in 2012 there were 5.6 million cases. New syphilis occurs among adolescents and adults aged 15-49 years worldwide and is followed by 350,000 pregnant women with positive syphilis results, including 143,000 stillbirths, 44,000 premature babies and 102,000 infected babies.[4,5] The incidence of syphilis in Indonesia based on the report of the Ministry of Health of the Republic of Indonesia an increase of 16.7% in 2011.[6]The Sanglah Central General Hospital (RSUP) in 2014, reported 20 new cases, namely 3 cases of primary syphilis, 11 cases of secondary syphilis and 6 cases of early latent syphilis.[7]

Syphilis diagnosed made based on the history, physical examination, serological examination (treponemal and non-treponemal) and radiology.[8] The treponemal test is a test that identifies immunoglobulin M (IgM) or IgG antibodies to the number of *T. pallidum* infections. Some of the tests included in the treponemal examination include *Treponema pallidum* haemagglutination assay (TPHA), *Treponema pallidum* particle agglutination assay (TPPA), fluorescent treponemal antibody absorbed (FTA-ABS). The non-treponemal test is a measurement of the levels of IgM and IgG antibodies produced by the host in response to lipoid material (mostly cardiolipin) released by spirochetes from damaged host cells, include Rapid Plasma Reagents (RPR), Venereal Diseases Research Laboratory (VDRL), Toluidine Red Unheated Serum Test (TRUST).[9]

Primary and secondary syphilis infections in untreated pregnancies result in stillbirth, abortion, low birth weight, premature birth, neonatal death or infection and disease in the newborn.[5] Pregnancy with syphilis infection can be treated by monitoring early in pregnancyor the first trimester, thereby reducing the rate of defects in the fetus or newborn.[4] The purpose of this paper is to increase knowledge and better understand the diagnosis and appropriate management of secondary syphilis that occurs in women with pregnancy.

## Case

A 39-year-old woman is a housewife, 16 weeks pregnant who comes to the skin and genital polyclinic of the Regional General Hospital (RSUD) Dr. Moewardi with complaints of reddish spots appearing on the palms of the hands and feet accompanied by lumps in the pubic area since 1 month ago which has been increasing and sometimes feels itchy.

The patient is currently 16 weeks pregnant with a history of having her first child born spontaneously assisted by a midwife with a birth weight of 2800 grams, her second and third pregnancies miscarried, then a curettage was performed. History of menarche at the age of 14 years and having sex for the first time at the age of 25 years with her husband after the patient married. The last sexual intercourse was 4 months ago with my husband without using a condom. The number of sexual partners up to now is 1, which is only with the husband. The patient did not know whether her husband had a history of previous penile injuries. My husband's job is as a delivery driver who returns home every 4 months. History of blood transfusion and previous tattoo use was denied. The patient denied having similar complaints before and the patient also denied having a history of allergies to certain drugs and foods, as well as other systemic diseases. The patient had never received treatment before.

Based on the results of the physical examination, the general condition was good, the nutritional status was adequate with a body mass index (BMI) in pregnancy of 33.01 including obesity category (TB 152 cm, weight 76 kg), vital signs within normal limits. Dermatovenereological status in the palmar manus and plantar pedis regions bilaterally showed multiple erythematous macules and plaques with overlying scales. Bilateral femur regions showed macules and multiple partially hyperpigmented erythematous patches. In the genital and perineal regions, condyloma lata appears with multiple skin-colored papules and plaques, round and oval in shape,

flat surface with erosions on it (Figure 1). There was no alopecia and regional lymph node enlargement.

Based on the results of autoanamnesis and physical examination, our patient was diagnosed with secondary syphilis, pompholyx and erythema multiforme (EM). Venereal disease research laboratory (VDRL) serology was reactive 1: 512, Treponema pallidum hemagglutination assay (TPHA) was reactive 1: 640 and HIV test was non-reactive.



**Figure 1. Dermatovenereological status. (AD) The palmar and plantar pedis regions show macules and multiple erythematous plaques with overlying scaling. (red arrows) (EF) Bilateral femur regions show macules and partially hyperpigmented multiple erythematous patches (yellow arrows) (G). ) The genital area shows condyloma lata with multiple skin-colored papules and plaques, oval round shape, flat and soft surface accompanied by erosions (green arrows).**

Based on autoanamnesis, physical examination and serological tests, the diagnosis in this case was secondary syphilis in pregnancy. The patient received a single dose of intramuscular injection of benzathine penicillin G 2.4 million IU and was followed up at the 1st and 3rd months after therapy at the Dermatology and Venereology Polyclinic, RSUD Dr. Moewardi Surakarta. The 3rd month evaluation showed no skin lesions (appendix) with a decrease in the number of VDRL titers, the results were 1:4 and TPHA 1:160.




**Figure 2. Follow up after 3 moths**

## Discussion

Syphilis is a sexually transmitted infection disease caused by *Treponema pallidum*.[10,11] Infection is transmitted through sexual intercourse (oral, anal and genital), the utero-maternal route, during delivery of a newborn and transmission through blood if the donor suffers from syphilis.[3]

Risk factors for syphilis in pregnant women include young women, history of sexually transmitted diseases, having more than 1 sexual partner in the past year, low economic status, lack of knowledge about sexually transmitted diseases and drug use.[10,12] Syphilis in pregnancy is frequent associated with the incidence of low birth weight, premature birth and miscarriage.[13] In this case, the patient was a 39-year-old pregnant woman with a history of two miscarriages, and in the family history it was found that the patient's husband had a job as a delivery driver outside Java and had a history of urinary complaints. Pregnancy and husband's history of working outside the city are risk factors associated with syphilis.[13]

Clinical manifestations of syphilis depend on the stage experienced. In primary syphilis, it is characterized by the appearance of chancre lesions in the form of solitary or multiple papules which then form ulcers, painless, there is induration with a clean wound bed and wound sizes ranging from 0.5 to 2 cm where these wounds can heal on their own (3- 6 weeks).[3]

Secondary syphilis or also known as spirochetemiaoccurs within 4 to 10 weeks after the chancre appears. Clinical manifestations of secondary syphilis include a non-pruritic maculopapular rash that appears on the body and then spreads to the extremities to the palms of the hands and feet, grayish-colored superficial mucous patches or erosions on the genital, anal and oral mucosa, and condyloma lata. Condyloma lata is one of the most infectious clinical signs of secondary syphilis with a grayish-white, elevated, and usually found in warm and moist areas such as the axilla, inguinal, perianal and perivaginal. The latent syphilis stage appears 2 to 6 weeks after the symptoms of secondary syphilis is characterized by reduced clinical signs and symptoms and is divided into early latent phase (<12 months) and late latent phase (>12 months).[10,12] Bilateral palmar manus and plantar pedis showed multiple erythematous macules with scaling overlying them, and the genital and perineal regions showed condyloma lata in the form of papules and multiple plaques of skin color, round oval shape, flat and moist surface accompanied by slight erosions (**Figure 1**). These clinical manifestations show the picture of secondary syphilis.

*Treponema pallidum* enters through the placenta (vertical transmission) can cause infection in the fetus. Syphilis in pregnancy can cause intrauterine growth retardation, spontaneous abortion, premature birth, stillbirth and hydrops fetalis.[14] In this case, the patient complained of small lumps in the pubic area and reddish patches on the palms of the hands and feet, so an initial screening was performed. The results initial screening VDRL/TPHAwere reactive so the patient was referred to the skin and genital polyclinic of RSUD Dr. Moewardi for further examination and appropriate treatment.

Maternal syphilis can be established through 2 examination principles, namely identification of *T.pallidum* in lesions or infected lymph nodes and serological tests. Identification of *T.pallidum* can be done by dark field microscopy and polymerase chain reaction (PCR).[15,16] Serological tests for syphilis can be classified into non-treponemal and treponemal tests. Non-treponemal examination such as VDRL and RPR. The results of the examination were reactive in 75% of cases of primary syphilis, whereas in secondary syphilis always a reactive VDRL result with a titer greater than 1/16 that indicates the disease is active, then if there is a fourfold decrease in the titer from the initial titer, it indicates the therapy is successful. Treponemal examination serves to detect interactions between serum immunoglobulins and antigens on the surface of *T.pallidum*. This examination consists of TPHA, TPPA, FTA-abs. Positive treponemal

examination indicates that a person currently has an active infection. [17] In this case, dark field microscopy and PCR were not performed because of limited equipment, but VDRL and TPHA were still examined. The results of the VDRL examination were reactive with a titer of 1:512 and reactive TPHA with a titer of 1:640, so it was concluded that this patient was in the stage of secondary syphilis with an active infection.

Differential diagnosis of patient is erythema multiforme (EM), pompholyx based on the history and physical examination. Erythema multiforme (EM) is an acute immune-mediated disease that affects the skin and/or mucosa that classificated into EM major (EMM) and EM minor (EMm). EM major has clinical manifestations involving the skin and at least 2 mucosal areas in different regions (lingual, buccal and labial) and may resolve in 1 to 6 weeks.[17] Clinical features of distributed typical or atypical cutaneous target lesions symmetrical,predilection for extensor surface areas of the extremities, multiple painful papules,vesicles, extensive ulcers and mucosal lesions, especially in the oral region.

Pompholyx or dyshidrotic eczema is an acute vesiculobullous disease that often affects the palms of the hands and feet, manifested by vesicles that spread on the palms of the hands to the tips of the fingers and sometimes can appear on the soles of the feet. Complaints usually accompanied by itching and discomfort. Usually acute lesions heal by themselves within 2 – 3 weeks, although they may recur.[18] Pompholyx has two clinical types, vesicular pompholyx commonly referred to as dyshidrotic eczema and bullous type pompholyx called cheiropodopompholyx.[19] In this case, a physical examination of the dermatological status of the region was obtained bilateral palmar manus et plantar pedis showed multiple erythematous macules and plaques with thin scales on top, no target lesions, vesicles, no tapioca appearance, painless lesions and no mucosal lesions. Based on the physical examination, the differential diagnosis of EM and pompholyx can be ruled out.

Based on the 2015 Center for Disease Control and Prevention (CDC) guidelines, it's recommended that therapy at the primary, secondary and early latent stages be given intramuscular injection (IM) of long-acting benzathine penicillin G in a single dose, while in late latent syphilis, guma syphilis and cardiovascular syphilis. According to WHO, the first-line treatment of syphilis in pregnant women, especially in the early stages (primary, secondary and early latent) is to give intramuscular injection of benzathine penicillin G2, 4 million units of a single dose or procaine penicillin 1.2 million units once a day for 10 days, if allergic to the penicillin group can be given erythromycin 500 mg four times a day orally for 14 days under close supervision or ceftriaxone 1 gram intramuscularly once a day for 10-14 days or azithromycin 2 g single dose orally. In late latent and tertiary syphilis, WHO recommends IM of benzathine penicillin G 2.4 million units once a week for 3 consecutive weeks or procaine penicillin 1.2 million units once daily for 20 days.[4] According to Kingston et al in the UK in 2015 explained that clinical examination and serological tests (RPR or VDRL) can be repeated 3 months, 6 months and 12 months after therapy (successful if there is a decrease in the RPR/VDRL titer four times the previous titer).[15] In this case, the patient was treated with a single dose of Benzathine Penicillin G injection, 3 months later, the patient was re-examined for a serological titer and the results of the VDRL titer were obtained. 1:4 and TPHA 1:160. These results indicate a therapeutic efficacy characterized by a more than four-fold decrease in the VDRL titer and a four-fold decrease in the TPHA titer.

Prognosis of syphilis in pregnancy depends on the administration of therapy that must be completed at least 30 days before delivery to prevent the occurrence of congenital syphilis. Infants born to mothers diagnosed and treated for syphilis during pregnancy required RPR/VDRL and IgM tests at birth and at three months of age, repeated every three months until negative. If this titer remains stable or increases, the child should be evaluated and treated for congenital

syphilis. Treatment for congenital syphilis is by administering benzyl penicillin sodium 60–90 mg/kg daily IV (in divided doses given 30 mg/kg every 12 hours for the first seven days after delivery and eight hours thereafter for ten days.[8,20]

## Conclusion

A 39-year-old pregnant woman who is 16 weeks pregnant comes to the Dermatology and Venereology Polyclinic, RSUD Dr. Moewardi with complaints of small lumps appearing in the pubic area since 1 month ago accompanied by reddish spots on the palms of the hands and feet. Based on the results of autoanamnesis, physical examination and serological tests the patient was diagnosed with secondary syphilis in pregnancy. The patient was given Benzathine Penicillin G injection therapy 2.4 million IU intramuscularly in a single dose. Monitoring therapy after 3 months by repeating serological tests revealed a decrease in VDRL and TPHA titers more than four times which indicated the success of therapy.

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# DNA Marker Screening for High-Risk Non-syndromic Hearing Loss Associated to Gene Mutations

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## Abstract

**Background:**Congenital hearing loss is a debilitating disease affecting 1–3 out of 1,000 live births. According to WHO's associated figures, in both ears, 278 million people globally suffer from moderate to extreme hearing loss. Most hearing-loss individuals live in developing countries. Many deafness and hearing problems cases were documented in our region. Those cases' exact cause is still unknown, so we performed this study and aimed to screen DNA in high-risk Non-syndromic hearing loss patients in Arbil city. **Methods:** This researchscreened 132 blood samples from (80 newborns and 52 individuals) **at** (Hiwa Institute for deaf and mutes); their ages patients from 14 to 22 years old**.***MTRNR1*genes were performed for molecular detection of mutant genes. The mutation gene wasamplified by multiplex tetra primer PCR. **Result:**G- mito-1555-F1, mito-1555-R1 (O), mito-1555-F2 (I), and mito-1555-R2 (I) hearing loss mutations were not observed in 132 blood samples from both classes and genotyped in MTRNR1. For mtDNA 12S rRNA mt.1555A>G, no mutant alleles were detected in all of the tests, and no false-positives were identified.Using all primers, fifty-two samples were easily separated on 2% agarose gel; two were outer primers, and others are inner primers. Two separate bands were observed with 52 molecular samples (wild type at 254bp and control at 341bp).Of 80 samples, 28 have control bands at 341 bp. We did not find any mutation in our 80 samples. **Conclusion**: MTRNR1 mutation genes were not present in collected samples in deafness-related mutation. Genetic tests for the deafness gene can better diagnose infant congenital NSHL cases than conventional screening procedures.

***Keywords:*** *NSHL, Gene, Mutation, Tetra-Primer ARMS PCR*

## Introduction

One of the most famous birth defects, the congenital birth defect deafness, is one of the most common sensory disorders of humans. The incidence before the age of 5 years is 2.7 / 1000, and in puberty, it rises to 3.5 per 1000.[1,2] Once a single gene mutation has occurred, it is unlikely that other genes are the primary culprit. Most likely, hybrid causes have one gene mutation and other environmental factors [3]. The role of both deafness's may be caused by biology and the climate. Fifty percent, in truth, all childhood deafness is known to be related to genetics.[4-6]

In the developed world, preinjury is the leading cause of death for babies younger than 37 weeks gestation.[7-8]Sensorineural infection is a widespread impairment in preterm infants.[9] Hearing deficiency or deafness is found in 7% of preterm babies.[8]A serious concern associated with the use of aminoglycoside

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is being present in infections, life support systems, and neonatal intensive care units.[10]As the perceptual, gestational age leads to hearing loss if an infant is born older, the mechanism remains underdeveloped. Extreme hyperbilirubinemia, which causes 80 percent of preterm hyperbilirubinemia, the risk of sensorineural hearing also increases for infants.[11] Hearing deficiency in premature babies may be progressive or progressive in the infant's hearing loss three years later; delayed-onset; at age.[12]The congenital hearing condition where there is no evidence of disease, such as with or without congenital deafness (NSHL). In certain circumstances, NSHL is caused by a single gene with the remaining 30 percent expressed in various signs.[8]Monogenic hearing loss can be hereditary. At 80 percent, autosomal recessive hearing loss (ARNSHL) occurs in usually pre-lingual. However, in about 20% of the cases, hearing deficits are caused by autosomal dominant non-syndromic and syndromic conditions. As part of a complicated succession mechanism of inheritance, 1% of cases (one in a thousand) are attributed to either the X-chromosome or the mitochondria.[11]Heteroplasmas Deferred extension, also known as the MTR1 12S mitochondrial ribosomal-s gene, is a hot spot for sensory hearing deficiency mutations.In total, it has been reported that over 30 mutations cause in MTRNR1[13], a condition such as m.1555A, non-syndromic hearing loss mutations of m.1494C>T and m.1095T>C.[14,15]Bilateral, incremental, and sensorineural hearing loss also occurs with mitochondrial mutations. A few additional neurological symptoms are observed. The severity of hearing loss can range from moderate to absolute lack of hearing.It has been estimated that m.1555A> G's penetrance is between 28-75 percent, with an average of about 60 percent. The date of birth ranges from early birth to adulthood.To date, it has been found that 46 genes are causally linked to NSHL. There are different frequencies of mutations in these genes across multiple ethnicities.[2,16]It appears to differ widely in the degree of mitochondrial hearing loss prevalence among populations. In Asian countries, the frequency of m.1555A>G has been estimated to be higher than in the rest of the world[6,17]however, one may also say that differences between countries and regions also exist.[18]Based on large-scale epidemiological studies, in a study in China were done in 28 provinces and municipalities, over 90% of those with a hereditary hearing impairment" (>80%).[13,15,19]

It is estimated that between 10 and 30% of Finnish people have non-syndromic hearing loss, the frequency of me.1555A>G,it is reported to be 2.6% Some research studies show that in Japan suggests that the percentage of deaf and hard of hearing individuals in Japan is about 5%. Among Spanish individuals with hearing loss, the prevalence is higher than 23%.[18] The mutation frequencies of M.1494C>T and m.1095T>C were similarly stated to remain hidden in Asian populations and go unnoticed by other populations.[8]

In some instances, aminoglycoside antibiotics can bind to the human mitochondrial DNA. It has been suggested that a mutation could be used to transform the human mitochondrial ribosome into a bacterial ribosome, improving the binding affinity of the drug molecules. A single dose of aminoglycosides can cause deafness at any age for the mutation carrier MTRNR1.[5]However, premature and newborns and infants are more sensitive. Often premature babies are born and develop viruses and infections, and sepsis, with aminoglycoside treatment being given to up to 90% of preterm infants.[7]The most important factor affecting a child's ability to acquire language is when hearing impairment is found.[7]It is also hampered by early identification, early diagnosis, and intervention may help to improve listening voice, and communication in children.[15]

In satisfying the needs of neonates with hearing damage, early diagnosis is of critical significance. Universal screening costs for the diagnosis of newborns with hearing damage are comparatively low[9]and can be economically advantageous even in developed countries.[20]Also, the discovery of mutations in aminoglycoside-induced mitochondrial

genes. Deafness may prevent these medications from being misused. Studies have demonstrated that concomitant gene screening for newborn hearing is an essential supplement to traditional hearing tests for better infant care[21] and can recognize infants whose hearing loss exists.[10]

For a small number of different versions and samples, PCR is exceptionally cost-effective, readily suitable at different health care distribution sites, and tests directly aimed at NSHL-associated variants have been licensed for use in clinical diagnosis.[2]However, standard PCR had difficulties in scaling to vast numbers of samples, with various approaches in play.[22]The most accurate and efficient way to recognize hereditary variations linked to hearing loss in public health efforts remains unanswered. [8,23]Therefore, this study aimed to investigate the same genetically mutant gene among newborns with Nonsyndromic Hearing Losscompared to healthy individuals.

## Materials and Methods

Patients and sample collection

This research has been carried out throughout the time between November 2019 till October 2020. **The study enrolled a total of** 80 newborns**with non-**syndromic hearing loss **and 52**healthy individuals**at** (Hiwa Institute for deaf and mutes) **their** ages patients from 14 to 22 years old**.** After gaining the Medical Research Center/ Hawler Medical University's ethics committee's approval, the study was undertaken. Samples were collected inpatients whom specialized physicians diagnosed. Three ml of peripheral blood samples wereperformed and aseptically collected by syringe and transferred intoan EDTA tube, then transferred to the laboratory for further tests.

### *Preparing the Primers*

Lyophilized forward and reverse primers of *MTRNR1*gene:

mt.1555A>G, mt.1555A>G-mito-1555-F1 (O) (TGTAGCCCATGAGGTGGCAAGAAATG), mito-1555-R1(O) (TTAGCTCAGAGCGGTCAAGTTAAGTTGAAA) mito-1555-F2(I) (TTTATATAGAGTAGG), mito-1555-R2 (I) (CACTTTCCAGTACACT TACCATGTTACGACGTGT).

This procedure was performed depending on the manufacturer's instruction by dissolving the lyophilized sample with nuclease-free water to give the final concentration (100 pM/µl) (as a stock solution) then rotating down briefly. To prepare 10µM of working primer (working aliquot), re-suspended tenpM/µl of stock primer in 90µl of deionized water to reach a final concentration of 10µM.These primers were synthesized by GeNet Bio Company (Korea).

### *Molecular Mutation Detection in MTRNR1 mt.1555A>G gene by Tetra-Primer ARMS PCR*

DNAwas collected from blood samples and genotyped using the genomic extraction kits (Addbio/ Korea), depending on the manufacturer's instructions. A Nanodrop spectrophotometer performed forthe extracted genomic DNA was tested to estimate the extracted DNA's concentration and purity of the absorbance reading at (260 /280 nm).

The *MTRNR1* mt.1555A>G mutation was identified by multiplex tetra primer amplification mutation system PCR. The total of 25 µl PCR master mix reaction volume was performed containing 3µl of genomic DNA, 12.5 µl of 2X GoTaqGreen Master Mix(Promega, USA), and 1.5µl was added for each of the forward and reversed primers of MTRNR1 mt.1555A>G-mito-1555-F1,mito-1555-R1(O),mito-1555-F2 (I),mito-1555-R2 (I)[24]then the mixture was completed by adding 3.5 µl of nuclease-free water.Amplification was initiated according to the manufacturers' instructionsan initial denaturation at 94°C for 5 minutes, 35 intervals accompany it at 94°C for 30 sec, 59°C for 30 sec, 72°C for 1 minute, and the last extension at 72°C for 5 minutes.The components of the PCR are lined with 2% agarose. The 254bp, 156bp, and 341bp signify wild form, mutation, and regulation presence, respectively.

## Result

A total of 132 (80 newborns and 52 individuals) were genotyped for mt.1555A>G, G-mito-1555-F1,mito-1555-R1 (O),mito-1555-F2 (I),andmito-1555-R2 (I) hearing loss mutations in MTRNR1. No mutant alleles were observed among all samplesfor mtDNA 12S rRNA mt.1555A>G,and no false-positive were found. Allowing just 52 samples to be run on a 2% agarose gel gives an almost transparent cut-and-and-dried result; two of them are outer primers, and others are inner primers. Two different bands were observed with 52 samples with different molecular sizes (wild type at 254bp and control at 341bp). 28 out of 80 samples have only control bands at 341 bp. In this study, we could not detect any mutation in our 80 samples.

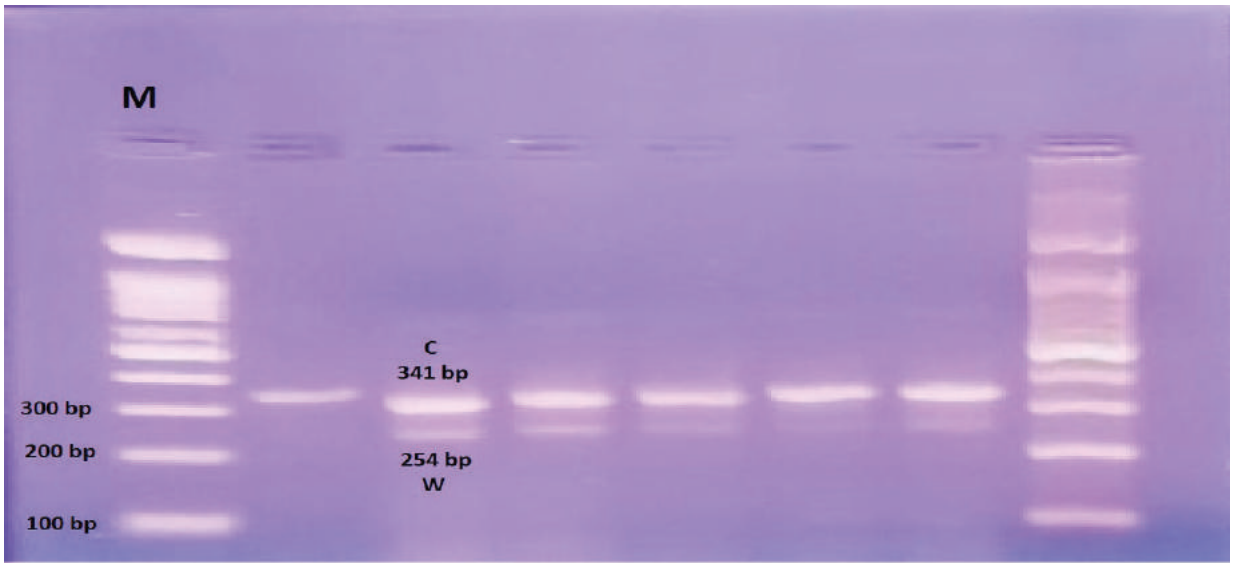


**Figure 1: PCR for gene MTRNR1 mt.1555A>G100bp M(marker) , 254 bp W(wild type), 341bp C(control)**

## Discussion

The investigation of hereditary deafness and other genetic conditions has increased significantly with the advent of genomics. Population-specific hearing loss mutations have been found by innovative genetic technology, which continues to change and become more widespread.[23,25]Many researchers have shown that the approximate figures range from 60 to 80% of pre-lingual (HL) and (NSHL) is caused by genetic causes, with empirical evidence from developing countries indicating that 60% of deaf people have inherited deafness.[8, 11, 14, 22, 24, 26, 27]Deaf disease gene translation and cloning have made significant strides since the human genome project was completed. The fundamental hereditary susceptibility to NSHL has been increasingly revealed by molecular genetics and its molecular epidemiology.[28]The positive screening figure has been 1% in China since the UNHS was broadly adopted in 2000, 2.5–3% were seen for the trials that had included 5-year-olds and juvenile respondents, and there has been other research that has seen prevalence estimates of 3.4–4% for 14-year-olds a sharp rise in the incidence of neonatal hearing loss and drug-induced hearing loss.[21-29]This scenario highlights the severe drawbacks of relying entirely on a newborn hearing screening to diagnose NSHL. This method does not diagnose the drug-induced deafness hearing gene carrier or those of delayed-type deafness. The MTRNR1 mt.1555A>G gene was performed for Nonsyndromic Hearing Lossgenotyping purposes; 132 samples (80 newborns and 52 individuals) were enrolled. For the mtDNA 12S rRNA mt.1555A>G, no

mutant alleles were found in any of the samples, and no false-positive were found. Two distinct bands were discovered with 52 samples of different molecular sizes (wild type at 254bp and control at 341bp). However, only control bands at 341 bp are found in 28 of the 80 samples. In a study by [2]in China, among 27573 newborn samples, 1810 carried pathogenic mutations. Furthermore,[2] recorded (0.298%) NSHL mutant MTRNR1gene, which does not agree with our findings. Our research findings for the infant and stable adults were distinct, most likely due to genetic trends in the two groups. The sample size may have impacted these effects.Since it's commonly correlated with non-syndromic hearing loss, the m.1555A>G entails a middle non-syndromic hearing loss in the mother and brother of the patient.[26]Furthermore, the m.1555A>G mutation results in a wide range of family members' clinical phenotypes. In reality, the penetrance of this mutation varies between families. Moreover,[26]detected MTRNR1gene in patients using PCR-RFLP using the restriction enzymeHaeIIIin Tunisia family members.

On a 2.5 percent agarose gel, all samples were quickly separated (Fig. 1).[24]screened 1181 newborns for NSHL mutant genes, they found 29 newborns had one or two mutant alleles; for MTRNR1 mt.1555A>G mutation, just one exception was found.[24]

As has been associated with genetic and acquired hearing loss, as well as an aminoglycoside-induced mutation.[9,30,31]Tetra-primer ARMS-R has been widely reported. Our study used point mutation genotyping methods have been the most commonly used.[24,30]The flow system used in the Tetra ARMS-R package, which includes running DNA through an agarose gel and examining it under a microscope for three separate lanes, has been significantly improved. We also found that a genotype usually takes about three hours.It has few necessary mutations and insertions, so it is an efficient and straightforward molecular classification technique that only requires a small quantity of material for typing. While the kit for large-scale population genotyping detection is successful, the Tetra-primer ARMS-PCR findings are less accurate and applicable. They have a hazy picture of the nucleotide they're looking for; additional recheck procedures are expected to better clarify the Tetra-primer ARMS-PCR kit's performance. This technique is a crucial factor in avoiding false positives and negatives.

Finally, we show that the Tetra-primer ARMS-PCR kit mentioned here is a suitable method for use in a wide range of smaller settings, especially in Iraq's underdeveloped rural areas. Larger-scale epidemiological research on inherited hearing loss in Iraq is required in the future to incorporate more diagnostic targets and develop molecular diagnosis and genetic therapy.

**Conflict of Interests:** None.

**Source of Funding:** Self.

**Ethical clearance:** The study was undertaken after gaining the Medical Research Center/ Hawler Medical University's ethics committee's approval.

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# The Role of Vitamin D3 in Improving Lipid Profile in Type 2 Diabetes Patients with Cardio Vascular Disease

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## Abstract

Hyperglycemia is the major risk factor for microvascular complications in patients with type 2 diabetes (T2D). Vitamin D a fat-soluble prohormone, has wide-ranging roles in the regulation of many physiological processes through their interactions with. This aim of this study to investigate the role of Vitamin D3 and other biochemical to associated with the risk of type 2 diabetes with CVD. This study was carried out at the National Diabetes Center (NDC) / Mustansiriyah University during the period from 1/11/2020 to 1/2/2021. group of type2 diabetes with CVD patients, including 80 patients from both sexes (male and female), and apparently healthy control group (n=40) were enrolled. The highest significantly were in values of FBS , HbA1c, TC , TG , LDL) were (195.33 - 89.45 mg/dl, P= 0.001; 8.75 - 4.86 %, P= 0.001; 234.08 158.9 mg/dl, P= 0.001, 221.25 versus 83.37 mg/dl, P= 0.001 and 132.62 - 87.40 mg/dl, P= 0.001 respectively). Vitamin D3 levels were significantly lower for patients group than that in control healthy people were. The conclusion of this study found Vitamin D3 Deficiency showed effective on lipid profile in Type2 diabetes patients with CVD .

***Key word:*** *T2DM, CVD, vitamin D3, Lipid profile, National Diabetes Center.*

## Introduction

Vitamin D deficiency and diabetes mellitus are two common conditions in the elderly population. Vitamin D deficiency is currently a topic of intense interest, and is widely prevalent across all ages, races, geographical regions, and socioeconomic strata. Suboptimal vitamin D status contributes to many conditions, including type 2 diabetes mellitus(TDM) [(1)]. Despite several advances in pharmacotherapy for type 2 diabetes, the increasing burden of the disease highlights the need for innovative and cost-effective prevention approaches. It is estimated that approximately one-third of adults in the United States are at increased risk of developing diabetes based on their having prediabetes, which is defined by the American Diabetes Association as impaired fasting glucose, impaired glucose tolerance, or abnormal hemoglobin A1c (HbA1c) [(2)]. Vitamin D, a sec-steroid, plays a pivotal role in the protection against numerous diseases, including cardiovascular diseases(CVD).

Vitamin D deficiency is associated with not only CVD itself but also cardiovascular risk factors [(3,4).] Low vitamin D levels could result in dyslipidemia, and lipid abnormalities that is, an increase in triglyceride (TG), total cholesterol (TC), and low density lipoprotein cholesterol (LDL-C) levels and a decrease in high-density lipoprotein cholesterol (HDL-C) level have been identified as important

risk factors for atherosclerosis and cardiovascular disease in adulthood [5,6]. vitamin D supplementation has become worldwide clinical practice. In line with this practice, the recently updated Kidney Disease Improving Global Outcomes guidelines on CKD-MBD suggests that vitamin D deficiency and insufficiency be corrected using treatment strategies recommended for the general population [7,8]. The aim of this study to investigate the role of Vitamin D3 and other biochemical to associated with the risk of type 2 diabetes with CVD.

## Materials and Methods

### Subjects and blood sample collection:

This study was carried out at the National Diabetes Center /Mustansiriyah University during the period from 1/11/2020 to 1/2/2021. This study was conducted on type2 diabetes with cardiovascular diseases group, including 80 patients with type2 diabetes with cardiovascular diseases of both sexes (male and female) , aged (40-65). Apparently the health control group consists of 40 healthy people. They were chosen on the basis of the diagnoses done by specialist . Each patient has a record of his disease and the analyzes he conducted at the National Diabetes Center. Samples were collected from the patients of type2 diabetes with cardiovascular diseases group was in a fasting state, as were samples from healthy people in a fasting state. 10 ml of venous blood samples were collected. includes the serum is obtained by placing the blood in a sterile tube gel and allowing it to clot at 37 ° C for 30 minutes before centrifugation . The tubes are centrifuged at 6000 revolutions per minute for a period of 5 minutes, then we collected the serum and distributed to several parts to make Lipid profile tests (TC , TG, HDL, LDL and VLDL ) , Kidney function Parameters( Urea and Creatinine), vitamin D3 levels ,Insulin level , Homa-IR and fasting blood sugar test.

### Biochemical analysis:

Biochemical analysis for Lipid profile tests (TC , TG, HDL, LDL and VLDL , Urea , Creatinine and FBS was performed by using Automatic biochemistry analyzer by the kenza tx240/biolab / French. The quantitative determination of vitamin d3 In vitro assay in serum. by the miniVIDAS auto analyzer, (bio Mérieux Company ) France and Insulin levels measured by the enzyme- linked immunosorbent assay (ELISA) DRG kit.

## Statistically Analysis

The data analysed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean , standard deviation and ranges. A level of P value less than 0.05 was considered significant.

## Result and discussion

The distribution of study groups by age, gender and BMI is shown in figures (1 ,2 and3). Study patients' age was ranging from 40 to 65 years with a mean of 54.36 years and a standard deviation (SD) of ± 6.39 years, while study patients' gender were (52.26% , 47.74%) male and female respectively, we founded that there 51% of patients 'group with 18-24.9 kg/m2 BMI . In comparison between study groups by age and gender, we noticed that there were no significant differences ($P \geq 0.05$) in age, and gender between study groups as shown in tables (1) and (2).

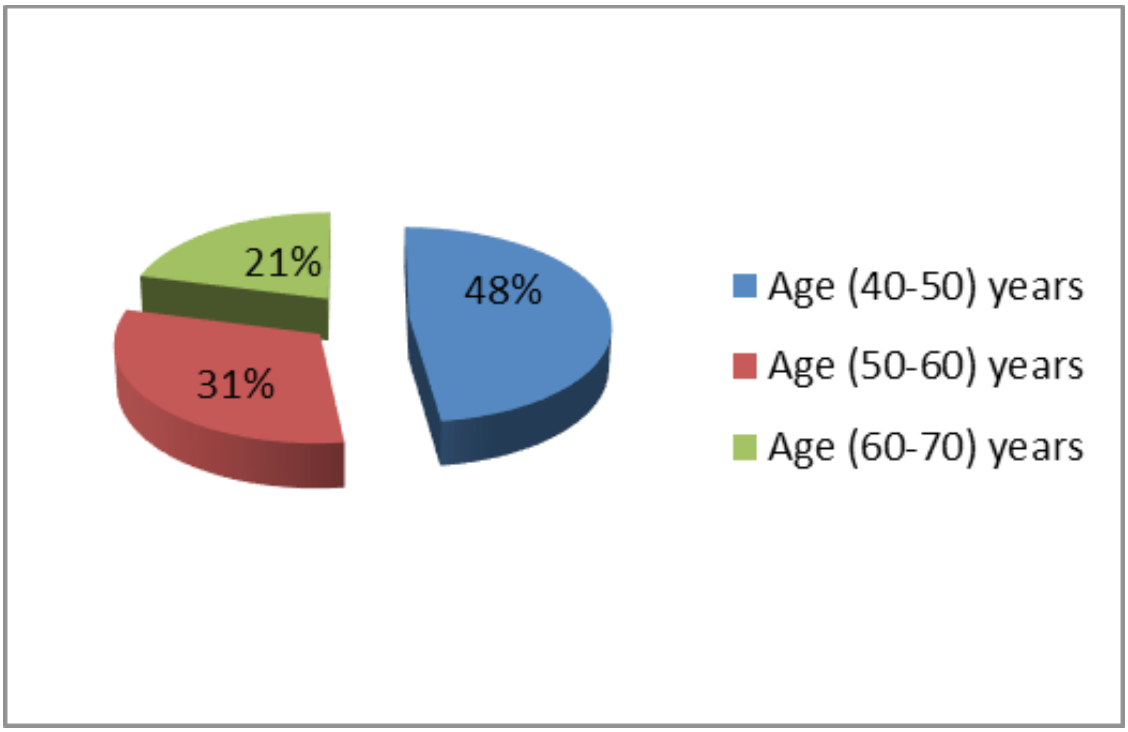


**Figure 1: Distribution of vitamin D3 according to age in T2DM with CVD.**

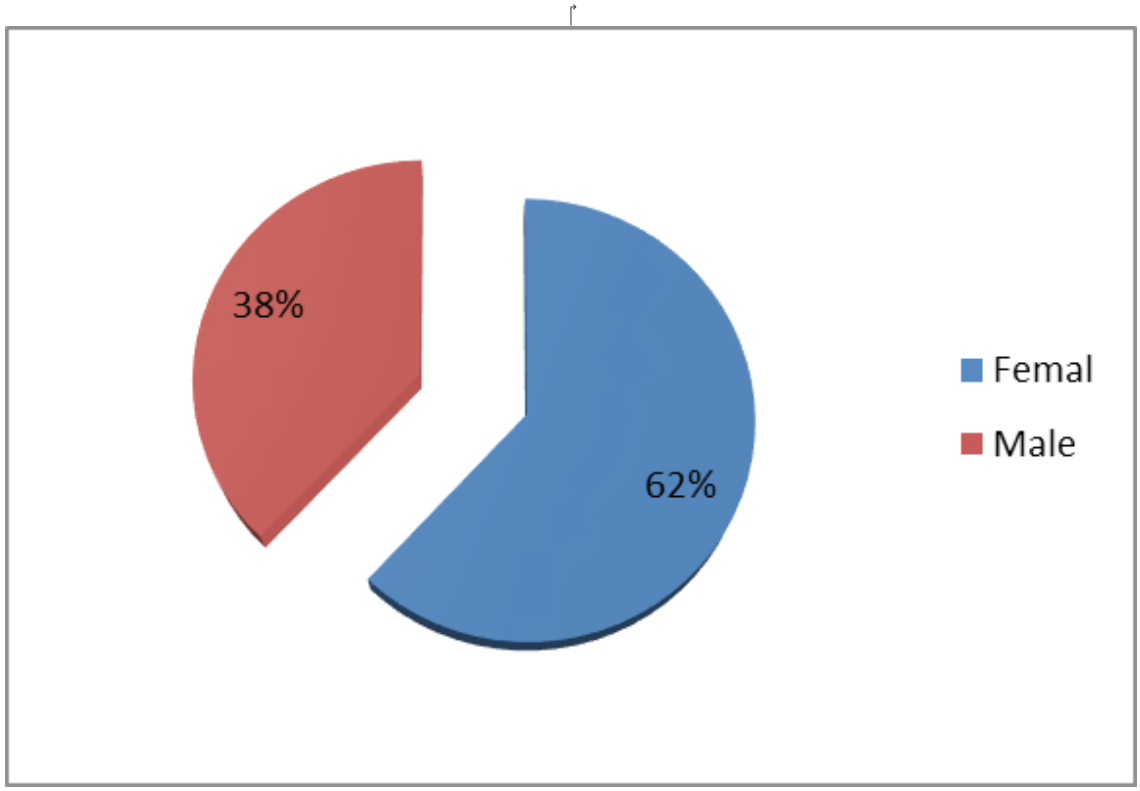


**Figure 2: Distribution of vitamin D3 according to gender in T2DM with CVD.**

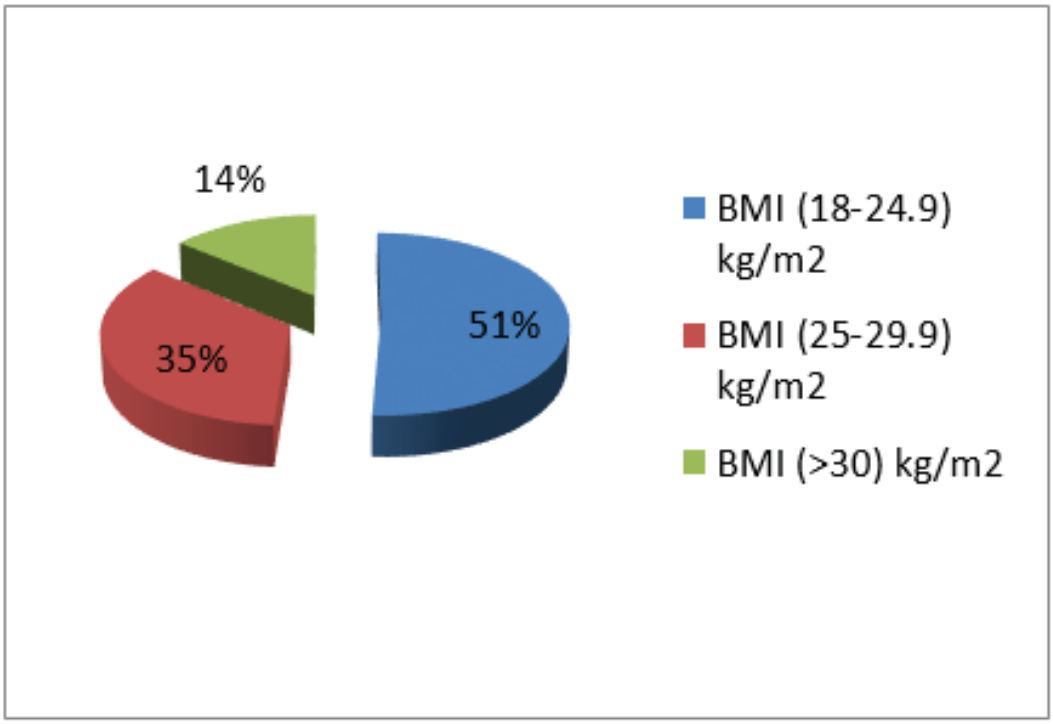


**Figure 3: Distribution of vitamin D3 according to BMI in T2DM with CVD**

The comparison between study groups by age and gender, we noticed that there were no significant differences ($P \geq 0.05$) in age, and gender between study groups as shown in tables (1) and (2).

**Table 1: Comparison between study groups by age.**

| | Study Group | | P – Value |
|---|---|---|---|
| Age (Years) | Patients Mean ± SD | Control Mean ± SD | |
| | 54.36 ± 6.39 | 38.12 ± 5.6 | 0.06 |

**Table 2: Comparison between study groups by gender.**

| Gender | Study Group | | Total (%) n= 120 | P - Value |
|---|---|---|---|---|
| | Patients n= 80 | Control n= 40 | | |
| Male | 41 (51.25) | 22 (55) | 63 (52.5) | 0.162 |
| Female | 39 (50.0) | 18 (60.0) | 57 (47.5) | |

Type 2 diabetes mellitus is a major risk factor for cardiovascular disease. However, compiled data suggest that type 2 diabetes affects the risk of cardiovascular disease differentially according to sex, large meta-analyses have confirmed that women with type 2 diabetes have a higher relative risk of incident coronary heart disease, fatal coronary heart disease, and stroke compared with their male counterparts. The reasons for these disparities are not completely elucidated. A greater burden of cardiometabolic risk in women was proposed as a partial explanation. Indeed, several studies suggest that women experience a larger deterioration in major cardiovascular risk factors and put on more weight than do men during their transition from normoglycemia to overt type 2 diabetes. This excess weight is associated with higher levels of biomarkers of endothelial dysfunction, inflammation, and procoagulant state [9].

The systematic review by Einarson et.al ,(2018) of 4,549,481 persons with T2DM, we estimated the overall prevalence of CVD at 32.2%. The most frequent type of CVD reported was CAD (21.2%) and lowest was stroke (7.6%). Males had higher rates of prevalent disease than females. CVD was responsible for 50.3% of all deaths in T2DM patients over the period of the review. Along with diabetes, cardiovascular disease is associated with several risk factors, obesity, and age[10].

**Biochemical, Vitamin D3 and Insulin levels**

Table 3 shows the Vitamin D3 and Insulin levels between patient group and control group. Means of FBS , HbA1c , Insulin , Homa-IR , TC,TG, LDL, VLDL and urea , were significantly higher for all values in patients group than that in control healthy people except the level of HDL and Vitamin D3 levels. There is no significance between both study groups in the level of creatinine, the highest significantly were in values of FBS , HbA1c, TC , TG , LDL) were (195.33-89.45 mg/dl, P= 0.001, 8.75 - 4.86 %, P= 0.001, 234.08 -158.9 mg/dl, P= 0.001, 221.25 - 83.37 mg/dl, P= 0.001 and 132.62 - 87.40 mg/dl, P= 0.001) respectively .Mean of Vitamin D3 levels were significantly lower for patients group than that in control healthy people were (12.27±2.71 - 34.37±2.43), P=0.001) respectively.

**Table 3: Comparison in Biochemical tests ,Vitamin D3 and Insulin levels between Patient and control group.**

| Variable | Study Group | | P-Value |
|---|---|---|---|
| | Patient Mean ± SD | Control Mean ± SD | |
| FBS (mg/dl) | 195.33±19.21 | 89.45±7.88 | 0.01 |
| HbA1C (%) | 8.75±2.19 | 4.86±0.36 | 0.01 |
| INSULIN (ng/ml) | 17.48±5.42 | 10.92±4.11 | 0.05 |
| HOMA-IR | 8.32±1.40 | 2.41±0.83 | 0.01 |
| TC (mg/dl) | 234.08±44.77 | 158.92±29.13 | 0.01 |
| TG (mIU/ml) | 221.25±17.95 | 83.37±9.27 | 0.001 |
| HDL (mg/dl) | 40.71±5.53 | 52.82±5.61 | 0.05 |
| LDL (mg/dl) | 132.62±5.23 | 87.40±2.31 | 0.01 |
| VLDL (mg/dl) | 44.25±4.99 | 18.76±1.94 | 0.05 |
| UREA (mg/dl) | 50.49±8.64 | 27.61±6.52 | 0.05 |
| CREATININE (mg/dl) | 1.17±0.49 | 0.76±0.24 | 0.012 |
| D3(ng/ml) | 12.27±2.71 | 34.37±2.43 | 0.01 |

We found that the level of vitamins D3 was decreased in patient group that's mean there was Vitamin D3 deficiency in patients with high level in most of biochemical test for lipid profile and kidney function parameters, This explain the role of Vitamin D3 in improving these parameters.

Lipid profile has long been considered among the most important risk factors for CVD in T2DM, and several trials have confirmed that lowering low-density lipoprotein cholesterol (LDL-C) via statins in T2DM was effective in reducing the risk of CVD [11,12], It is also well known that statins also have a TG-lowering effect [13, (14)] . This paradox could mean that low triglyceride is not necessarily associated with good clinical outcomes in all people with T2DM and that there are subgroup associations with CVD in patients with different durations of T2DM. Furthermore, Clua-Espuny et al. (2018) , suggest that the relative importance of risk factors wanes in complex chronic patients with T2DM with advancing age [ 15] . In a cohort study of almost 3500 complex chronic patients above the age of 80 of whom 53% had diabetes and a high prevalence of associated classical risk factors, the researchers found that all causes mortality was more affected by aging factors than by specific complications of diabetes. Type 2 diabetes mellitus is associated with a cluster of lipid abnormalities (diabetic dyslipidemia) that include elevated triglyceride levels, decreased (HDL) cholesterol levels, and an increase in (LDL) particles. most people with T2DM [16]. It appears to be more frequent in women with T2DM after the age of 60 years than in men with T2DM of the same age group [17], this may be due to declines in estrogens' levels and signaling after menopause. In a large sample of people with T2DM from Sweden, women had significantly higher levels of total, LDL, and HDL cholesterol (P<.001 for all) when compared with men. Triglyceride levels were lower in women in the youngest age group (40-54 years) but higher in elderly women (≥70 years) when compared with age-matched men[18].

Several studies reported the relationship between vitamin D3 and type 2 diabetes and cardiovascular diseases such as the review of Zhiguo et.al, (2020) , was dedicated to reveal the correlation between vitamin D and adipo genesis, with emphasis on the diseases related to adipose metabolic disorders. Obesity is a common occurrence worldwide, and it can lead to diabetes. Many studies have indicated that vitamin D deficiency or insufficiency plays an important role in the development and process of obesity and diabetes [19]. Vitamin D deficiency is associated with incident cardiovascular disease. Further clinical and experimental studies may be warranted to determine whether correction of vitamin D deficiency could contribute to the prevention of cardiovascular disease.

## Conclusions

The results of this study revealed that Vitamin D has important effects on insulin action, and may impact on a number of pathways which may be of importance in the development of type 2 diabetes. The vitamin D deficiency is a risk factor for various diseases with Decreased levels of vitamin D may cause insulin resistance and impaired insulin secretion . The lack of consensus in definitions of both 25(OH)D deficiency and of renal hyperfiltration, the definitions chosen are common but not universal. As with all epidemiologic studies, this investigation does not establish causality. The Vitamin D3 Deficiency showed effective on lipid profile in Type2 diabetes patients with CVD.

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# The Protection of Traditional Knowledge of Medicinal Herbs for Just Health and Welfare Access for the Traditional Communities: A Comparison between India and Indonesia

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## Abstract

The purpose of this study is to identify a model in the protection of traditional knowledge (TK) of medicinal herbs by adopting a comprehensive protection instrument, comprising positive and defensive protection. Based on the regulation of TK in international conventions and the national laws, the best practice for the protection of TK of medicinal herbs is by implementing a sui generis regulation that stems from the principle of justice in fulfilling the traditional communities' rights over access to health and welfare.

***Keywords****: Protection, TK, Medicinal Herbs, Health, Welfare, Justice*

## Introduction

Currently, one important topic in regard to the progress of intellectual property (IP) rights is the protection of genetic resources and traditional knowledge (TK) of medicinal herbs. This protection is vital given that they are the sources of knowledge related to human lives that can be commercialized. The value of product sales that embraces TK in the form of genetic resources worth approximately 800 billion USD each year.[1] This figure is according to the results of research by pharmaceutical products, wherein the annual market value of medicinal herbs traded in countries that joined the Organization for Economic Cooperation and Development (OECD) reaches 800 billion USD.[2] In addition, based on data by the Secretariat of the Convention on Biological Diversity (CBD), the profit made from the sales of medicinal herbs globally in 2000 amounted to 60 billion USD.[3] In the same year, the trade value of medicinal herbs in Indonesia summed up to 1.5 trillion IDR, equivalent to 150 million USD.[4] Without a doubt, these enormous values would require the necessary legal protections.

The demand rate of medicinal-herb-based products is closely related to the widespread usage level across nations and the economic aspects for improving people's economy. On the other hand, TK of medicinal herbs has been unrightfully misused by multiple companies, especially those from the developed countries in efforts to manufacture pharmaceutical, medicinal, and cosmetic products. As a result, the traditional communities in developing countries filed claims for the violations on TK. The filed lawsuit by the Council of Scientific and

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Industrial Research of India (CSIR) to revoke U.S. Patent No. 5.401.504 on behalf of University of Mississippi Medical Centre (1995) was regarding the use of turmeric in wound healing. According to Agarwal and Narain, turmeric powder is a traditional remedy passed down by their ancestors from the Ayurveda.[2] Another one is the revocation lawsuit for U.S. Patent No. 4.946.681 and 5.124.349 on behalf of W. R. Grace & Co. regarding the extraction technique of the Neem tree and fruit for the production of Azadirachtin and the pesticide composition derived from the extract of Neem seeds and revocation of U.S. Patent No. 5.663.438 on behalf of Rice Tec., (1997) for rice lines and grains from Basmati.[2] No less important, the patent case of Indonesian herbs and vegetations consisting of pulowaras (*Anethum foeniculum*), sintok (*Cinnamomum sintoc*), kayu rapat (*Parameria laevigata*), cubeb (*Piper cubeba*), pluchea (*Pluchea indica*), masoyi (*Massoia aromatica becc*), blackboard tree (*Alstonia scholaris*), and field milk thistle (*Sonchus aevensis*) by a Japanese cosmetic company, Shisedo, in which the patent was subsequently revoked based on the lawsuit filed by one of the Indonesian non-government organizations.[2,5]

Based on these elaborations, TK of medicinal herbs is a part of nation's intellectual creativity products that must be protected from the act of misappropriation. Therefore, it is of the utmost importance to provide protection of TK of medicinal herbs for just and equitable access to health and welfare for the traditional communities.

## Materials and Methods

This study employs the normative legal research method, which by definition is a know-how activity in legal studies for solving an examined legal issue.[6] Legal research requires the ability to identify legal issues, conduct legal reasoning, analyze problems, and provide a solution to said problems. Morris L. Cohen and Kent C. Olson stated that: "Legal research is the process of finding the law that governs activities in humans society".[7] To find an answer to a problem, primary and secondary sources are used as expressed by Enid Campbell:[8] "Law books may be divided into two broad categories: primary and secondary sources. The primary resources consist of authoritative records of the law made by the law-making authorities. The secondary sources comprise all of the publications that pertain to the law that are not themselves authoritative records of legal rules". Then, the entire collected law sources are analyzed normatively to obtain prescriptions according to the research problem.

## Results and Discussion

1. Classifications and Characteristics of Traditional Knowledge

The 2001 WIPO Fact-Finding report states that: "TK in the narrow sense refers to knowledge as such, in particular the knowledge resulting from intellectual activity in a traditional context, and includes know-how, practices, skills, and innovations. Traditional knowledge can be found in a wide variety of contexts, including agricultural knowledge; scientific knowledge; technical knowledge; ecological knowledge; **medicinal knowledge, including related medicines and remedies**; and biodiversity-related knowledge".[9]

Based on this basis, TK is established, derived from, developed, and practiced by the TK holders, which are a part of the heritage concept, classified as follows:[10]1) agricultural knowledge; 2) scientific knowledge; 3) technical knowledge; 4) ecological knowledge; 5) **medical knowledge, including related medicine and remedies**; 6) biodiversity-related knowledge; 7) expressions of folklore; 7) handicrafts; 8) design; 9) stories and artwork; 10) element of language; 10) movable cultural properties. According to Johanna Gibson in Zainul Daulay, the fundamental concept of TK is placed upon the fact that TK rests on tradition, which is "the ways that reflect the tradition of people, which are the ways by which knowledge is created, preserved, and spread".

[10] TK focuses on the use of knowledge, such as the knowledge of traditional technical skills, traditional environment, science, agriculture, or medicine—which includes the knowledge of medicinal herbs and medical techniques.[10]

According to the aforementioned description, the characteristics of TK as mentioned by several international conventions:

**Table 1**

| WIPO-IGCGRTKF Draft | 2003 UNESCO Convention for The Safeguarding of The Intangible Cultural Heritage | 1992 Convention on Biological Diversity |
|---|---|---|
| 1. generated, preserved, and transmitted in a traditional and intergenerational context;<br>2. distinctively associated with a traditional or indigenous community or people which preserves and transmits it between generations;<br>3. integral to the cultural identity of an indigenous or traditional community;<br>4. tangible and intangible. | 1. is passed down between generations;<br>2. develops dynamically;<br>3. becomes an integral part of the community identity;<br>4. is the source of creativity;<br>5. intangible. | 1. Knowledge, innovation, and practice of the traditional community;<br>2. Developed by the indigenous people and local communities across numerous generations;<br>3. A sustainable traditional lifestyle stemming from biodiversity. |

**2. Instruments for the Protection of Traditional Knowledge of Medicinal Herbs**

According to the WIPO-IGCGRTKF Glossary, protection "refers to the protection of traditional knowledge and traditional cultural expressions against some form of unauthorized use by third parties".[11] Instruments for the protection of TK of medicinal herbs in this study include positive and defensive protection. As stated in the WIPO-IGCGRTKF draft, positive protection "…grants IP rights in the TK and TCEs. These rights may be used to prevent unauthorized or inappropriate uses by third parties. It may also enable active exploitation of TK and TCEs by the originating community itself, for example, to build up its own handicraft enterprises".[12]

Positive protection involves the creation of law by granting exclusive rights over TK to prevent its exploitation of unauthorized or inappropriate uses by third parties. The reason why it is named as such is that there are new legal provisions that act as positive law through implementations.[13] The implementation here means both the ratification of international conventions and the legislative process within the national legal system.

On the other hand, there is defensive protection. As stated in the WIPO-IGCGRTKF draft, defensive protection "does not grant IP rights over the subject matter of TK but aims to stop such rights from being acquired by third parties".[14, 15] Defensive protection is a strategy to ensure that no illegitimate claims are filed over TK, which may be achieved by making an inventory and documentation. This defensive protection

can also mean "preservation" and "safekeeping" as the manifestation of comprehensive protection over TK, described in the WIPO-IGCGRTKF draft as such:[16] "... non-IP laws and programs dealing with the safeguarding and promotion of living heritage can play a useful role in complementing laws dealing with IP protection". These efforts aim to guarantee that TK does not cease to exist in the traditional communities' everyday life, but instead, endures and be carried out.

4. The Protection of Traditional Knowledge of Medicinal Herbs within National Laws

a. India

India governs the protection of TK in The Biological Diversity Act No. 18 of 2002 (Act No. 18/2002), enacted in 2003. The exercise of this Act is further regulated in the executive regulation through the Ministry of Environment and Forests Notification and contained in "the Gazette of India Extraordinary", on 15 July 2004. This Act is a form of positive protection of TK, explicitly mentioned as such: "An act to provide for the conservation of biological diversity, sustainable use of its components and fair and equitable sharing of the benefits arising out of the use of biological resources, knowledge and for matters connected therewith or incidental thereto".[17]

Furthermore, India provides protection of TK in the scope of biodiversity, expressly put in Article 2 (b) Act No. 18/ 2002 that "The Central Government shall endeavour to respect and protect the knowledge of local people relating to biological diversity". Based on this stipulation, India places biodiversity as a resource that must be protected by the government due to having high economic value. Therefore, the country imposes regulations concerning the access to benefit sharing, using terms such as "benefit claimers", defined as "… the conservers of biological resources, their by-products, creators and holders of knowledge and information relating to the use of such biological resources, innovations and practices associated with such use and application".[18]

On a related note, the distribution of benefits gained from TK becomes the authority of the National Biodiversity Authority, by whom the amount of money payable to the TK holder—individual or group—is determined, thereby ensuring that the traditional communities receive the benefits of the TK belonged to them as a form of access to equitable health as regulated in Article 21 section (3): "Where any amount of money is ordered by way of benefit sharing, the National Biodiversity Authority may direct the amount to be deposited in the National Biodiversity Fund: Provided that where biological resource or knowledge was a result of access from specific individual or group of individuals or organisations, the National Biodiversity Authority may direct that the amount shall be paid directly to such individual or group of individuals or organisations in accordance with the terms of any agreement and in such manner as it deems fit". In addition, the National Biodiversity Authority can take steps in delivering the agreed sum of money directly to the traditional communities through the local governments. In the event that the individual or individual groups of the traditional communities cannot be identified or found, then the payment is reserved in the National Biodiversity Fund as regulated in Article 20 (8) Ministry of Environment and Forestry Notification 2004.

India's experience represents the general tendency of developing countries in managing genetic natural resources and TK legacies. The country's strategic measure in regard to defensive protection is to set up the Traditional Knowledge Digital Library (TKDL), an electronic database of TK in the field of medicinal herbs. The purpose of which is to prevent any registration of a patent for TK. The existing database will allow patent offices across the globe to search and examine every common usage and, thus, preventing any grants of wrong patent based on the knowledge in the public domain.[19, 20] Additionally, India has also made revisions on its Patent Law that has put into effect since 1970 with The Patent (Amendment) Act No. 15 of 2005. India's Patent Law provides positive

protection of its traditional communities' TK through the Law's implementation within the national legal system of India.

b. Indonesia

In regulating the protection of TK of medicinal herbs for just and equitable access to health and welfare for the traditional communities, Ronald Dworkin's principle of justice serves as the basis, which revolves around the provision of facilities, infrastructure, and access for the people based on an equal opportunity, regardless of the genetic luck that results in "the different talents" and "the different ambitions".[21] In Dworkin's criteria, a just law should 'take rights seriously'. He argues that this is fundamental start as a demand to the government to provide equal respect and concern to the people, to which he further added, "anyone who professes to take rights seriously, and who praises our Government for respecting them, must have some sense of what that point is. He must accept, at the minimum, one or both of two important ideas. The first is the vague but powerful idea of human dignity…".[22] This line of thinking is pivotal in the regulation of TK, i.e., a legislative process consistent with said principle will produce a just law to realize the protection of TK.

Indonesia grants positive protection of medicinal herb TK, by enacting international conventions that have been ratified into national law through the Law No. 5 of 1994 on the Ratification of United Nations Convention on Biological Diversity, Law No. 11 of 2013 on the Ratification of Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity, Presidential Regulation of the Republic of Indonesia No. 78 of 2007 on the Ratification of the 2003 Convention for the Safeguarding of the Intangible Cultural Heritage. Moreover, the Law No. 13 of 2016 on Patent explicitly states that "Indonesia is a country with genetic resources and traditional knowledge oftentimes utilized by domestic and foreign investors to produce new inventions. Therefore, this Law contains regulations regarding clear and honest mentions about the materials used for the invention should it pertain to and/or is derived from the genetic resources and/or TK in its description". However, as part of positive protection, the most ideal protection of TK is through a sui generis system. Currently, Indonesia has a Draft of Traditional Knowledge and Traditional Cultural Expression (TKTCE) Law as an effort to provide sui generis protection. The context of TK protection for the traditional community's access to health and welfare should encompass a number of essential points, which are:

1. General Scope of Subject Protection

Article 4 (1) notes that "The Scope of Traditional Knowledge comprises technical knowledge in the traditional context, traditional skills, innovations in the traditional context, traditional practices, traditional education, and knowledge inherited from generation to generation that underlies a lifestyle, including **Traditional Knowledge regarding genetic resources, traditional medicines**, and other intellectual properties". This article accommodates TK of medicinal herbs within a wide scope.

2. Beneficiaries of Protection

Article 3 of Draft of TKTCE Law mentions that "the country holds sovereignty to governs the regulation of TKTCE for the utmost prosperity and welfare of the people". This means that the regulation of TK is more appropriate to be passed on to the country for the utmost prosperity and welfare of the people that ultimately addresses the traditional community, in line with Article 11 of Draft of TKTCE Law—governing that "every citizen, whether they are individual, community, or business entity, has the right to make use of TKTCE to be used as and converted into raw materials for creative economy".

3. Fair and Equitable Benefit-Sharing

Access benefit sharing (*ABS*) is crucial for the

welfare of traditional communities, both in the form of monetary and non-monetary compensation. As monetary compensation, ABS can be provided through cash payment or continuous stipend, whereas non-monetary compensation is a model long practiced in Indonesia that has enriched Indonesian TK, in which the new products or works that are made based on it must be "returned" or available to those who have preserved the relevant TK.

Regarding ABS, the Draft of TKTCE Law dictates:

1) The caretaker communities receive benefits from the use of TKTCE;

2) Should the use of TKTCE be unknown to the caretaker communities, the government and/or local government acts as the caretaker for the benefit of the Indonesian people.

Furthermore, the Draft of TKTCE Law, Article 17 (1) stipulates that:

1) The distribution of benefits referred to in Article 14 section (4), is accomplished according to the agreements made between the User and the beneficiaries.

2) The agreement in section (1) is contained in written form unless otherwise specified contingent on the customary law.

A designated legal instrument is necessary to protect the traditional communities from the use of TK by third parties, especially in circumstances related to misappropriation. For that reason, provisions regarding obligations of "adequate disclosure (or disclosure of origin requirement)" are closely associated with ABS and Material Transfer Agreement (MTA).[2] As a result, the distribution of economic and other benefits calls for provisions that lay down several preconditions, which are:[2] *first*, establishing the stages to attain commercial benefit distribution through participation, partnership, and public-private sector collaboration. *Second*, determining the MTA standard that imposes the resource beneficiary that commercializes products received from the multilateral system to pay according to the agreed mechanism—with equitable and fair benefit distribution—unless the products is readily available without limitations for subsequent research and advancements. This may serve as the basis of improvements for ABS provisions for TK within the Draft of TKTCE Law.

## Conclusion

The protection of TK of medicinal herbs can be attained comprehensively using positive and defensive protection. Positive protection is implemented by ratifying international conventions in the field of IP and non-IP law in national law thus becoming a positive law. Defensive protection comprises the preservation and safeguarding by making inventory and documentation, compiled in a centralized database so that strategic measures can be made to prevent misappropriation of TK of medicinal herbs. These protection instruments are built on the principle of justice to fulfill the rights of traditional communities over access to health and welfare.

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# How is the Effect of Health Services on Toddler Diarrhea?: Ecological Analysis in Indonesia

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## Abstract

**Background:** About 4 billion cases of toddler diarrhea occur worldwide each year. As many as 70% of toddler deaths in the world were caused by diarrheal infections. This study was intended to analyze the relationship of health services factors on the prevalence of diarrhea of infants in Indonesia.

**Methods:** Ecological analysis was conducted using secondary data from the Ministry of Health of the Republic of Indonesia report in 2018. All provinces were taken as samples. Apart from prevalence of children under five with diarrhea, 4 other variables analyzed as independent variables were, the availability of health workers, the coverage of diarrhea services and the coverage of oralit services to the patients, the coverage of zinc services to the patients, and the poverty factors in each provinces. Data were analyzed using cross-tabulation and spearman test.

**Results:** The results show from 34 provinces, the highest prevalence of toddlers is in the Papua Province. The results showed that the higher the percentage of poor people in the province, the higher the prevalence of diarrhea in toddlers (r=0.363). While the low availability of health resources, coverage of oralit use, and coverage of zinc use in patients were mostly occurring in provinces that had a high prevalence of toddler diarrhea. This means that there was an inverse relationship between the availability of health resources (r=-0.430), the coverage of oralit use (r=-0.149) and the coverage of zinc use in patients (r=-0.013) with the prevalence of diarrhea in toddlers.

**Conclusion:** It was concluded that according to bivariate analysis on the prevalence of diarrhea of toddlers showed that increasing availability of health resources in the provinces can help to reduce the prevalence of toddlers by assuming other variables remain.

***Keywords:*** *ecological analysis, secondary data, diarrhea, toddlers, good health, well-being*

## Background

Diarrheal disease is one of the most common diseases affecting children around the world including developing countries such as Indonesia. This is due to its high morbidity and mortality. Diarrhea in children can cause fatal things if not balanced with good fluid and nutritional inputs or sudden diarrhea occurs and does not get proper treatment by caregivers, otherwise diarrhea actually also includes mild illnesses if the sufferer gets adequate care. Diarrhea is defecation with a more fluid feces consistency with a frequency

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of >3 times a day, except in neonates (infants< 1 month) who get breast milk usually defecate with a frequency more often (5-6 times a day) with good consistency is considered normal[1].

An estimated 4 billion cases diarrhea occur each year in toddlers worldwide. Every year 1.5 million toddlers die from diarrhea. Diarrhea leads to faster deaths in children than adults due to dehydration and malnutrition. Based on data released by WHO almost 1 trillion and 2.5 billion deaths from diarrhea in the first 2 years of life. Diarrhea also causes 70% of the deaths of the toddlers in the world. A record proved, 1.8 billion die each year from diarrhea, many of which have complications such as malnutrition, growth retardation and immune abnormalities[2]. Although mortality from diarrhea can be lowered by rehydration/ fluid therapy programs, the pain rate remains high. The death rate due to diarrhea was 3.8 per 1000 per year, the overall incidence median in children under 5 years of age was 3.2 episodes of children per year[3].

The prevalence rate of diarrhea in Indonesia is still fluctuating. The period of diarrhea prevalence in Indonesia is currently 6.8% higher than in 2013 (3.5%) but smaller than the Basic Health Research (Riskesdas) 2007 which is 9.0%. This high decrease in prevalence period is possible due to unequal sampling times between 2018, 2013, and 2007[1]. On the other hand, through the SGDs program the government can improve and improve the quality of the health in toddlers, as contained in the target SDGs program until 2030, in the third goal is to target the reduction of deaths in the number of newborns to 12 per 1000 live baby births and the death of toddlers 25 per 1000 live toddler births[4].

The target coverage of services for toddlers who come to health facilities of 20% of the estimated number of diarrhea sufferers (Incidence of Diarrhea toddlers multiplied by the number of toddlers in one work area within one year). In 2018 the number of diarrhea sufferers of toddlers served in health facilities as much as 1,637,708 or 40.90% of the estimated diarrhea in health facilities[5].

Control of diarrhea disease itself has long been attempted by the Indonesian government to suppress the incidence of diarrhea. Efforts made by the government such as the provision of clean water and total community-based sanitation programs with the aim of improving the quality of life of the community and lowering deaths caused by diarrheal disease. However diarrheal disease is still the highest cause of death in toddlers after Acute Respiratory Tract Infection[6].

Five Steps To Resolve Diarrhea Program recommends that all diarrhea sufferers should get oralit then the target of using oralit is 100% of all cases of diarrhea that get services in puskesmas and cadres. In 2018 nationally the use of oralit of all ages is still below the target 0f 90.48%. The achievement is still lacking because the service providers in puskesmas and cadres have not provided oralit in accordance with the standard of governance that is as much as 6 packs/ diarrhea sufferers.

Zinc is a micronutrient that serves to reduce the length and severity of diarrhea, reduce the frequency of defecation, reduce the volume of stool and decrease the recurrence of diarrhea incidence in the next three months. The use of zinc for 10 days in a row when the toddler diarrhea is diarrhea therapy toddlers. In 2018, the coverage of zinc administration in diarrhea toddlers was 93.23%[5].

Indonesia is a country of low health. This is due to health care factors, one of which is the lack pf availability of health worker[7]. The placement of health workers in the village is expected to increase the coverage of health services and reduce the incidence of diarrhea[8]. The previous study have shown that families with high poverty rates are more at risk of having children with diarrhea than families with low poverty rates[9]. Therefore, this study is intended to identify the association of diarrhea prevalence in infants in Indonesia with poverty factors, availability of health workers, and the use of zinc and oralit.

## Materials and Methods

### Study Design

This study used an ecological analysis approach. Ecological studies focus on comparisons between groups, not individuals. The data analyzed is aggregated data in certain groups or levels, which in this study is the provincial level. Variables in ecological analysis can be aggregate measures, environmental measures, or global measures[10,11].

### Data Source

This study was carried out by utilizing secondary data from reports of the 2018 Indonesia Basic Health Survey and the 2018 Data and Information of Indonesia Health Profile. The two reports were officially issued by the Ministry of Health of the Republic of Indonesia. The variable prevalence of diarrhea in children under five were obtained from the 2018 Indonesia Basic Health Survey[5]. Whereas, the other variables were obtained from the 2018 Data and Information of Indonesia Health Profile[1]. The unit of analysis in this study is the province. All provinces in Indonesia were analyzed, as many as 34 provinces.

## Data Analysis

Data were analyzed to be univariate and bivariate. Univariate analysis were obtained to identify variable statistical description. While other variables will be categorized into 2 strata with statistically the same cut of points (by the mean of each variable). Bivariate analysis were obtained by using cross-tabulation to identify the number of distributions between two variables dan spearman test to identify the value of the relationship and the direction of the relationship between the two variables. Because the data processed was a total sample of the entire province, it was not necessary to see the level of significance. All analysis processes utilized software SPSS 21.

## Results

Table 1 shows a fairly wide disparity in the prevalence of toddlers diarrhea in Indonesia 2018. It was recorded that Papua Province had the highest prevalence of toddlers diarrhea at 15.80%. While the lowest prevalence of toddlers diarrhea was in Riau Islands Province at 6.00%.

**Table 1. Descriptive statistics variables of prevalence of toddler diarrhea and other related variables in Indonesia**

| | Prevalence of Toddler Diarrhea | Coverage of Toddler Diarrhea Services | Coverage of Patients Receiving Zinc | Coverage of Patients Receiving ORS | Poor Population | Availability of Health Workers |
|---|---|---|---|---|---|---|
| N | 34 | 34 | 34 | 34 | 34 | 34 |
| Mean | 11.37 | 37.93 | 89.78 | 88.89 | 10.61 | 72.25 |
| Median | 10.85 | 37.37 | 95.53 | 93.48 | 8.90 | 51.70 |
| Mode | 9.70 | 9.77 | 100.00 | 100.00 | 3.55 | 16.30 |
| Std. Deviation | 2.57 | 14.01 | 18.45 | 18.33 | 5.70 | 61.06 |
| Variance | 6.59 | 196.26 | 340.49 | 336.03 | 32.523 | 3728.49 |
| Range | 9.80 | 66.11 | 100.00 | 100.00 | 23.88 | 321.34 |
| Minimum | 6.00 | 9.77 | .00 | .00 | 3.55 | 16.30 |
| Maximum | 15.80 | 75.88 | 100.00 | 100.00 | 27.43 | 337.64 |

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

**Table 2. Prevalence of toddler diarrhea according to the coverage of toddler diarrhea services**

| Coverage of Toddler Diarrhea Services | Prevalence of toddler diarrhea | | | | Total | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | n | % | n | % | n | % |
| Low | 12 | 66.7 | 6 | 33.3 | 18 | 100.0 |
| High | 7 | 43.8 | 9 | 56.3 | 16 | 100.0 |

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

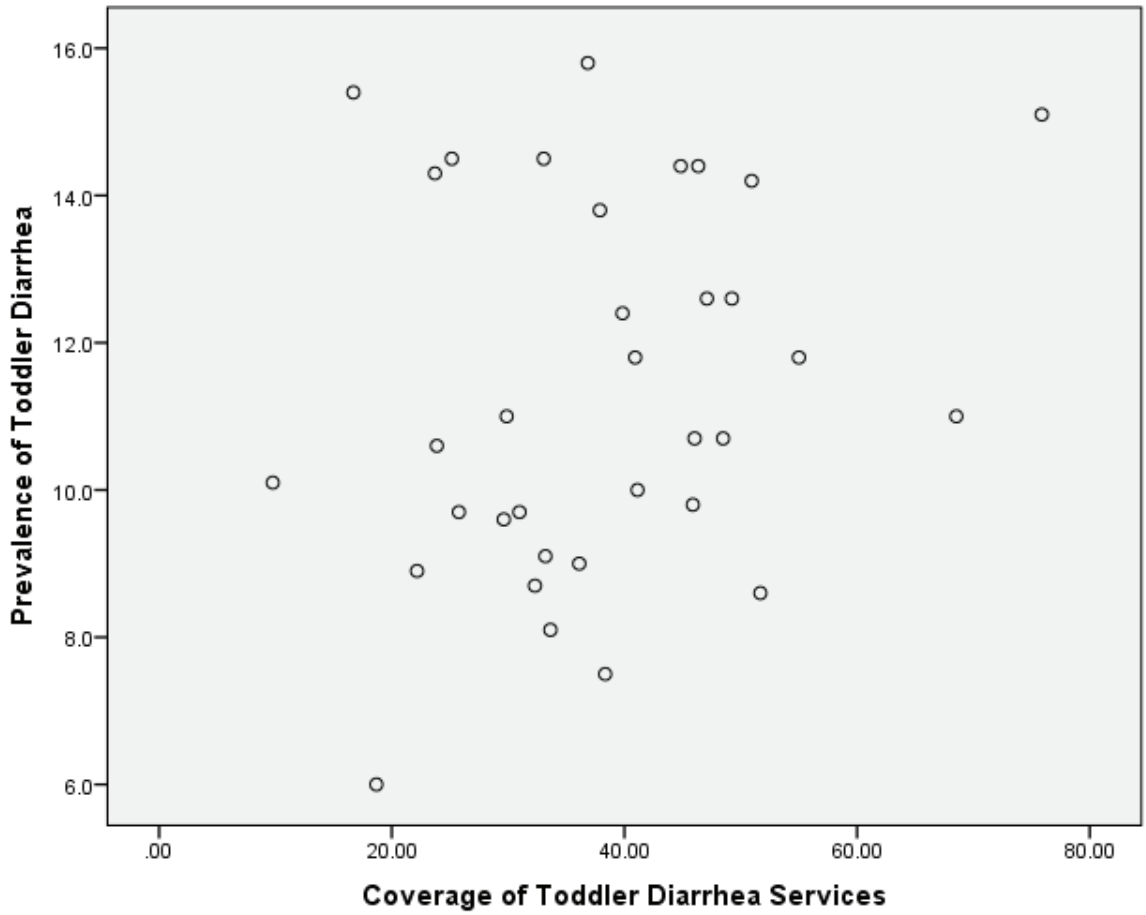
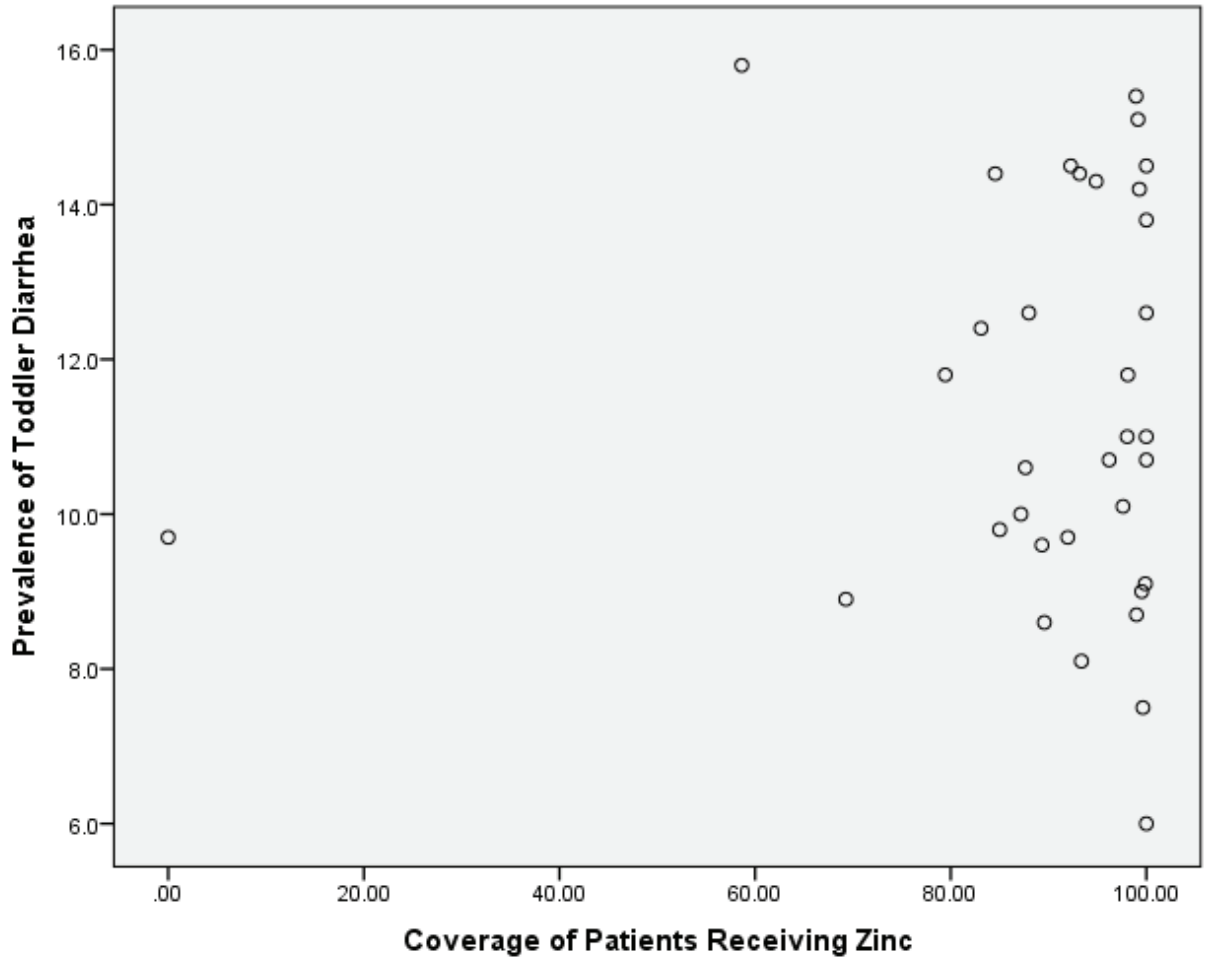



**Figure 1. Scatter Plot of Coverage of Toddler Diarrhea Services and Prevalence of Toddler Diarrhea in Province of Indonesia 2018.**

Based on Table 2, it can be seen that the prevalence of diarrhea is high, many occur in provinces with high diarrhea service coverage, which is 56.3% compared to provinces with low service coverage. Scatter plot results show that the higher the health care coverage and prevalence, the higher the prevalence of diarrhea of toddlers with r = 0.208 (Figure 1).

**Table 3. Prevalence of toddler diarrhea according to the coverage of patients receiving zinc**

| Coverage of Patients Receiving Zinc | Prevalence of toddler diarrhea | | | | Total | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | n | % | n | % | n | % |
| Low | 7 | 58 | 5 | 42 | 12 | 100 |
| High | 12 | 55 | 10 | 46 | 22 | 100 |



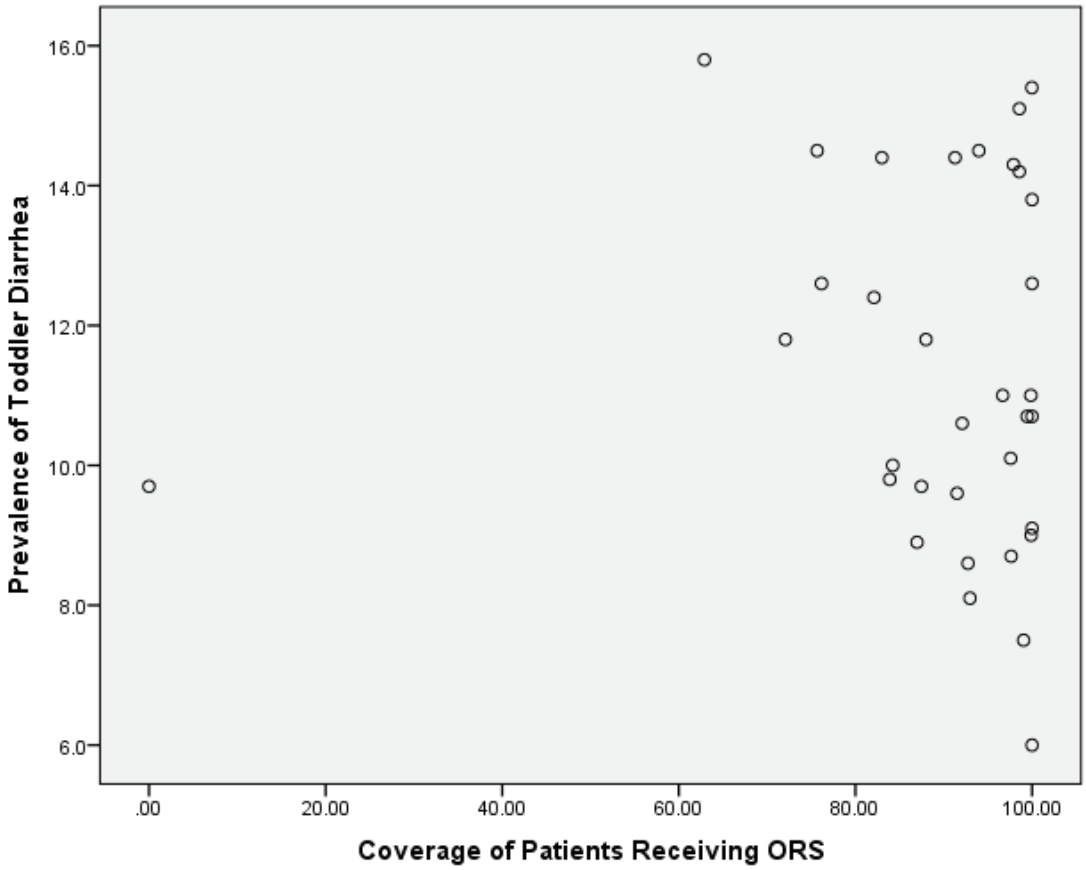

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

**Figure 2. Scatter Plot of Coverage of patients receiving zinc and Prevalence of Toddler Diarrhea in Province of Indonesia 2018.**

Based on Table 3, it can be seen that the prevalence of diarrhea is high, many occur in provinces with high zinc coverage, which is 46% compared to provinces with low zinc coverage. Figure 2 also shows that the lower the coverage of sufferers getting zinc the higher the prevalence of diarrhea in toddlers (r = -0.013).

**Table 4. Prevalence of toddler diarrhea according to the coverage of patients receiving ORS**

| Coverage of Patients Receiving ORS | Prevalence of toddler diarrhea | | | | Total | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | n | % | n | % | n | % |
| Low | 5 | 41.7 | 7 | 58.3 | 12 | 100.0 |
| High | 14 | 63.6 | 8 | 36.4 | 22 | 100.0 |




Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

**Figure 3. Scatter Plot Coverage of Patients Receiving ORS and Prevalence of Toddler Diarrhea in Province of Indonesia 2018.**

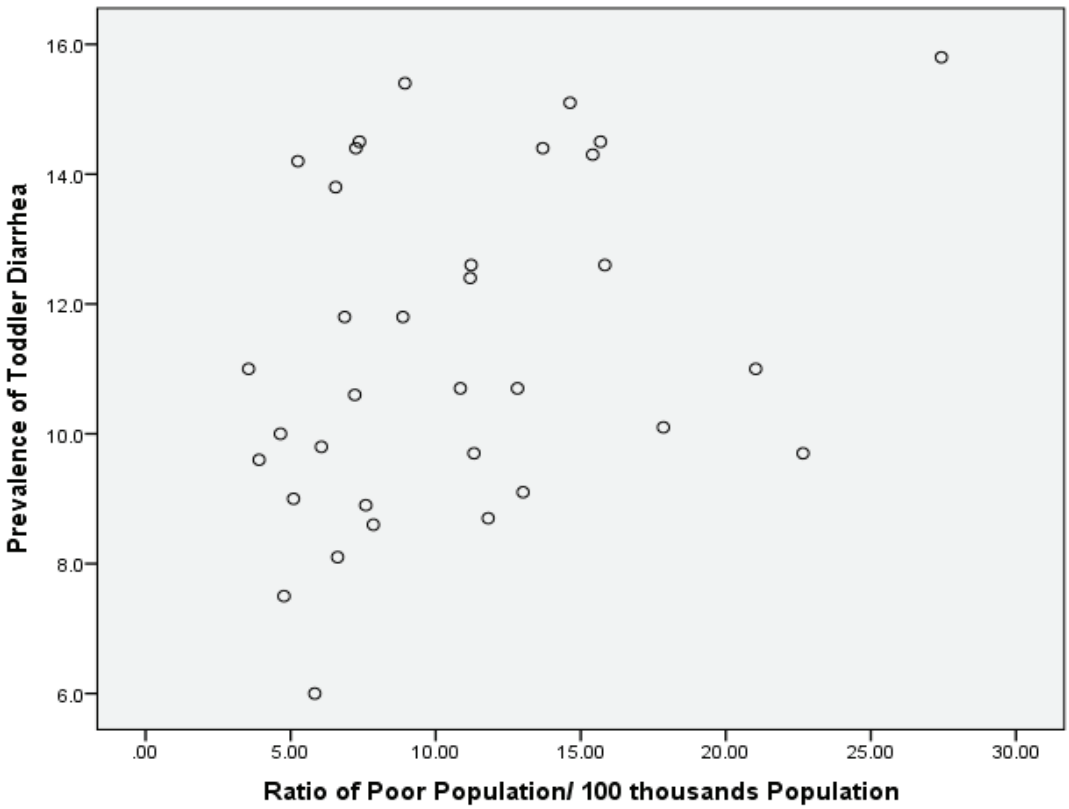
Based on Table 4, it can be seen that the prevalence of diarrhea is high, many occur in provinces with low coverage of oralit, which is 58.3% compared to provinces with low coverage of oralit (36.4%). Based on the results of the scatter plot it can be known that the lower the coverage of patients getting oralit the higher the prevalence of diarrhea of toddlers (r = -0.149) (Figure 3).

Based on Table 5, it can be seen that the prevalence of diarrhea is high, many occur in provinces with a high percentage of poor people, which is 50% compared to provinces with low service coverage. Based on the

results of the scatter plot it can be known that the higher the percentage of poor people in the province the higher the prevalence of diarrhea of toddlers with r = 0.363 (Figure 4).

**Table 5. Prevalence of toddler diarrhea according to the poor population**

| Poor Population | Prevalence of toddler diarrhea | | | | Total | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | n | % | n | % | n | % |
| Low | 11 | 61.1 | 7 | 38.9 | 18 | 100 |
| High | 8 | 50.0 | 8 | 50.0 | 16 | 100 |




Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

**Figure 4. Scatter Plot Poor population and Prevalence of Toddler Diarrhea in Province of Indonesia 2018.**

**Table 6. Prevalence of toddler diarrhea according to the availability of health workers**

| Availability of Health Workers | Prevalence of toddler diarrhea | | | | Total | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | n | % | n | % | n | % |
| Low | 9 | 43 | 12 | 57.1 | 21 | 100 |
| High | 10 | 77 | 3 | 23.1 | 13 | 100 |

Source: The 2018 Indonesia Basic Health Survey and The 2018 Indonesia Health Profile

Based on Table 6, it can be seen that the prevalence of diarrhea is high, many occur in provinces with a low number of workers per 100,000 inhabitants, which is 57.1%. Based on the results of the scatter plot it can be known that the lower the availability of health workers in the province the higher the prevalence of diarrhea of toddlers with r = -0.430 (Figure 5).

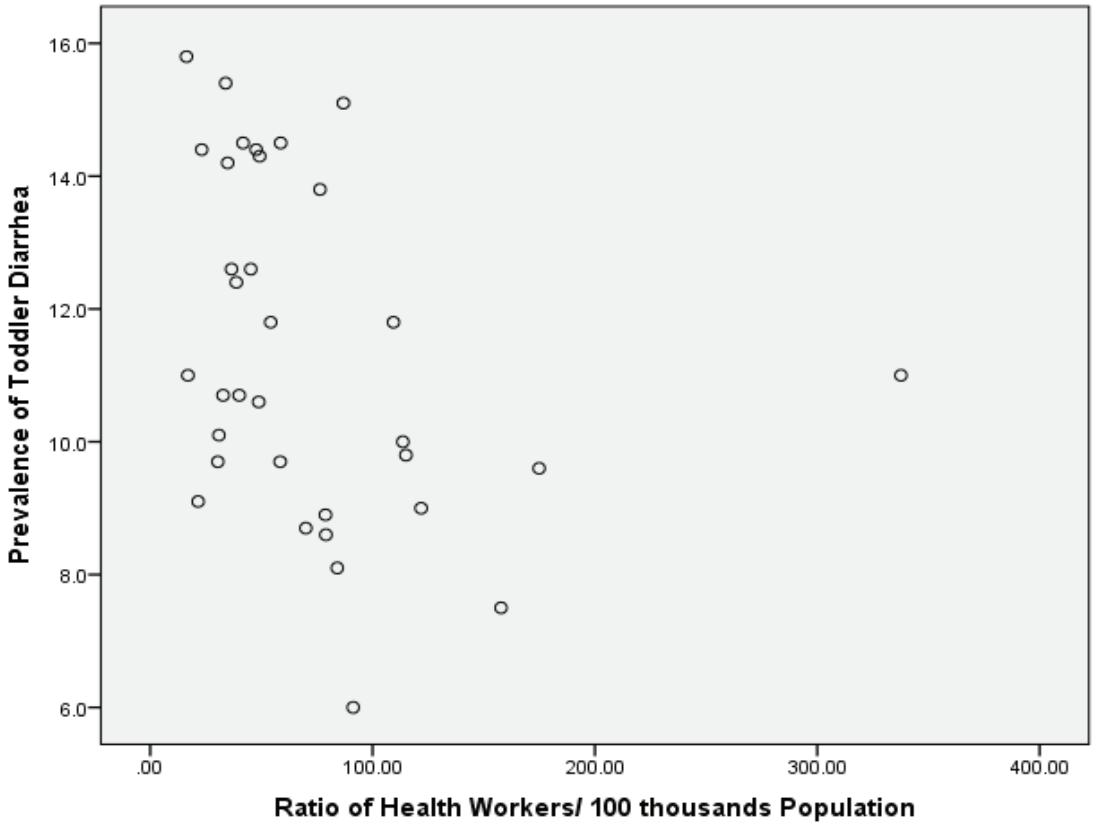



**Figure 5. Scatter Plot Availability of Health Workers and Prevalence of Toddler Diarrhea in Province of Indonesia 2018.**

## Discussion

The prevalence of diarrhea in toddlers in Indonesia increased from 2013 to 2018 by 6.7% to around 11%. This figure increases compared to the prevalence in 2013 which is around 6.7%. However, the prevalence rate of diarrhea in Indonesia is still relatively low when compared to Ethiopia which reached 21.5%[12]. In addition, research conducted by Gupta (2015) showed that the prevalence of diarrhea in India reached 22.36%. There were 57.69% cases of diarrhea in children in the 7-12 month age group, followed by 25.71% in the 13-24 months age group. With age, the prevalence of diarrhea gradually decreases[13]. Based on Demographic and Health Surveys (DHSs) at 2001-2011 in 51 low- and middle-income countries, it is known that the overall prevalence of diarrhea reaches 14.3%. The prevalence of diarrhea varies widely between countries ranging from 4.5% in the Maldives to 26.2% in Bolivia[14].

The average coverage of toddler diarrhea services in Indonesia in 2018 is 38%. This figure is very low compared to the coverage of toddler diarrhea services in Wolaitta and Soddo (Ethiopia) which is 95% and 86%[15]. However, the coverage of diarrhea health

services in Indonesia still meets the target of coverage of diarrhea services in Indonesia, which is more than 20%. Results showed the higher the coverage of diarrhea services of toddlers the higher the prevalence of diarrhea of toddlers. This happens because the more coverage of diarrhea services toddlers can detect the more diarrhea that toddlers experience. Not that the province needs to lower coverage to lower the prevalence of diarrhea in toddlers.

Receiving zinc during diarrhea is proven to reduce the length and severity of diarrhea, reduce the frequency of defecation, reduce the volume of stool, and decrease the recurrence of diarrhea incidence in the next 3 months[16]. Similarly, research by Restuti (2019) which showed a significant relationship between zinc intake level and the incidence of diarrhea, namely zinc intake level with less category can be at risk of 3,095 times diarrhea compared to subjects with sufficient zinc intake level[17].

The results of frequency distribution showed a high prevalence of diarrhea in infants in provinces with high zinc coverage. But the correlation value showed an inverse relationship between zinc administration coverage and the prevalence of infant diarrhea. This contradiction means that there was no meaningful relationship between zinc administration in toddlers suffering from diarrhea and the prevalence of diarrhea of toddlers, the correlation value also shows a very small number (r = -0.013). Another study conducted by Arnisam, et al (2013) showed similar results that there was no link between Zinc intake and the incidence of diarrhea in toddlers[18].

Oralit is a drug in the form of salt powder to be liquefied as a substitute for minerals and fluids that come out due to vomiting or crackling. Oralit is administered as an intervention against impaired balance of sodium and potassium concentrations due to dehydration[19]. Based on the results of the analysis, showed that in the prevalence of diarrhea of toddlers are high, many occur in provinces that have coverage of sufferers get low oralit but not much different compared to provinces that have coverage of patients getting high oralit. Prawati research, et al (2019) also showed that there is a link between taking oralit respondents and the incidence of diarrhea in the last 3 months[20]. In addition, research by Puji and Yuniar (2017) showed that there is an effect of oralit 200 on the consistency of feces and decreased frequency of defecation in infants with acute diarrhea of mild-moderate dehydration[21].

The results of the analysis showed that the higher the percentage of poor people, the higher the prevalence of diarrhea in the province. Previous research at Puskesmas Babakansari Bandung mentioned that there is a significant relationship between parental income and the incidence of diarrhea in toddlers[22]. The low family income makes individuals choose to use public latrines because of the lack of land to make latrines and the lack of funds to build family latrines that can affect individuals and family members[23]. Poverty is a factor in parents reducing capacity to support good health care for children, less likely to have good hygiene and poor education[24].

This study shows that the lower the availability of health workers, the higher the prevalence of diarrhea in a province. Previous studies have also stated that the more the number of medical personnel and paramedics, the more qualified the available personnel[25]. In line with research in West Sumatra, Indonesia shows where the better the support provided by posyandu will be lower morbidity of children to diarrheal infections[26]. However, in contrast to the research conducted in the West Alas Subdistrict, Indonesia which stated that there was no meaningful relationship between the role of health workers and the incidence of diarrhea of toddlers[27]. Health workers have an important role in the health service to improve the level of public health.

The study has limited recommendations for macro policy because the analysis used aggregate data at the provincial level. Therefore, further research is still needed to find out the factors that affect the incidence

of diarrhea in toddlers at the individual level both from the history of nutritional status of toddlers and parenting patterns. Future studies are expected to be used as the basis for more detailed policy decision making at the micro level.

## Conclusions

Based on the results of the study, it can be concluded that the highest prevalence of diarrhea in Indonesia in 2018 is in the Papua Province. However, the distribution of high and low prevalence of toddlers is fairly evenly distributed throughout the island in Indonesia. Among the factors seen cross-tabulation distribution shows that the availability of health resources in the province is the most related factor in the prevalence of diarrhea of toddlers. Efforts to increase the availability of health resources are expected to reduce cases of diarrhea in the province in Indonesia.

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**Conflict of Interest**: None declared.

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**Ethical Clearance**: This study was conducted by utilizing secondary data from published reports. For this reason, there is no need for ethical clearance in the implementation of this research.

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# The Role of Soluble HLA-G Serum Level in Therapeutic Response of Chronic Myeloid Leukemia Patients

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## Abstract

Human leukocyte antigen-G molecules (HLA-G) have been suggested to play a role in immune evasion and progression of different malignancies by their tolerogenic activity, through interaction with inhibitory receptors on surface of immune cells.

The aim is to evaluate the role of the s HLA-G serum level in the prognosis and therapeutic responses to TKIs in CML patients.

Serum level of soluble HLA-G was measured for a total of 61 adult patients with CML, who were on regular TKI for at least 6 months whom attended the out-patient's clinic of the Hematology Center in Basra, compared with 20 apparently healthy controls matched in ages and sexes to the patients using Enzyme Linked Immuno-Sorbent Assay (ELISA) technique.

Serum levels of HLA-G in CML patients was significantly higher than that in healthy controls (p=0.006). Elevated serum level of s HLA-G was significantly correlated with sex, BMI, duration of disease, Sokal scoring system.On the other hand, low s HLA-G serum level was significantly correlated with event free status (EF) of CML patients.

Lower level of serum soluble HLA-G in CML patients compared to healthy controls and it might be proved as a prognostic biomarker for CML patients.

***Keywords:*** *CML, soluble HLA-G, TKI, ELISA.*

## Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1-2 cases per 100 000 adults. It accounts for approximately 15% of newly diagnosed cases of leukemia in adults. CML is characterized by a balanced genetic translocation, t (9;22) (q34;q11.2), this translocation result in the generation of a BCR-ABL1 oncoprotein which has a tyrosine kinase activity. [(1)]

CML has a variable clinical course and prognosis. Therefore, the identification of prognostic factors to recognize risk groups of CML patients would be of major interest and one of the studied factors was HLA-G antigen. The expression of which was evaluated previously in different solid and hematologic tumors[(2,3)].

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Human leukocyte antigen G (HLA-G) is a nonclassical MHC class I antigen with very low polymorphism and limited expression in physiological conditions [4,5]. HLA-G exerts multiple immunoregulatory functions such as inhibition of natural killer (NK) cell or T-cell–mediated cytolysis, induction of T-cell apoptosis, or inhibition of trans endothelial NK cell migration.[6,7] Since the net result of these effects is immunosuppression, the ectopic HLA-G expression in tumor cells may favor their escape from antitumor immune responses, thus allowing tumor progression.[8]

All above high lighting the possible role of soluble HLA-G as a prognostic parameter in CML patients. So, we tried to investigate the impact of s HLA-G level on CML patients as it may serve as a possible marker for tumor sensitivity to chemotherapy and as a prognostic indicator for advanced disease and clinical outcome.

## Material and Methods

### Study population:

This case-control study was conducted at outpatient clinics in Hematology Consultation in Basra city during the period from October 2019 to the end of April 2020. Peripheral blood samples were collected from 61 patients aged ranges between (18-70) years, who were 32 males and 29 females diagnosed as CML, on regular use of TKI treatment for at least six months were included in this study.

Patients who were newly diagnosed, irregularly use the treatment, had history of transplantation, or viral infection were excluded from the study. All patients were assessed for hematologic response by doing complete blood count test (CBC), BMI was calculated for each patient, the most recent RT- PCR tests (within 3months) were recorded. Grouping of patients according torisk stratification scores using Sokal, Hasford or EUTOS scoring systems was done. Also, 20 apparently healthy subjects were included in the study.

### Blood sample collection and preparation

five mls of blood were collected from each subject under a septic technique. The blood samples were evacuated into gel tubes, centrifuged to get serum then the supernatant was carefully transferred to Eppendorf tubes and immediately frozen in aliquots at -20°C till use for the s HLA-G ELISA test.

### s HLA-G Enzyme-linked Immunosorbent Assay (ELISA)

s HLA-G serum level was measured according to the manufacturer's directions using s HLA-G ELISA kit (CUSABIO/ China).

## Statistical Analysis

Statistical Package for Social Science program (SPSS version 18, Chicago, IL, USA) was used for statistical analysis of the results. Chi. Square test, ANOVA test and t-test were used to determine the significance of the differences. Logistic regression analysis was done to detect association of s HLA-G level in CML patients with their variables. P value $\leq$ 0.05 was considered statistically significant.

## Results

In the present study the most frequent age group of CML patients was (40-49) years old which comprised (31.1%) of the patients, followed by the age group (50-59) years old and ( $\geq$ 60) years old which constituted (29.5% and 21.3 %) of the patients respectively. The number of males were 32 (52.5%) while females were 29 (47.5%) (table 1& fig. 1).

This study shows significant statistical difference in the serum level of s HLA-G between patients and controls with a mean ± SD of (4.85± 5.01) among cases compared to (1.60± 0.37) of healthy controls with (P= 0.006) (Fig. 2). However, serum level of s HLA-G increased with age although difference among age groups was statistically not significant (P=0.591) (Table 2).

As well as this work revealed a female sex predominance over male sex but the difference was statistically not significant (p= 0.373) (table 3).

On the other hand, elevated level of s HLA-G with a mean value (8.89±9.70) was detected among CML patients received first line treatment with nilotinib 150 mg without statistically significant difference compared to other groups (P=0.117).

Furthermore, no statistically significant differences in the level of s HLA-G were detected among optimum, warning and failure molecular responder group in the current study (p=0.532) (table 3). However, this study revealed that the serum level of s HLA-G in patients survive event free status was significantly lower than that in those who developed bad events (became accelerated) (p=0.012) (table 3)

On the other hand, a logistic regression analysis was done, where the dependent variable s HLA-G was significantly correlated with the five variables Sex, Duration of disease, BMI, Sokal score and Event development that were independent predictors which are significantly affected serum level of s HLA-G with p-values (0.017, 0.023, 0.024, 0.033 and 0.040) respectively. While age, type of treatment, CCI, Hasford, EUTOS scoring systems and MR were non significantly affected s HLA-G level as shown in Table 4.

**Table1: Comparison of cases and controls according to age groups**

| Age group | Patients | | Controls | | Total | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| < 30 | 6 | 9.8 | 1 | 5 | 7 | 8.6 |
| 30-39 | 5 | 8.2 | 4 | 20 | 9 | 11.1 |
| 40-49 | 19 | 31.1 | 9 | 45 | 28 | 34.6 |
| 50-59 | 18 | 29.5 | 5 | 25 | 23 | 28.4 |
| ≥ 60 | 13 | 21.3 | 1 | 5 | 14 | 17.3 |
| Total | 61 | 100 | 20 | 100 | 81 | 100 |

**Table 2: comparison of s HLA-G levels in different age groups of patients**

| Age groups | Mean ±SD of s HLA-G | No. of cases |
|---|---|---|
| <30 | 1.82± 0.449 | 6 |
| 30-39 | 4.97± 3.02 | 5 |
| 40-49 | 4.62 ±5.20 | 19 |
| 50-59 | 5.31± 5.89 | 18 |
| ≥ 60 | 5.91 ±5.49 | 13 |
| Total | 4.85 ±5.1 | 61 |

**Table 3: Serum level of s HLA-G among patients according to their different clinical and sociodemographic characteristics**

| Patient characteristics | No. | s HLA-G mean ±SD |
|---|---|---|
| Sex | | |
| Male | 29 | 4.234 ±4.493 |
| Female | 32 | 5.4084± 5.585 |
| Type of treatment | | |
| Imatinib 400 mg | 29 | 4.25± 4.11 |
| Nilotinib 200 mg | 26 | 4.59±4.47 |
| Nilotinib 150 mg | 6 | 8.89±9.70 |
| MR (molecular response) | | |
| Optimum | 36 | 5.408± 5.83 |
| Warning | 2 | 1.78 ±0.134 |
| Failure | 21 | 4.39 ±4.001 |
| Event | | |
| EF patients | 50 | 4.09 ± 4.596 |
| Patients had event | 11 | 8.3 ±5.987 |

**Table 4: Logistic regression analysis to predict value of s HLA-G**

| Variable | Regression coefficient | P value | CI | |
|---|---|---|---|---|
| | B | | Lower | Upper |
| Significant | | | | |
| Male Sex | -3.260 | 0.017 | 0.003 | 0.558 |
| Duration of disease | 0.047 | 0.023 | 0.917 | 0.994 |
| BMI | 0.270 | 0.024 | 1.036 | 1.655 |
| Sokal score | 3.234 | 0.033 | 1.301 | 494.734 |
| ED | 4.796 | 0.040 | 1.246 | 11749.966 |
| Insignificant | | | | |
| Age | -0.168 | 0.146 | 0.674 | 1.060 |
| Type of treatment | -0.762 | 0.320 | 0.104 | 2.098 |
| CCI | 2.409 | 0.307 | 0.109 | 1136.891 |
| Hasford | 0.847 | 0.504 | 0.194 | 28.023 |
| EUTOS | -2.023 | 0.053 | 0.017 | 1.029 |
| MR | -1.220 | 0.084 | 0.074 | 1.179 |

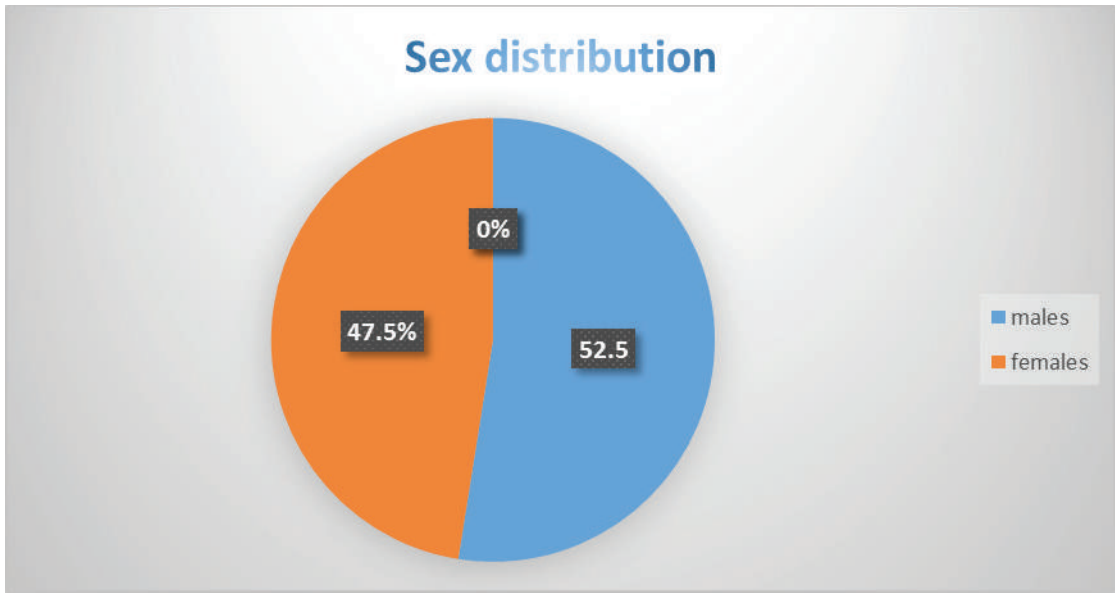


**Fig 1: Sex distribution of CML patients**

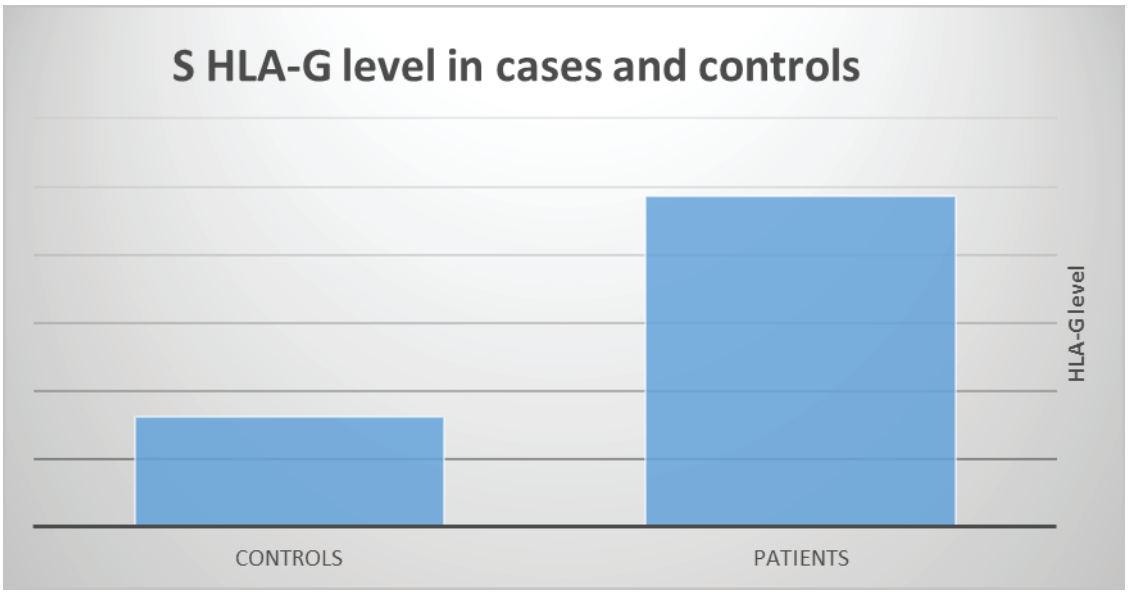


**Figure 2: S HLA-G level in cases and controls**

## Discussion

In current study the mean age of patients with CML was 48.7± 0.597 yearsthat is agreed with previous studiesreported that CML is detected in younger age population (median age 47 years) in developing countries than that in developed countries (median age 72 years) [9, 10].

On the other hand, the incidence of CML was higher in males than females which is comparable with previous literatures [2, 3, 10].

Data regarding s HLA-G secretion in CML are limited and controversial despite the relatively small number of patients, the current study is the second one in the world to assess the association between s HLA-G level and clinical outcome of CML patients.

We detected a statistically significant higher level of s HLA-G in CML patients compared to controls. This result is compatible with previous studies on hematological and solid malignancies [13,14,15,16]. So, the elevated s HLA-G level in CML group is

consistent with speculations on type of secreting cells.

The univariant analysis of the association between s HLA-G level and different clinical parameters revealed absent significant relation of s HLA-G level with age and sex, whereas in multiple regression test a significant correlation was detected between sex and s HLA-G level of CML patients. Similarly, Zidi et al (2014) reported absence of association between s HLA-G level and age or sex of patients, but Calini et al (2013) reported a significant correlation [17, 18].

Furthermore, the highest mean value of s HLA-G level was detected in obese patient group and in multiple regression test a significant association between s HLA-G level and BMI was reported. The effect of obesity on s HLA-G level may be hormonally directed [19]. This result is agreed with previous studies done by Solini et al (2010) and beneventi et al (2016) [20, 21].

Regarding the type of treatment, we noticed that the highest level of s HLA-G was among patients using nilotinib (150 mg) which is higher than that among patients using imatinib (400 mg) and nilotinib (200mg) but we cannot depend on such result that's may be related to the small number of patients using nilotinib (150 mg).

In the current study, different levels of s HLA-G were detected among different scoring system groups and the most accepted one was that seen among low-risk group of Sokal scoring system where the lowest s HLA-G level was seen in single test and in multiple regression test it was significant association.

Furthermore, patients with higher s HLA-G level were seem to have significant lower event free survival in both single and multiple regression test. Also, a negative correlation was found between s HLA-G level and molecular response (MR) in multiple regression test, but not in univariant analysis that is agreed with Cocci et al (2017) [22].

## Conclusion

The higher level of s HLA-G may play a role in suppression of immune response to CML cancer cells, while its lower level may account for the event free survival of CML patients and their benefit of TKI program discontinuation.

**Declarations**: Conflicts of interest related to the study.

**Source of Funding:**Nil

**Ethical Clearance:** This research has exemption as it aroutine treatment (none materials were used).

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# Different Patterns and Distribution of Skull Fractures in Road Traffic Accidents

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## Abstract

**Background:** The head is a common site of trauma in road accidents, and despite safety initiatives, the mortality rate for head injuries has not decreased. Despite the existence of a mandatory helmet law, both mortality and morbidity rates are on the rise. Head injuries are also associated with injuries to the neck, spine, stomach, abdomen, and pelvic cavity.

**Objectives:** To study different patterns and distribution of skull fractures in different kinds of road accidents.

**Methods:** In the Department of Forensic Medicine at Osmania General Hospital 4,213 post-mortem Examinations were carried out during the period ( August 2019 to January 2021. In 784 cases, death was attributed to Road Traffic Accidents. Head injury was present in 634 cases. In 471 cases out of 784, skull fractures were found. The data has been collected from PME reports and inquest reports, relatives and friends of the deceased

**Results:** Most frequently noted type of fracture is fissured in 59.8% of the cases. Injuries in RTA's are almost always due to blunt force on cranial vault. Highest number of fractures are seen in temporal 100 (21%), Fractures occurring in cranial vault alone is 336(71.3%), BOS alone is 28(5.94%). Fractures extending into BOS are 308(65.3%). In the base of skull, overall highest frequency of fracture is found in MCF (alone in BOS) with 109 (23.1%), then in PCF (alone in BOS) is 71 (15%), least in ACF 39(8.28%). Only fracture MCF in all over skull is seen in 11(2.33%) cases, PCF 11(2.33%) cases

**Conclusion:** Commonest fracture of skull in road traffic accidents is the fissured fracture in the cranial vault, mostly in temporal bone. Fractures of cranial vault most frequently extend to middle cranial fossa and posterior cranial fossa till foramen magnum which are responsible for immediate deaths. Victims who survived till hospital admission are almost half in number. Strict enforcement of road safety regulations and improving emergency medical services may prevent untimely deaths and disabilities caused by RTAs.

***Keyeords:*** *Road Traffic Accidents, RTA, Base of Skull, BOS, MCF, ACF, PCF*

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## Introduction

The skull is an important part of the body because it protects the brain from external hazards. The skull prevents our brain from getting damaged every single

time we bump our heads. Thanks to the thickness of the bones of the skull and the skull's round shape, our brain is well protected. However, some times the skull will receive such a blow that it will break, just like the shell of an egg may crack, so the human skull. The head and brain are most vulnerable to injury in a variety of situations. A head injury is any injury that results in trauma to the skull or brain. The study of skull fractures is important as head being the most exposed and prominent part of the body; it becomes most susceptible to injuries, most commonly in road traffic accidents.

The history of trauma parallels the history of the evolution of man. Injury to brain without fracture of skull is not uncommon, though fracture of skull is usually accompanied by some degree of injury to the brain[1]. It has been truly said by Polson, that no injury to the head is too trivial to be ignored or so serious as to be despaired of.[1,2] Now – a- days cases of head injuries in road traffic accidents are increasing at an alarming rate in world communities, especially more densely populated areas with fast and heavy traffic flow along with rapid growth of industrialization. Hyderabad occupies the top position in highest vehicular density in India. Head injury is one of the leading causes of death and more than half of these deaths are as a result of road traffic accidents.

Expansion in road network, motorization and urbanization in the country has been accompanied by a rise in road accidents resulting in increased fatalities, disabilities and hospitalizations, mostly by head injuries with severe socio-economic costs across the country[1].

Poor road conditions such as missing guard rails, erosion, pot holes and faulty designs and dangerous conditions are significant contributors of road traffic accidents. While driver errors such as speeding, using mobile phone while driving, driving under the influence of alcohol etc are also other major factors.

Road traffic accidents result in tremendous loss of lives and prolonged morbidity besides causing sufferings to relatives and friends. Loss of lives and useful working hours inflict heavy damage to nation's economic activity, loss of bread winner to family, and deprivation of family bond of love and security. Increasing traffic accidents and subsequent trauma creates heavy burden on our scarce funds and already overburdened hospitals. Road traffic accidents are one of the major causes of death and illness which is preventable. There is tremendous rise in RTAs due to increase in vehicular volumes on our roads, speeding of vehicles, poor driving skills, drunk-driving, bad roads, poor traffic controls and lack of public awareness, rampant indiscipline of road users in competent authorities and lack of implementation of existing laws to tackle the menace of disrespect to the laws and rules.

The main victims of RTAs are pedestrians, two wheeler riders, pillion riders and cyclists. The two wheeler riders in India rarely use protective and secure helmets. At times to escape the law, the riders merely use unsafe thin head gear. This group is called vulnerable road users.

As per the latest 2020 report last published on road accidents in India was for the year 2019, published by Transport Research Wing under Ministry of Road Transport and Highways, Government of India, the country recorded at least 4,49,002 accidents in 2019 leading to 1,51,113 deaths.[3]

## Materials and Methods

In the Department of Forensic Medicine at Osmania General Hospital 4,213 post-mortem Examinations were carried out during the period (August 2019 to January 2021. In 784 cases, death was attributed to Road Traffic Accidents. Head injury was present in 634 cases. In 471 cases out of 784, skull fractures were found.

The data has been collected from PME reports and inquest reports, relatives and friends of the deceased during the period of August 2019 to January 2021

**Inclusion criteria :** All autopsy cases of road traffic accidents with a finding of skull fracture.

**Exclusion criteria :** All autopsy cases of road traffic accidents without skull fracture.

**Place of study :** Department of Forensic Medicine and Toxiclogy, Mortuary, Osmania General Hospital, Afzalgunj, Hyderabad.

**Duration of study :** 1.5 year ( August 2019 to January 2021) (Since lockdown affected and extended the duration of our study)

**Statistical Analysis:** SPSS software version 22 has been used for statistical analysis. Data were presented as statistical tables and charts.

## Results and Discussion

The study sample included 471 autopsy cases in one year duration. After analyzing inquest, PME reports of all the 471 cases with skull fractures in deaths due to road traffic accidents the results and discussion are described and depicted below.

The frequency distribution of all the variables of the study is depicted in tables here under.

GENDER and AGE WISE DISTRIBUTION

**Table 1. Gender and Age group wise distribution of victims.**

| Gender | Frequency |
|---|---|
| Males | 397 |
| Females | 74 |
| Age Group | |
| 0 to 10 | 14 |
| 11 to 20 | 39 |
| 21 to 30 | 119 |
| 31 to 40 | 103 |
| 41 to 50 | 90 |
| 51 to 60 | 55 |
| 61 to 70 | 42 |
| 71 to 80 | 8 |
| 81 to 90 | 1 |

Incidence of skull fractures is 397(84.3%) in males and 74 (15.7%)in females.

The M : F ratio is 5.36:1.

Majority of the cases were in the age group of 21 to 30 yrs with 119(25.2 %) cases and 31 to 40 yrs age group with 103(21%) in 31 to 40 yrs of age group, 90(19%) in 41 to 50 yrs of age group, 55(11.6 %) in 51 to 60 yrs of age group, 42(8.9%) in 61 to 70 yrs of age group, 8(1.69%) in 71 to 80 yrs of age group, 1(0.2%) in 81 to 90 yrs of age group.

**Table 2: Distribution of different types of fractures single bone fracture**

| Fracture | Frequency |
|---|---|
| Fissured | 282 |
| Depressed | 20 |
| Sutural | 8 |
| Comminuted | 42 |
| Fissured, Depressed | 14 |
| Depressed, Comminuted | 10 |
| Fissured, Sutural | 29 |
| Sutural, Comminuted | 1 |
| Compound, Comminuted | 34 |
| Mosaic | 9 |

Most frequently noted type of fracture is fissured in 282 (59.8%). Depressed fracture is found in 20 (8%), Comminuted fracture in 42 (8.9%), Sutural in 8 (1.6%).

***Cont...*** **Table 3: Distribution of single bone fractures and Frequency of different bones fractured**

| Single bone fractures in vault | Frequency |
|---|---|
| Frontal | 19 |
| Temporal | 100 |
| Parietal | 9 |
| Occipital | 56 |
| Orbit | 3 |
| SKULL BONE FRACTURE | |
| All facial bones | 3 |
| All cranial bones | 5 |

***Cont...*** **Table 3: Distribution of single bone fractures and Frequency of different bones fractured**

| | |
|---|---|
| All skull bones | 52 |
| Frontal Alone | 19 |
| Frontal + Others | 62 |
| Temporal Alone | 100 |
| Temporal + Others | 116 |
| Parietal Alone | 9 |
| Parietal + Others | 81 |
| Occipital Alone | 56 |
| Occipital + Others | 61 |
| Orbital Alone | 3 |
| Orbital + Others | 13 |
| Nasal Alone | 1 |
| Nasal+ Others | 12 |
| Zygoma | 2 |
| Zygoma + Others | 10 |

Injuries in RTA's are almost always due to blunt force on cranial vault. Highest number of fractures are seen in temporal 100 (21%), then in occipital 56 (11.9%), frontal 19 (4%), least single bone fracture is seen in 9 (1.9%) cases.

**Table 4: Frequency of Fractures of BOS, frequency of involvement of BOS, Frequency in Vault and BOS.**

| Fractures of base of the skull | Frequency |
|---|---|
| ACF | 39 |
| ACF+ | 29 |
| MCF | 109 |
| MCF+ | 22 |
| PCF | 71 |
| PCF+ | 24 |
| Entire | 66 |

***Cont...*** **Table 4: Frequency of Fractures of BOS, frequency of involvement of BOS, Frequency in Vault and BOS.**

| **Involvement of base of skull** | |
|---|---|
| Extension into BOS | 308 |
| No BOS Involvement | 135 |
| BOS Alone | 28 |
| **Frequency In Vault And Base** | |
| Cranial Vault alone | 336 |
| BOS Alone | 28 |

Fractures occurring in cranial vault alone is 336(71.3%), BOS alone is 28(5.94%). Fractures extending into BOS are 308(65.3%). In the base of skull, overall highest frequency of fracture is found in MCF (alone in BOS) with 109 (23.1%), then in PCF (alone in BOS) is 71 (15%), least in ACF 39(8.28%). Only fracture MCF in all over skull is seen in 11(2.33%) cases, PCF 11(2.33%) cases

**Table 5: Frequency in different survival intervals**

| **Survival Interval** | **Frequency** |
|---|---|
| Brought Dead | 40 |
| Hospital Death | 246 |
| Spot Dead | 185 |

246 (52.2%) cases survived for a duration in hospital and died in hospital, 185(39.2%) cases died on the spot, 40(8.5%) cases died on the way to hospital.

## Discussion

Road traffic injuries are a major public safety issue. These not only result in death, but also in disabilities among survivors, who can become a burden to the society. India, as a developing nation, is undergoing a demographic, epidemiological, and economic transition that has had a significant impact on health outcomes. This change led to the rise of non-communicable disorders, such as road traffic accidents resulting in serious injuries, as a major health-care problem. Since road traffic collisions are a leading cause of morbidity and mortality.

Males are predominant as they are head of a family and go out for earning livelihood, whereas most females takes care of house hold chores. This finding matches with a similar study.[4] When compared to another study[5] female victims has an increase in number, this may be due to more number of females going out for earning livelihood supporting the family.

According to WHO estimates, The leading cause of death for children and young adults aged 5-29 years

are road traffic injuries account for 73% of global road traffic deaths and Males are about three times more likely than young females to be injured in a road traffic accident.[6]

Highest number of victims are seen in age group of 21 to 30 yrs, then 31 to 40 yrs of age. The simple explanation is that they are part of the mainstream working community and therefore are more vulnerable. This finding is in coherence with other studies.[7,8,9]

In a study done by Kanchan et al[10] in Manipal, 89.8% of the victims was 38.7 years. In another study done by Goyal et al[11] in Jaipur, maximum number of cases was in the age group of 21 – 40 years and 87.1% of the 69 victims were males. Janine jagger et al[12] in a study found that maximum occurrence of head injury occurred in the age group of 20-29 years. The age and gender distribution in our study match well with that of all the other studies

Most frequently noted type of fracture is fissured in 282 (59.8%). Depressed fracture is found in 20 (8%), Comminuted fracture in 42 (8.9%), Sutural in 8 (1.6%).

Maximum frequency is of fissured type of fracture. This finding matches with other studies[13,14,15] not coherent with another study.[8]

Temporal bone is the bone that is fractured the most, individually in 100 cases, with extension to surrounding in only 16 cases, while parietal is fractured separately in only 9 cases, as an extension from surrounding bones(temporal or frontal or occipital) in 72 cases mostly from temporal(48 of 72) , the reason being for it may be due to anatomical location of these bones, thickness of bones[16-19]. Temporal bone (squamous portion) is present at sides of the skull (lateral cranial vault), parietal on top of skull, this anatomical presence makes the temporal bone most vulnerable, parietal the least. In RTA'S the victim may get hit by a vehicle or fall on the ground as a primary impact, but this scenario is most likely when the victim is either a pedestrian or two wheeler rider/pillion rider wherein he would fall on ground and vulnerable anatomical areas of skull come in contact with ground, and the likely regions i.e, the frontal, temporal or occipital rather than parietal get affected and then lead to casualty. Most frequently fractured bone is temporal, this finding is in coherence with other studies,[8,20] doesn't match with another study.[21]

Fractures occurring in cranial vault alone is 336(71.3%), BOS alone is 28(5.94%). Fractures extending into BOS are 308(65.3%). In the base of skull, overall highest frequency of fracture is found in MCF (alone in BOS) with 109 (23.1%), then in PCF (alone in BOS) is 71 (15%), least in ACF 39(8.28%). Only fracture MCF in all over skull is seen in 11(2.33%) cases, PCF 11(2.33%) cases – this might be as per reference.[7] Highest frequency is of cranial vault, this finding is similar to other study.[22]

This matches the findings of Jacobsen et al.[23] in Copenhagen, who found that linear fractures were the most frequent, accompanied by comminuted, depressed, ring, and spider web fractures. Linear fractures were the most frequent in the Jaipur study by Goyal et al,[11] followed by depressed and then comminuted fractures. Frontal and temporal fractures were much more frequent than parietal and occipital fractures when it came to the most common sites of skull fractures. This is due to the fact that most road traffic collisions involve the fronto-temporal region, which is more vulnerable to injuries than the parieto-occipital region.

There was a mix of fractures of the vault of the skull, intracranial hemorrhages, and fracture of the base of the skull in the majority of fatal head injury cases. This is due to the fact that fracture begins at the point of greatest contact and radiates downward to the base of the skull.

Devadiga and Jain et al[24] studied at 20 cases of fatal head injuries and found that 12 of them had fractures of both the vault and base of the skull. Six of the remaining cases had a base of the skull fracture

and eight had a vault of the skull fracture.

In a study by Davidson et al who discovered a combination of vault and base of the skull fractures.[25]

The current study's results are in accordance with the previous findings.

The time it takes for patients to be transported to the closest help station has an impact on their chances of surviving a head injury. The role of pre-hospital and in-hospital treatment in determining survival time is critical. The significant variation between the rate of mortality within 12 hours and instantaneous death may be due to the nature of rapid post-trauma care, the patient's physical ability to survive injury, and any pre-existing or related diseases and injuries.

According to Simon Sivett, 16% of RTA victims died on the spot, 44 % within 24 hours, 28% within 6 hours, 9 % within an hour, and 16 % between 6 and 24 hours.[26]

## Conclusion

Commonest fracture of skull in road traffic accidents is the fissured fracture in the cranial vault, mostly in temporal bone. Fractures of cranial vault most frequently extend to middle cranial fossa and posterior cranial fossa till foramen magnum which are responsible for immediate deaths. Victims who survived till hospital admission are almost half in number.

Strict enforcement of road safety regulations and improving emergency medical services may prevent untimely deaths and disabilities caused by RTAs. Awareness campaigns concerning safety rules targeted at the high-risk groups and by improvement of the roads. The fact that the economically productive age-group were mostly involved an urgent public policy response with special reference to education, engineering, environment, and emergency care of road accident victims.

**Ethical Clearance:** Ethical clearance was obtained from the College institutional ethics committee of Osmania Medical College and General Hospital prior to the commencement of the study.

**Source of Funding:** Self

**Conflict of Interest:** Nil

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# Classification Study of Solid Medical Waste in Heet General Hospital

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## Abstract

Is rated about (10-25 %) Of these wastes as hazardous and can affect the public health environment and pollution in particular, the medical waste m n this hospital if it is not handled properly can cause health problems for health workers in the hospital and for the patients and the community. Medical waste consists of hazardous waste and non - hazardous wastes include waste and hazardous waste infectious , disease, drugs , sharp tools, chemicals, toxic waste genetic and radioactive either non - hazardous waste fats included garbage and general daily waste of residues of food , materials , office and other.

***Keywords:*** *Waste, medical, solid, Hit General Hospital*

## Introduction

The medical waste resulting from the diagnosis and treatment in health institutions of the important topics due to the danger to public health and the environment as a not managed well and that the presence of non - integrated program leads to imbalance in the management. Is rated about (10-25 %) Of these wastes as hazardous and can affect the public health environment and pollution in particular, the medical waste m n this hospital if it is not handled properly can cause health problems for health workers in the hospital and for the patients and the community. Medical waste consists of hazardous waste and non - hazardous wastes include waste and hazardous waste infectious, disease, drugs, sharp tools, chemicals, toxic waste genetic and radioactive either non - hazardous waste fats included garbage and general daily waste of residues of food , materials, office and other. And the World Health Organization reports that 85% of hospital waste is non-hazardous. The remaining 10% of waste is infectious and 5% of waste is non-infectious but hazardous, thus about 15% to 35% of waste for hospitals is infectious waste.

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## Literature Review

### 1. Types of medical waste

**Hazardous medical waste:** It is the waste that is produced from polluted sources or the possibility of contamination with chemical, infectious or radioactive factors, which constitutes the least percentage of the total health care waste (20%). As for the types of hazardous medical waste

**a. Infectious waste materials:** are all material to be blunt disposed of after patients contaminated with blood or body fluids take care of the patient , such as blood, sputum, saliva, spinal fluid sample cord .

**b. Remnants of pathology:** Remnants of human tissues, remains of human or animal organs,

remnants of surgical operations from blood and body fluids excised, and parts rose in surgical operations sent for tissue transplantation**.**

**Chemical residues:** all liquid chemical residues used in health centers, such as disinfectants and sterilization materials used to clean wounds and surgical devices.

**Non-hazardous medical waste:** It can be defined as ordinary waste that does not contain infectious, hazardous chemical or radioactive waste. It constitutes the bulk of the total medical waste, up to about (80%), and this type is treated like household waste such as kitchen waste, laundries, administrative offices , covers and furniture waste if Mixed with some hazardous medical waste (radioactive materials, needles, blood) treated as hazardous wast

**Table (1) the approximate proportions and quantities of each type of medical waste:**

| **non-infectious waste** | **80%** |
|---|---|
| Pathological waste and infectious waste | 15th% |
| sharp waste | 1% |
| chemical or pharmaceutical waste | 3% |
| Pressurized cylinders, broken thermometers | less than 1% |

Used bags and special containers with fixed colors indicate the nature of the waste inside them, according to the following colors:

General waste ● black or ● blue

Waste metal ● yellow or ● red

Waste contaminated with cytotoxic substances ● orange

Chemical waste ● airtight iron containers

Waste contaminated with radioactive materials ● yellow

Medicines and medical consumables ● yellow

Sharps ● Custom container ● Yellow or ● Red.

**WHO classification and characterization of medical waste**

**Table (2) WHO classification and characterization of medical waste**

| T | waste type | Description |
|---|---|---|
| 1 | Ordinary | Waste similar to household waste such as food scraps and metal cans Plastic and paper |
| 2 | infectious | Waste containing germs such as bandages, bedding, patient clothes, doctors' clothes used in surgical operations, gauze and paws |
| 3 | pathological | The patient's tissues, fluids, organs, and blood |
| 4 | Sharp or sharp | Needles, knives, surgical scissors, laboratory glassware |
| 5 | pharmaceutical | Medicines and expired drugs and the remains of their boxes and containers |
| 6 | Toxic to fetus | Substances capable of destroying human cells (cancer drugs) |
| 7 | chemical | Sterilization materials, laboratory and radiological solutions, and the like |
| 8 | Heavy metals container | Batteries and pressure devices (lead and mercury) |
| 9 | radiological | Radioactive materials from research laboratories, analyzes, and clothes of patients and therapists |
| 10 | pressurized containers | Oxygen cylinders and gas canisters, for example |

**Classification of solid medical waste according to its main components:**

This classification depends mainly on the main component of each type of solid medical waste, as stated by

· **Plastic:** bags, syringes, tubes, paws, etc.

· **Glass:** includes glassware, empty medicine vials, medicine or chemical containers , and glassware contaminated with the patient's body blood or waste.

· **Textile materials:** include cotton waste, gauze and laces (Bandj) and contaminated panels, etc.

· **Metals:** Needles, metal cans filled with drugs or chemicals, staples, scalpels, metallic surgical threads, etc.

· **Organic material:** includes all parts of the human body that are cut or amputated during surgery and includes the results of the birth process.

· **Paper and cardboard:** All paper waste includes medical examination forms, treatment papers, chemical boxes, medical devices, and all papers used in the management of hospital affairs.

· **Food leftovers: It** includes the leftovers of meals provided to patients and the leftovers of food produced during the process of cooking food in the kitchen.

has I showed a study at Peruvian that descent weight for ingredients aste medical as such follow :

15.6-37 % Paper

0.0-9.8 % carton

15.8-9.1% plastic

3.7%-0.0% Plaster

0.0-21.7% Parts removed

%14-4.0 glass

26.5 - 12.3% cotton

33.5-21.5% other %

**Sources of medical waste:**

Has ranked Resources Waste medical to me Resources main and sources high school as it came.

A / the main sources:

Hospitals, ambulance points, popular clinics, nursing homes for the elderly, centers, specialized clinics, private clinics and sanatoriums, emergency services, primary health care centers, maternity clinics, dialysis centers, blood banks, military medicine services, medical analysis laboratories, medical research centers, human medicine centers and research centers Animal laboratories and veterinary hospitals.

B/ Secondary Sources:

Doctors' offices used for periodic examinations, small dental clinics, psychiatric clinics, beauty centers, funeral services, acupuncture treatment, physiotherapy clinics and care for the disabled.

**Table (2) the color coding of medical waste according to the World Health Organization (Abu Mohsen , 2014).**

| waste type | Container color and tag | container type |
|---|---|---|
| highly infectious waste | Yellow with the words highly contagious | Durable leak-proof plastic bag or container that can be sterilized by autoclaving |
| Infectious waste and anatomical waste | yellow | Leak-proof plastic bag or container |
| Sharps | Yellow with the words Sharps | Puncture Resistant Container |
| radioactive waste | - | A pencil box marked with the symbol of radiation |
| Ordinary medical waste | black | Plastic bag |
| Chemical and pharmaceutical waste | brouwn | plastic bag or container |

## Methodology

**Materials and working methods:**

**Sorting, weighing and treating solid medical waste.**

The process of sorting solid medical waste was carried out in the places of its initial collection in the surgical wards, blood withdrawal centers, recovery rooms, surgeries, sections of the radiology unit, pharmacy unit, laboratory, dialysis unit, isolation rooms for people with corona and other departments in Hit General Hospital, each according to the type of waste that results from that section where Vzr each type of waste type of bags or special containers with this type if the acute waste containers cartoony collection against the hole either infectious waste is collected bags special either Pathological waste mostly are sent parts taken from patients to implant tissue after putting them solution (formalin) The pharmaceutical waste collected from stores if they expired shelf life and then landfilled especially large glass bottles either ointments and substances allowed entry for a waste treatment such as bottles of needles, grains and other of the intervention of the device after collecting them from their locations and when this collection of species of by workers hygiene are taken to room waste chopping (Almthermh) Steril Wave 250 Figure (3) as each of these types is weighed with a regular scale.

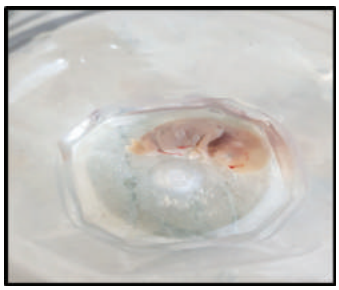

**Figure (1) Pathological medical waste**



**Figure (2) Sharp medical waste**



**Figure (3) shows the waste disposal device Steril wave 250**

## Results and Discussion

**Classification and weight of solid medical waste:**

It showed the results of the classification and the weight of solid medical waste in Hit General Hospital and for a period of one month from the date (2020/8/27 ) up to ‘2020/9/27) The results of the table showed (3) that the largest productive amount is medical waste infectious and was at a rate (7.5 - 22 kg) per day and the waste weight for one month (360.3 kg) while the medical waste weight sharp between (05/01 to 04/04 kg ) day one and it was the weight of the entire month (71.9 kg) , while the range of pharmaceutical and medical waste weight between (0 - 24.5 kg) per day per was the waste weight of the entire month (49.5 kg) of either Pathological medical waste weight ranged between (0 - 7.5 kg ) per day and the weight of the total during the month (31.7 kg), either the weight of the chemical and medical waste ranged between (0 - 2.5 kg) per day either total during the weight of the month was (16.1 kg) there are types of waste are not available due to lack of section The specialist for its production in Heet General Hospital, such as fetal toxic waste, waste with a high content of mercury, and compressed containers, the total total of medical waste of all kinds during a month was (529.9 kg). This result is close to what it came with Tuama, NH(2019)

The weight of waste in Al-Zahraa General Hospital for a month was (444 kg), because it is considered the largest hospital in the city of Kut and has a number of beds of 400 beds, and completely different from what Jawad Abdul Wahed Faydallah (2018) had, as the weight of solid medical waste was The result from Shendi Teaching Hospital (961.6 kg) within a month, and this is due to the large capacity of the hospital , as well as the population density in that area more. It is also considered a teaching hospital that differs from Heet General Hospital. The high production of infectious medical waste in Hit General Hospital is that most of the hospital departments produce this type of waste from patients' clothes, bed covers, and hallways, as well as surgical waste such as gauze, cotton, orthopedic waste, and any waste contaminated with blood or patients' fluids. These are considered infectious waste. The result is in agreement with several studies, including:

As shown in Figure (4) , as was the proportion of medical waste infectious (81%) in this study, the highest proportion of different types of medical waste and the t ratio (16%) of acute waste and (3%) of waste trochanter of .

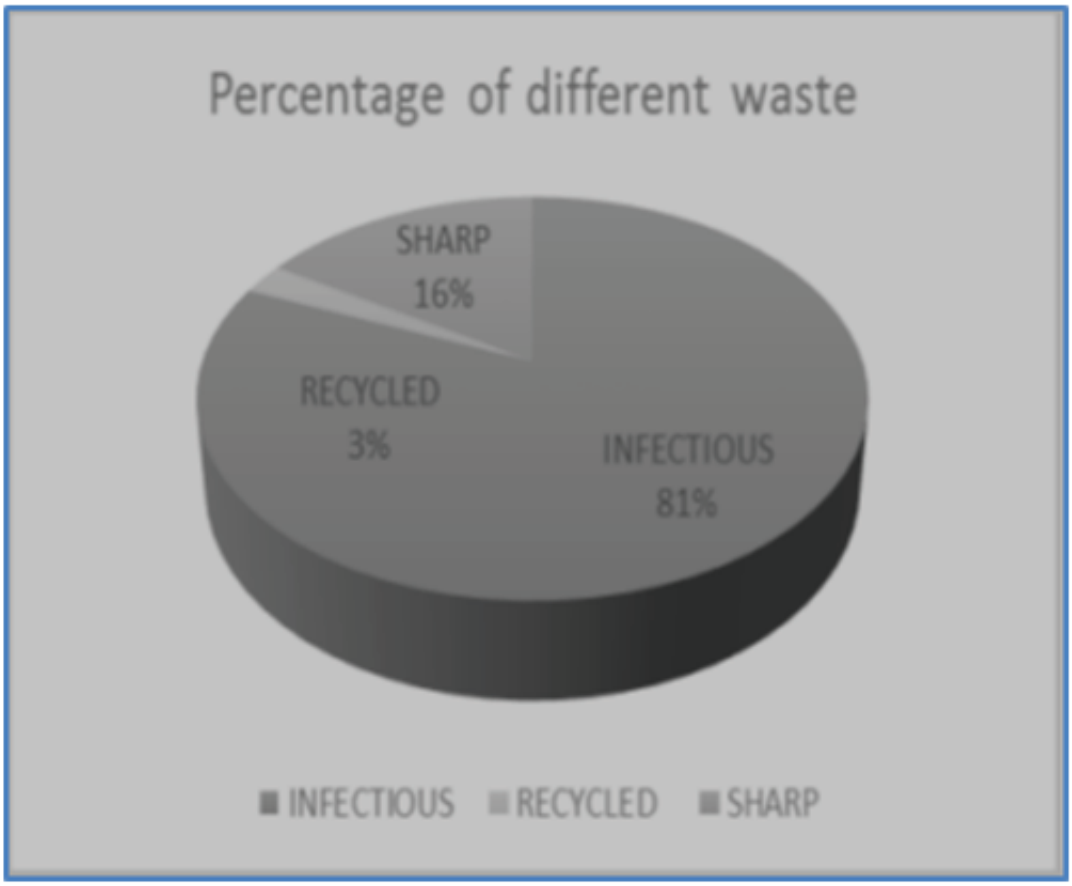


**Figure (4) Percentage of solid medical waste distribution according to its classification**

**Table (3) Weight and type of solid medical waste for a month**

| waste type | Weight / kg |
|---|---|
| sharp | 71.9 |
| infectious | 360 .3 |
| pharmaceutical | 49.5 |
| radioactive | 0 |
| pathology | 31.7 |
| toxic | 0 |
| chemical | 16 .1 |
| mercury | 0 |
| compressed | 0 |
| Total | 529.5 |

**Average waste collection between days of the week:**

Figure shows (4The average collection of solid medical waste by days, where there was a difference in the percentage of waste collection between days of the week, and this indicates a difference in the number of patients visiting Hit General Hospital according to the days of the week, as the average waste collection on Sunday, Tuesday and Thursday was by (19%) and saturday (12% ) and monday (16% ) and wednesday (15th%).

**Conflict of Interest** – Nil

**Source of Funding**- Self

**Ethical Clearance** – Not required

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# Postoperative Incidence of Iatrogenic Gallbladder Perforation During Laparoscopic Cholecystectomy in Sulaimaniyah Teaching Hospital

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## Abstract

**Background and Objective:** Iatrogenic perforation of the gallbladder has been reported in 28% of those patients who undergo laparoscopic cholecystectomy. It has been pointed out that gallbladder perforation can result in formation of gallstones and spillage of bile. The present study was carried out in order to investigate the postoperative incidence of iatrogenic gallbladder perforation during laparoscopic cholecystectomy in SulaimaniTeaching Hospital.

**Patients and Methods:** The present investigation was a single center, retrospective observational study that was carried out in Sulaimani Teaching Hospital in 2018-2019. The study sample included 99 patients who had undergone elective cholecystectomy. Required data on the possible risk factors and early outcomes and the patients' demographics including age, BMI, and gender were collected. The collected data were analyzed through the Statistical Package for the Social Sciences (version 22.0).

**Results:** The results revealed that 80% of the patients were females. Also, 42.4% had no chronic diseases, while diabetes mellitus (DM), DM along with hypertension (HT), and HT were found to be the most prevalent chronic diseases among them with 18.2%, 16.2%, and 10.1% of prevalence, respectively. Only 17.2% of the patients had abdominal operation before, and acute cholecystitis and chronic cholecystitis were seen respectivelyin 13% and 17% of them. The most common causes of gallbladder perforation (GP) were found to be electrocautery(16.2%) and grasper (7.1%). Moreover, 33.3% of the patients were overweight (BMI between 25 and 29.9), 45.5% were obese (BMI between 30 and 34.9), and 20.2% had BMI of over 35. Most of the patients aged 30 to 49 (62.6%). A majority of the patients (94.9%) were found to have gallstones.

**Conclusion:** Iatrogenic gallbladder perforation is prevalent among patients who undergo laparoscopic cholecystectomy. However, laparoscopic cholecystectomy is still a better choice and associated with fewer complications compared to open cholecystectomy. Required measures need to be adopted for patients with perforated gallbladder in order to minimize spillage and remove as much spilled gallbladder content as possible.

***Keywords:*** *iatrogenic gallbladder perforation, laparoscopic cholecystectomy, gallstone, spillage of bile*

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## Introduction

As a small hollow pear-shaped organ, the gallbladder receives,collects, and stores bile produced by the liver through the common hepatic duct. Later, it releases the bile into the duodenum in which the bile helps with the digestion of fats [1]. The gallbladder has a length of about 7 to 10 cm, can hold about 30 to 80 milliliters of fluid, and is located at the upper right quarter of the abdomen and in an indentation under the liver[2].

Benign gallbladder disease is mainly treated through laparoscopic cholecystectomy as the first treatment choice instead of open surgery [3]. However, surgeons have pointed out that this procedure is associated with a higher risk of injury than open cholecystectomy [4]. That is why cholecystectomy is the second most widely conducted abdominal operation in general surgery practice [5]. Iatrogenic perforation of the gallbladder (PGB) has been reported to be 28% prevalentduring laparoscopic cholecystectomy (LC). PGB can result in formation of gallstones and spillage of bile [6].According to Neimeir's classification, acute cholecystitis is a spontaneous preoperative gallbladder perforation, which is a rare life-threatening condition. .

The surgeon's judgment, skill, experience, and training and the quality of the instruments used for the operation can remarkably affect the outcome of laparoscopic cholecystectomy. Stopping bile leak during laparoscopic cholecystectomy and examining its source can have a great effect on the outcomes, which should be done by the surgeon[7].In this surgical procedure, a few small incisions are made on the right side of the abdomen. Then, one of the incisions is used to insert a laparoscope which shows the gallbladder on a screen. Afterwards, the gallbladder is removed through another small incision. Compared with open cholecystectomy, laparoscopic cholecystectomy is less invasive[8].

In comparison with conventional techniques, laparoscopic cholecystectomy has some advantages, including early return to daily activities, shorter hospital stay, lesser postoperative pain, and better cosmetic results. Moreover, laparoscopic cholecystectomy might be associated with some severe complications, such aspancreatitis, abscess, bleeding, and bile duct injury [9].The patients' health status, the surgeon's experience, and post-operative care can remarkably affect the complications of this treatment. However, this procedure is believed to result in fewer postoperative complications [10]. Elevated risk of GP has been reported to be correlated with a difficult operation, an inflamed or non-visualized gallbladder, the use of a laser, a history of acute cholecystitis or previous laparotomies, and male sex [11].

The present study was aimed at investigating the postoperative incidence of iatrogenic gallbladder perforation during laparoscopic cholecystectomy in SulaimaniTeaching Hospital.

## Patients and Methods

***Study design and setting:***The present single center, retrospective observational study was carried out in Sulaimani Teaching Hospital located in Sulaimani, Kurdistan-Iraq in 2018-2019.

***Study sample and sampling method:***The study sample consisted of 99 patients who underwent an elective cholecystectomyinSulaimani Teaching Hospital and were analyzed retrospectively. The inclusion criteria were all age groups with gallstone and all cases who underwent laparoscopic cholecystectomy not open cholecystectomy. The exclusion criteria were patients who underwent open cholecystectomy, cases who did not respond to our phone call, and gallbladder perforation that was not recorded in the operation note. The selected patients were assigned into two groups based on the presence of GP.

***Data collection:*** Required data on the possible risk factors and early outcomes were collected from the patients' profiles. Moreover, the patients' demographics including age, gender, and BMI was also gathered.

***Statistical analysis:*** The collected data were analyzed through the Statistical Package for the Social Sciences (version 22.0). For this purpose, descriptive statistics was employed, and the results were presented as frequencies and percentages in appropriate tables.

## Results

The results of the present study indicated that most of the patients (80.8%) were females, and 19.2% were males. The results also revealed that 42% of them did not have any chronic diseases, and the most frequent chronic diseases were respectively diabetes mellitus (DM) in 18.2% cases, DM and hypertension (HT) in 16.2%, and HT in 10.1%. It was observed that 17 patients (17.2%) had abdominal operations. Moreover, chronic and acute cholecystitis was seen in 17 and 13 patients, respectively (See **Table 1**).

**Table 1. The patients' gender and other variables**

| | Frequency (N) | Percentage (%) |
|---|---|---|
| Gender | | |
| Male | 19 | 19.2 |
| Female | 80 | 80.8 |
| Total | 99 | 100.0 |
| Chronic diseases | | |
| Normal | 42 | 42.4 |
| DM | 18 | 18.2 |
| HT | 10 | 10.1 |
| Respirator | 1 | 1.0 |
| DM and HT | 16 | 16.2 |
| DM, HT and Valvular | 3 | 3.0 |
| DM and Respirator | 1 | 1.0 |
| HT and Valvular | 2 | 2.0 |
| HT and Respirator | 6 | 6.1 |
| Total | 99 | 100.0 |
| Abdominal operation | | |
| No | 82 | 82.8 |
| Yes | 17 | 17.2 |
| Total | 99 | 100.0 |
| Acute and chronic cholecystitis | | |
| Normal | 69 | 69.7 |
| Acute | 13 | 13.1 |
| Chronic | 17 | 17.2 |
| Total | 99 | 100.0 |

According to the results, endoscopic retrograde cholangiopancreatography (ERCP) was used for 5 patients (5.1%). A large number of the patients (72.7%) did not havegallbladder perforation (GP), while the causes of GP were electrocautery in 16% of the cases, grasper in 7.1%, clip in 2%, and removal in 2%. In terms of BMI, 65.7% of the patients were obese with a BMI ranging from 30 to 39.9, and 33.3% were overweight with a BMI of 25 to 29.9. The patients' age ranged from 20 to 69 years, with 33.3% and 29.3% being 40-49 and 30-39 years, respectively (See **Table 2**).

**Table 2.The patients' age, BMI, and other variables**

| | Frequency (N) | Percentage (%) |
|---|---|---|
| **ERCP** | | |
| No | 94 | 94.9 |
| Yes | 5 | 5.1 |
| Total | 99 | 100.0 |
| **Causes of GP** | | |
| No GP | 72 | 72.7 |
| Electrocautery | 16 | 16.2 |
| Grasper | 7 | 7.1 |
| Clip | 2 | 2.0 |
| Removal | 2 | 2.0 |
| Total | 99 | 100.0 |
| **BMI** | | |
| 18.5 - 24.9 Normal weight | 1 | 1.0 |
| 25 - 29.9 Overweight | 33 | 33.3 |
| 30 - 34.9 Obesity class 1 | 45 | 45.5 |
| 35 - 39.9 Obesity class 2 | 20 | 20.2 |
| Total | 99 | 100.0 |
| **Age group** | | |
| 20 - 29 | 13 | 13.1 |
| 30 - 39 | 29 | 29.3 |
| 40 - 49 | 33 | 33.3 |
| 50 - 59 | 13 | 13.1 |
| 60 - 69 | 11 | 11.1 |
| Total | 99 | 100.0 |

The ultrasound findings demonstrated that 94.9% of the patients had stone, 3% had polyp, and 2% had sludge (See **Figure 1**).

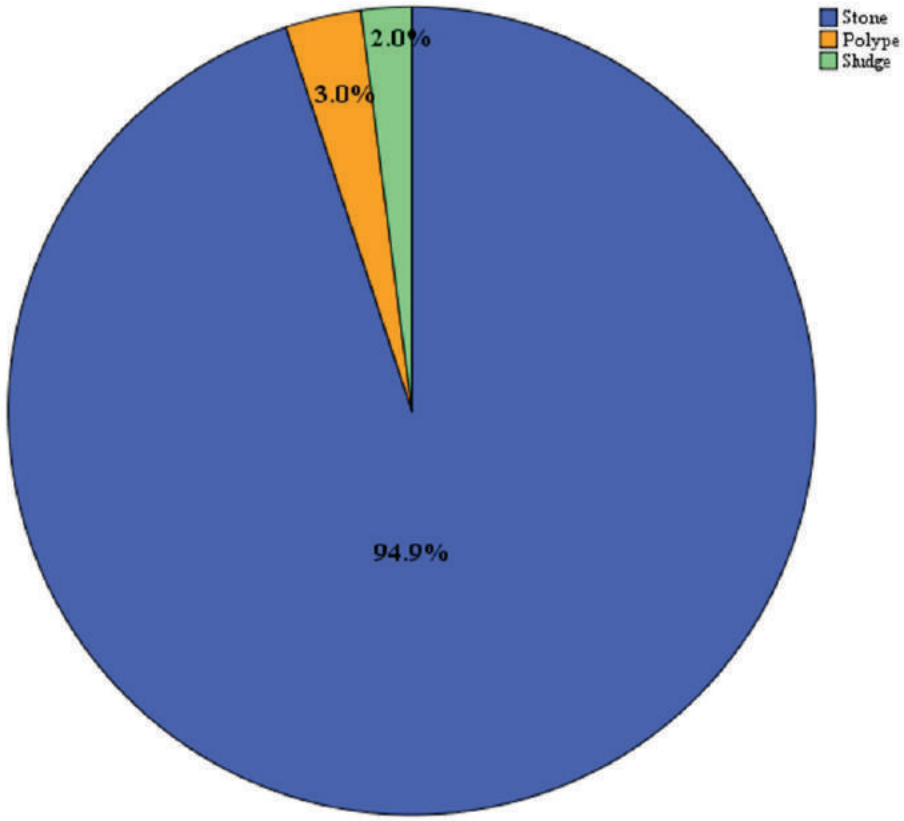


**Figure 1. Ultrasound findings**

According to the results, the patients' mean age was 42.10±11.46 years with a minimum of 20 and a maximum of 69 years. Their mean weight was 87.28±9.83 kg with a minimum and a maximum weight of respectively 68 and 110 kg. Their mean height was 166.36±4.97 cm with a minimum and maximum height of respectively 158 and 178 cm. Their mean BMI was 31.56±3.50 with a minimum and a maximum BMI of respectively 22.98 and 38.46 (See **Table 3**).

**Table 3.Mean and standard deviation of the patients' age, weight, height, and BMI**

| | **Age (years)** | **Weight (kg)** | **Height (cm)** | **BMI** |
|---|---|---|---|---|
| Mean ± SD | 42.10± 11.46 | 87.28 ± 9.83 | 166.36 ± 4.97 | 31.56 ± 3.50 |
| Min - Max | 20- 69 | 68 - 110 | 158 - 178 | 22.98 - 38.46 |

## Discussion

Laparoscopic cholecystectomy was introduced in 1985, and since then, it has widely been employed as a technique to treat gallbladder perforation (GP). Through this technique, complications associated with open surgery and the high cost of hospital stay can be tackled. In spited of being the first chosen treatment technique for GP, laparoscopic cholecystectomy can causes GP [12]. It is highly essential to classify and manage perforated cholecystitis appropriately. In addition, it has been shown that morbidity in gallbladder perforations can safely and feasibly be reduced through laparoscopic cholecystectomy [13].

As shown by the results of the present study more than four-fifths of the participants were females, and less than half of them did not have any chronic diseases. While, less than one-fifth of participants were detected with DM. In this regard, Whiting et al [14]demonstrated that diabetic patients are typically diagnosed with clinically silent gallbladder disorders which can lead tosudden catastrophic complications which need emergency surgery. In their study, they noticed a positive correlation between the duration of diabetes mellitus and the prevalence of gallbladder dysfunction and subsequent gallbladder diseases.

In their retrospective study, Gunasekaran et al[15] found that 18 patients with gallbladder perforation had comorbid diseases, among whom 12 patients had diabetes mellitus (DM), 4 hadischemic heart disease, and 2 had both DM and hypertension (HT). In this regard the findings of the present study showed that about 17% of the patients had abdominal operations, and chronic and acute cholecystitiswas seen in 17 and 13 patients, respectively.

Düzenli et al[16] showed that endoscopic retrograde cholangiopancreatography (ERCP) is a very useful technique in the detection of most biliary tract diseases. They also demonstrated that gallbladder and intrahepatic duct system diseases can be diagnosed through endoscopic selective biliary cannulationdespite its preponderance in extra hepatic biliary tract conditions. The results of the current study indicated that ERCP was used as a diagnostic method for about 5% of participants, revealing that over two-thirds of them did not suffer from GP. Also, electro cautery was the main cause of GP in 16% of the cases. In a similar study, Ahmad et al [17]showed that the harmonic scalpel can be used to safely and effectively dissect gall bladder and hemostasis in laparoscopic cholecystectomy. They also referred to electro cautery as an appropriate alternative for this purpose.

The results of this study demonstrated that patients with a higher BMI were at a higher risk of gallbladder perforation. A similar study by Enami et al [18]reported a higher risk of gallstones in adults with a higher BMI. Female gender and elevated BMI have been referred to as definitive risk factors for gallstone growth [19]. Elevated BMI has also been reported to cause symptomatic gallstone disease [20]. Obesity can have a remarkable effect on most pathogenic mechanisms of gallstone formation, includingdefective gallbladder emptying, stone aggregation, and supersaturation of bile with cholesterol increased propensity to cholesterol crystallization [21]. A remarkable increase in the risk of gallstones has been reported during rapid weight loss (>1.5 kg/week) [22, 23]. Similar to these studies, nearly two-thirds of the patients in the present study were obese and the other one-third were overweight. According to the results of some similar studies, high prevalence of obesity can lead to an elevated incidence of benign gallbladder conditions [24]. The early outcomes of open surgical procedures can be unfavorably affected by obesity and obesity-related comorbidities; however, this influence changes in case of utilizing laparoscopy [25, 26].

The results of the present study demonstrated that nearly 95% of patients were detected with stone through conducting ultrasound examinations. Similarly, in a study by Salih[10] in Iraq, it was shown that iatrogenic gallbladder perforation and spillage of gall stones are correlated, and this association might result in abdominal infections which in turn can cause a number of abdominal problems. Therefore, the probability of more abdominal infections and problems can rise as a result of the presence of any kind of stones. The results of another study conducted by Hanashe et al [27] in Iraq revealed that the risk of gallbladder perforation can drop by precisely detecting the presence of any kind of stones. They also stated that gallbladder perforation can result in gallstone spillage and, in many cases, an unsuccessful retrieval of the stones. Most of the spilled stones are clinically asymptomatic, but 0.04% to 19% of the cases experience adverse events. Intra-abdominal abscess formation has been reported as the most widespread complication when there is any kind of stones.

In this study, it was observed that the mean age of patients was 42 years with a maximum of 69 years.

Similarly, Hanashe et al [27]showed that significant risk factors that cause gallbladder perforation includethe patient's factor such as male gender, older age, and obesity and the surgeon's experience and the difficulty of the surgery (including palpable gallbladder preoperatively, pain >96 hours before surgery, adhesions in the right upper abdomen, and acute cholecystitis). Among these factors, the patient's age has been referred to as one of the most decisive factors that could affect gallbladder perforation. Similar findings were reported by Akmoosh et al [2]who carried out a study in Iraq and concluded that both old age and female gender are significant risk factors for development of gallbladder perforation. This finding is in agreement with those of the present study.

## Conclusion

One-third of the patients undergoing laparoscopic cholecystectomy experience iatrogenic gallbladder perforation.Laparoscopic cholecystectomy is not always the only good procedure to follow; however, itis associated with less pain and possible complications. It has been reported to be accompanied by various complications due to variety of factors including the patient's health status. Iatrogenic gallbladder perforation can be caused by factors like inflamed gallbladders,old ages, and overweight male with acute inflammation. Moreover, patients with a higher BMI were at a higher risk of gallbladder perforation. The incidence of benign gallbladder conditions increases as the prevalence of obesity soars. In spite of low incidence of iatrogenic gallbladder perforation during laparoscopic cholecystectomy, the morbidity associated with this complication can be serious. As a result, it is necessary to make sufficient attempt to prevent iatrogenic PGB intra-operatively. In case ofperforated gallbladder, measures need to be taken to minimize spillage and to remove as much spilled gallbladder content as possible.

**Conflict of interests:**None.

**Source of funding:**Self.

**Ethical clearance:**The protocol of the study was approved by the Research Protocol Ethics Committee of Kurdistan Board of Medical Specialties. Moreover, informed consent was obtained from the patients during our phone calls with them. In addition to these, required permission was obtained from the authorities of Sulaimani Teaching Hospital.

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# The Role of Biochemical Parameters in Prediction of Retinal Diseases and their Relationship to Cataract, Diabetes, and Hypertension, in Ibn Al Haytham Hospital, Baghdad, Iraq

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## Abstract

This work summarize multiple protective roles serum Zeaxanthin, and Malondialdehyde, in association with metabolic profiles and as a risk of retinal disorder disease with cataract, diabetes, and hypertension, totally thirty healthy control group, and seventy-five patients group for both genders were studied. Retinal disorder disease subdivided after clinical diagnosis, into three major -subgroups, the first cohort was twenty-five patients suffers of cataract, the second twenty-five of diabetic patients, while the third group is twenty-five hypertensive patients. Lipid-profile, Fasting-insulin-levels, serum zeaxanthin and malondialdehyde, have been done to all groups. Important findings presented in the roles of serum zeaxanthin, and malondialdehyde, by similarities and differences, in retinal diseases with cataract, diabetes, and hypertension. The activity levels of serum zeaxanthin in hypertensive retinopathy patients (32.80±30.56 ng/mL), was non-significantly ($P>0.01$) reduced compared to healthy control (88.85±139.31 ng/mL), in compression with the same patients for serum malondialdehyde MDA which expressed the highest level of MDA (2.456±2.149 μg/mL) among the rest groups, which was significantly higher ($P<0.05$) than that in control (0.783±0.937 μg/mL), but the differences were non-significant ($P>0.05$) compared to diabetic retinopathy group (1.839±1.515 μg/mL).

The activity levels are negatively associated with malondialdehyde levels in retinal disease patients with cataract, diabetes, and hypertension. Retinal disease patients with cataract, diabetes, and hypertension pathogenesis aren't legitimately ensured. But multiple protective roles may be adopted in clinical diagnosis of retina, also in response to higher levels of oxidative stress, including serum malondialdehyde, and zeaxanthin, by fasting at least eight hours pre sophisticated lens surgery.

***Key words:*** *Zeaxanthin, Malondialdehyde, Retinal disorder diseases, Cataract, Diabetes, Hypertension.*

## Introduction

The retina is the light-sensitive part of the eye and responsible for converting the focused image into nerve action potentials, which are then relayed to the brain via the visual pathway. There are many layers of nerve cells within the retina, which provide complex connections between the light-sensitive cells located toward the posterior parts of its surface [1]. Lens is external part of the eye, a bright, transparent focuses light on the retina dark part, and refract the

reminder. It is noteworthy that pacity of lens is a direct outcome of oxidative stress, lens cells undergoing oxidation, crosslinking, and insolubilization form lens fibers accumulate in the lens center that *fibrosis* are progressively compressed result in lens nuclear sclerosis leading to opacity. A plaque-like opacity grows in the axial posterior cortical layer in posterior subcapsular cataract. In most patients, over one type of cataract is found [2]. Under hyperglycemic conditions, part of the excess glucose reacts non-enzymatically with proteins or other tissue or blood constituents, leading to the formation of advanced glycation end products. Progressive accumulation of advanced glycation end products in the diabetic lens has been shown to contribute to the acceleration of cataractogenesis in hyperglycemic animals and diabetic humans [3]. The crystalline lens does not require insulin for glucose and other simple sugars to enter into the lens through the capsule. In the case of diabetes, high concentrations of glucose in the aqueous humor can passively diffuse into the lens. The lens aldose reductase enzyme converts excess glucose to sorbitol or galactose to galactitol. These sugar alcohols (polyols—sorbitol or galactitol) cannot passively diffuse out of the lens, and they accumulate inside the lens. The accumulation of polyols inside the lens results in an osmotic gradient, which facilitates diffusion of water from the aqueous humor to the crystalline lens. The water drags sodium with it, and the lens swelling and electrolyte imbalances result in lens fiber disruption and cataract formation. [4]

Photochemical insult, which is intraocular penetration of light and the consequent generation of reactive oxygen species, such as superoxide and singlet oxygen, and their derivatives, such as hydrogen peroxide and hydroxyl radical, induces damage to the epithelial cell deoxyribonucleic acid (DNA) of the lens, thus triggering a sequence of events leading to cataracts. Potential sources of oxidative stress to the lens include UV light, oxidants in the ocular fluids, endogenous oxidants produced in lens cells, and smoke constituents [4]. Diabetic retinopathy divided into two stages: nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) [5]. Non-proliferative diabetic retinopathy involves basement membrane thickening, pericyte loss, microaneurysms, intraretinal hemorrhages, cotton wool spots, hard exudates, venous beading, venous dilation, capillary acellularity, capillary non-perfusion, and intra-retinal microvascular abnormalities. Early on, capillary basement membrane thickening is seen with increased levels of collagen and laminin. Basement membrane thickening may affect capillary autoregulation as well as interactions with proteins and neighboring pericytes. Pericyte loss is hypothesized to be one of the initial pathologic alterations in DR, and their loss leads to altered microvascular autoregulation, a disrupted blood-retinal barrier, and proliferation of endothelial cells [6].

Proliferative diabetic retinopathy is the advanced type of diabetic retinopathy. When abnormal new blood vessels, (lacking mature endothelial cell tight junctions), began to grow on the surface of the retina and the optic nerve, it would result the onset of Proliferative diabetic retinopathy. If left untreated, proliferation of these vessels (termed retinal neovascularization) can lead to severe vision loss from vitreous hemorrhage and/or tractional retinal detachment [7].

While, for hypertensive retinopathy patients with higher levels of blood pressure, focal ischemic areas of the nerve fiber layer are clinically visible as “cotton wool spots”. Breakdown of the blood-retinal barrier causes secondary exudation of blood and lipid “hard exudates”, both of which are visible on clinical examination. At very high blood pressure levels there may be variable degrees of optic disc swelling [8].

Arachidonic acid is converted by cyclooxygenase, COX, lipoxygenase LOX, and and cytochrome P

CYP pathway into eicosanoids [9,10,11,12]. These lipid mediators can contribute considerably to oxidative stress, inflammation[10,13], and vascular function [14,15]. A type of functional, and bioactive lipid mediators known as Eicosanoids. They derived through long-chain polyunsaturated fatty acids metabolism, and mediated by three types of enzymes including cytochrome enzymes (CYPs), cyclooxygenases (COXs), and lipoxygenases (LOXs).

## Patients and Method

Patients are examined for retina disorder conditions by common Diagnostic A-Scan, Fluorescein angiography used to diagnose and monitor the impact of macular degeneration, diabetic retinopathy, and also for Fundus photos. Nerve fiber layer analyzer, and Visual field testhave been done by a ophthalomegalist according according to Amsler grid test, and Fundus Auto Fluorescence (FAF), doctor checked whether patients have the symptoms for retinal disorders diseases with each group of cataract, diabetes or hypertension patients. Biochemical Analysis done for 105 control and patients of both genders, at morning fasting at least for eight hours pre surgery gets started by artificial intraocular len for blood specimens and data were collected directly in Ibn Al Haytham Hospital, stored in dark place and directly analyzed, all the measured individual ages are between (16-65 year), 30 subjects are diagnosed as normal controls, and 25 subjects Cataract, 25 diabetic subjects, and 25 subjects having hypertension. The biochemical tests have been done are serum Zeaxanthin level was determined according to Stahl et al method [16], by HPLC technique. Malondialdehyde, by an enzyme-linked immunosorbent assay, also called ELISA using kit from My Bio Source, its a test that detects and measures blood antibodies. This test can be used to determine if you have antibodies related to certain infectious conditions. ELISA, Fasting Blood Sugar (FBS), insulin resistance test & glucose level we use Cobas c 111 Analyzer for estimation assay, blood pressure by Blood Pressure Monitor, and Blood Picture Film in Ibn Al Haytham Hospital Baghdad Iraq.

## Statistical Analysis

Statistical analysis have been done according to SPSS-27 (Statistical Packages of Social Sciences-version 26). The data are simple measurement for frequency, percentage, mean, standard deviation SD, the range (minimum – maximum values). The difference significance of different means (quantitative data) has been tested by Students-t-test for difference between two independent means, or ANOVA test, for difference among more than two independent means. Difference significance of different percentages (qualitative data) were tested using Pearson Chi-square test ($c^2$-test) with application for Yate's correction or Fisher Exact test if applicable. Statistical significance was considered by P value equal or less than 0.05. Pearson correlation was calculated between two quantitative variables with its t-test for testing significance for correlation.The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. Also, to correlation the $r^2$ was calculated (The coefficient of determination), i.e., when value of r=0.58, then $r^2$=0.34, this means that 34% of variation of y may be accounted for by knowing values of x or vice versa. [17-21].

## Result and Discussion

This is the first study are demonstrated that early clinical diagnosis based on determination of biochemical concentrations for serum zeaxanthin, and malondialdehyde to gain successful treatments and theraphy may lead to preserve the retina and macula from degradation, also as a risk of various eye diseases, especially for retinal disease patients with diabetes miletus, cataract, and hypertension,

and starving patients at least eight hours also pre lens surgery. All age related retinal diseases, especially cataract development is associated with degradation then loss transparency of crystalline lens related to interactions between concentrations for serum zeaxanthin, malondialdehyde and environmental factors.

In general, serum Zeaxanthin, and Malondialdehyde concentrations as a risk of retinal disorder disease with cataract, diabetes, and hypertension, are determined in associated with sample collection of Iraqi volunteers, so a direct collection for blood specimens done data were collected, and directly analyzed, to avoid sample components destruction by light depending on the risk of male sex, older ages, in Ramadan and out of Ramadan time, at morning fasting at least for eight hours before lens replacement surgery gets started by artificial intraocular lens, for blood specimens collection, fasting glucose concentrations at baseline, antihypertensive drug use location of residence. Table 1 shows the level of MDA in µg/mL expressed as mean ± SD (range). Patients with hypertensive retinopathy have expressed the highest level of MDA (2.456±2.149) among the rest groups, which was significantly higher (P<0.05) than that in control (0.783±0.937), but the differences were non-significant (P>0.05) compared to diabetic retinopathy group (1.839±1.515), and cataract group (2.026±2.367). Furthermore, both of diabetic retinopathy group, and cataract group have shown significant (P<0.05) higher levels of MDA than control group. The results are confirmed with Ateset al (2010), Gönenç et.al. (2013), Kumar et al.(202020) , Sanz-González et (2012), and And Kaliaperumal et (2020).

**Table 1: The level of MDA in µg/mL in patients and control.**

| | Malondialdehyde (µg/ml) | P value compared to | | |
|---|---|---|---|---|
| | | Control | Cataract | Diabetes |
| **Hypertension (n=25)** | 2.456±2.149 (0.080-8.920) | 0.0001# | 0.505 | 0.247 |
| **Diabetes (n=25)** | 1.839±1.515 (0.083-4.962) | 0.003# | 0.741 | - |
| **Cataract (n=25)** | 2.026±2.367 (0.125-8.916) | 0.011# | - | - |
| **Control (n=30)** | 0.783±0.937 (0.026-4.231) | - | - | - |
| -Data were presented as Mean±SD (Range)<br>#Significant difference between two independent means using Students-t-test at 0.05 level. | | | | |

The ocular lens which is constantly exposed, to light and atmospheric oxygen, is vulnerable to the risk of photo-oxidative damage which results, in a cataract. The ROS seem to cause an impairent to the lens crystallins which result, in its aggregation and precipitation, forming opacities, and also, to the proteolytic enzymes, which involved, in the elimination, of the damaged proteins. Oxidation is known, to be a quite early, or initiating step in the comprehensive process in the sequantial steps which lead, to cataractogenesissuggested that it involved in cataractogenesis. [22] MDA is a form of lipid peroxidation, which has been have suggested that it involved in cataractogenesis, mainly because its cross-linking ability. According to Cekic *et al* (2010), The MDA lens may result from lipid peroxidation of the membranes of the lens cells or may be the result of its migration from the readily peroxidized retina or the central body compartment.[23] Kaur *et al* (2016), have suggested that the increase of MDA concentration

in senile cataract patients is a probable indicate to the redox imbalance (in the privilege of oxidative stress), and generating cataracts [24]. Drumond *et al* (2017), have found that serum MDA level is increased in patients with hypertensive, and diabetic retinopathy compared to control, and this increasing was comparable between patients with hypertensive retinopathy, and those with diabetic retinopathy. [25]

In the diabetic state, oxidative stress plays a crucial role. Via multiple interacting pathways and reactive oxygen species ROS generation, oxidative stress harmfully affects the activity of insulin. This could degrade the β-cell islets of the pancreas, resulting in reduced insulin release. Furthermore, free radical creation by non-enzymatic protein glycation, oxidation of glucose, and increasing of lipid peroxidation leads to enzymes damage, sabotage the machinery of cell injury, cell membrane changes, and increased resistance to insulin that is at risk for diabetes .[26] Hypertensive retinopathy is an important condition and a potential source of damage to the target organ due to hypertension. Autoregulation of the retinal circulation is known to fail when blood pressure increases above a critical level. However, the rise in blood pressure alone does not completely account for the degree of hypertensive retinopathy. [27] Hypertension, oxidative stress, resulted either from enhances in ROS production or decreases in antioxidant defenses, is associated with increase in blood pressure, endothelial dysfunction and vascular remodeling.[28]

For Zeaxanthin the level of zeaxanthin in serum of hypertensive retinopathy patients (32.80±30.56 ng/mL), diabetic retinopathy patients (86.38±40.81 ng/mL), and cataract patients (52.58±5.05 ng/mL) was non-significantly (P>0.01) reduced compared to healthy control (88.85±139.31 ng/mL), all demonstrated at Table 2. Dherani *et al* (2008), have found that serum zeaxanthin level is inversely correlated with age-related cataract.[29] Also, Kappi et al (2012), have reported significant decrease of plasma zeaxanthin concentration in ARC patients compared to healthy control, suggesting that zeaxanthin is a protective agent against cataract.[30]

In Liu *et al* (2014), meta-analyses study they have reported significant inverse correlation between blood zeaxanthin and nuclear cataract, as well as blood zeaxanthin is associated with lowering the risk of cortical cataract and sub-capsular cataract.(31) Jiang *et al* (2019), have reported that the increase of zeaxanthin consumption by 10mg per day has significantly decrease the risk of age- related cataract by 26%.[32] The results of zeaxanthin in the current study DR subjects are agreed with She *et al* (2017), who have found a comparable non-significant difference in serum zeaxanthin concentration between DR patients and healthy nondiabetic control. [33] Yet the study of Giehrat and Kowluru (2006), have revealed that supplementation of zeaxanthin is significantly inhibited the development of retinal oxidative damage in diabetic rats and could represent a supplemental therapy to inhibitthe development of retinopathy in diabetes [34] Hozawa *et al* (2009), have found significant negative relationship between serum zeaxanthin level and hypertension, whereas as blood pressure rise up the level of zeaxanthin reduced significantly. [35]

**Table 2 : The level of zeaxanthin distributed on age groups and gender.**

| Groups | | Zeaxanthin (ng/mL) | | | |
|---|---|---|---|---|---|
| | | Hypertension (n=25) | Diabetes (n=25) | Cataract (n=25) | Control (n=30) |
| Age (years) | 20---29 | - | - | 53.63±2.87 | 72.19±98.72 |
| | 30---39 | - | 79.04±0.0 | 52.67±2.63 | 88.86±85.81 |
| | 40---49 | 36.93±21.42 | 69.62±33.73 | 52.01±2.44 | 95.60±111.8 |
| | 50---59 | 25.19±8.83 | 116.72±42.16 | 50.91±2.10 | 94.61±106.21 |
| | 60---69 | 35.50±19.66 | 83.36±49.85 | 53.61±2.22 | 91.83±0.0 |
| | **P value** | **0.313** | **0.213** | **0.274** | **0.996** |
| Gender | Male | 38.85±19.83 | 83.91±43.93 | 52.44±2.68 | 103.87±101.33 |
| | Female | 27.02±14.40 | 88.42±48.46 | 52.51±2.46 | 73.56±102.5 |
| | **P value** | **0.072** | **0.792** | **0.939** | **0.422** |

-Data were presented as Mean±SD (Range)
#Significant difference between two independent means using Students-t-test at 0.05 level.
^Significant difference among means by using one way ANOVA.

Table 2 shows the mean ± SD of zeaxanthin distributed on age categories for each of the study cohorts. The variations of zeaxanthin level was non-significant (P>0.05) neither among age categories nor between males and females in all of the study cohorts. Thus age does not consider as risk factor for zeaxanthin in hypertensive, diabetic, cataract, or control subjects of the present study.

In the present study, these biochemical parameters are risk of various eye diseases, especially zeaxanthin. It is a nutritional, not synthesized during development, present in the external membrane of macula and retina cells at high concentrations, act as ocular antioxidants for retardation of age-related cataracts and macular degeneration. These carotenoids are found in the human lens, retinal pigment epithelium/choroid (RPE/choroid), the macula, the iris, and the ciliary body.[36-37] The substituted beta carotene with oxygen as hydroxyl group produce xanthophyll rich with oxygen to cross blood-ocular and blood-brain barriers, as in zeaxanthin. Other carotenoids ($\beta$-carotene and lycopene) contain only carbon and hydrogen atoms and do not cross the blood-brain or ocular barriers, and Zeaxanthin also inhibits the proliferation and induces apoptosis in human.[38-39]

## Conclusion

The study concludes the followings:

1. Malondialdehyde antioxidant concentrations are significantly high levels with low levels for normal individuals, invers relation for diabetes related to starvation for more than eight hours and increased oxidative stress for metabolic disorder pre surgery resulting in this state, causing inflammations and soreness.

2. Increased concentration of serum Malondialdehyde antioxidant for hypertension shows decreased liver storage of vitamin A, this seems to be very clear for low levels of Zeaxanthin concentration leading to development of cardiovascular diseases, advance stages for liver diseases affecting development and adult tissue regeneration.

3. These results suggest that improvement of Zeaxanthin and Malondialdehyde antioxidant concentrations as important diagnostic risks factor of potential usefulness for all retinal diseases with related states as hypotheses for further study, and a monitor for these cases also.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: None

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# Effect of Thermocycling on Surface Roughness and Shear Bond Strength of Acrylic Soft Liner to the Surface of Thermoplastic Acrylic Treated with Ethyl Acetate

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## Abstract

**Objective**: To enhance bonding strength between thermoplastic denture base and acrylic soft liner through ethyl acetate surface treatment.

**Materials and Methods**: Modifications of thermoplastic acrylic denture base surface were investigated with SEM. FTIR was used to detect whether there was a chemical bond between thermoplastic acrylic and the organic solvent. A total of 80 samples were prepared and divided into 20 samples for the surface roughness test and 60 samples for the shear bond strength test. Failure type was assessed visually.

**Results**: Shear bond strength and surface roughness values of un treated samples were lower in comparison to surface treated groups; the greatest post thermocycling bond strength value was recorded for the samples treated with ethyl acetate following 1500 cycles.

**Conclusions**: Bonding strength was improved following ethyl acetate surface treatment.

***Key words**: Thermoplastic acrylic, ethyl acetate. toxicity*

## Introduction

There are various types of thermoplastic denture base materials used in dentistry; polyamide, polyester, acrylic resin, and polycarbonate[1]. Thermoplastic acrylic resins have a main advantage over the classical acrylic resins which is the absence of residual monomer. The material is non-toxic and it is stable in water, alcohol, saliva and stomach acids. It has high wear resistance, mechanical strength and aesthetics [2].

Gradual oral tissue changes allow alveolar ridge sensitivity to the applied functional pressure during functional activities[3, 4]. Therefore, using of soft liners for removable prostheses will provide cushioning layer on the denture intaglio surface resulting in decreased transmission of traumatic occlusal forces[4, 5].

Resilient denture liners can be divided into acrylic based liners, [such as: poly (methyl methacrylate)] and silicone based, (such as poly dimethyl siloxanes) known as silicone[6]. Ideal denture relining strongly dependent on the high bond strength between the two bonded materials which is an essential requirement to attain an intact interface; it can overcome leaching out of relining material from the surface of denture base. In contrast, poor bond strength will cause mechanical strength deterioration[7, 8].

Several attempts were made to enhance adhesion of soft liners including chemical and mechanical surface modifications[9]. Among the chemical surface

preparations were organic solvents, such as ethyl acetate[6]. Ethyl acetate is a popular organic solvent that is not listed in the classification of the International Agency for Research on Cancer (IARC) and selected as a safe surface treatment mediator that can swell the denture base surface increasing the bond between the denture base and soft liner[10].

The denture base with soft liner is subjected to thermal variations intraorally which affect negatively on bonding strength. Therefore, thermocycling is used to evaluate bonding durability of adhesive interface against thermal stress as it represents oral function in vitro[11].

This study aimed to assess the effect of surface preparation using ethyl acetate on surface roughness and shear bond strength of thermoplastic acrylic denture base to acrylic soft liner.

## Materials and Methods

Heat polymerized acrylic soft liner (vertex–soft / Netherlands), injection molded thermoplastic acrylic denture base material (Deflex \ Argentina) and ethyl acetate solvent were used in this study.

In order to select the most effectual time, pilot study of shear bond strength and surface roughness tests were performed and 4 application periods of ethyl acetate were used (60, 120, 180 and 240 sec.). According to the results of pilot study for each test, 180 sec of ethyl acetate surface treatment was the most appropriate time because it showed improvement in shear bond strength between thermoplastic acrylic and soft liner.

SEM (Inspect S50, FEI, Japan) was taken to detect topographical changes to the denture base material surface following ethyl acetate surface treatment.

FTIR analysis (Tensor 27, Bruker, Germany) was also accomplished to explore the chemical interaction between ethyl acetate solvent and the surface of thermoplastic acrylic material.

### Samples grouping

Twenty Samples of injection molded thermoplastic acrylic denture base were fabricated to perform surface roughness test. Injection molded thermoplastic acrylic and heat cured acrylic soft liner was used to fabricate 60 samples used for shear bond testing. According to the number of thermal cycles, thermoplastic acrylic samples were divided into 3groups and these groups were segmented into 2subgroups of 10 samples depending on ethyl acetate surface treatment. The first group was tested for shear bond strengths without thermal cycling while other two groups were thermocycled for 1500 cycles and 3000 cycles in water before testing.

### Samples design

20 rectangular samples of thermoplastic acrylic with dimensions of (65mm x 10 mm x 2.5 mm) were fabricated for surface roughness testing according to ADA Specification No.12, (1999).

For shear bond testing, acrylic blocks with the measurements of (75mm x 25mm x 5) mm with a stopper of 3mm depth were constructed. Two acrylic blocks were put over each other to produce one sample with a space of (25mm x 25mm x 3 mm) between blocks[4].

### Samples preparation

In order to obtain the acrylic blocks for shear bond and surface roughness test, plastic and metal patterns were constructed in accordance with dimension of each test and invested in silicon duplicating material (Addition Silicon, putty consistency Zhermack- Italy) .Silicone molds were then invested in dental stone in a flask. Preformed wax tubes were then connected to the pattern to allow material injection. Wax tubes were removedby performing wax elimination process. According to manufacturer's instruction, injection of

the thermoplastic acrylic samples was done under (5-7 Bar) for (15 min) and at temperature (265ºC ± 10ºC). The finished acrylic samples were stored in distilled water at 37ºC for 48 hours according to ADA Specification No.12, (1999).

**Surface conditioning**

Surface roughness samples were cleaned ultrasonically [6]and were then air dried for 15 minutes[12, 13].

Shear bond test samples were abraded by using 400-grit silicon carbide paper[14]. This procedure was performed manually [15, 16], and under running water to overcome heat generation[17]. . Samples were then cleaned ultrasonically [13] and air dried for 15 minutes[12, 13].

**Surface treatment with ethyl acetate**

Thermoplastic acrylic blocks were surface treated by its immersion a closed clean glass beaker of ethyl acetate solvent for 180 sec. Samples were then left to dry for 2 min[18].

**Soft liner mixing and application**

Each shear bond test sample consisted of two thermoplastic acrylic blocks which were secured together in a way that a space dimensioned 25mm length, 25mm width and 3mm depth was formed. Silicon putty duplicating material was used to invest the sample which was then invested in dental stone inside a custom-made flask.

The soft lining material was proportioned and mixed in a clean glass container and left till the dough stage and then applied in custom flask with gradual application for achieving even distribution. After flask closure, curing process was done using thermostatically controlled water bath by heating up to 70ºC for 90 minute and then boiled up to 100ºC for 30 minutes.

**Thermocycling procedure**

40 samples were thermocycled (20 samples for each number of cycles) in a thermocycler at 5°C and 55°C (± 2°C) with 1 minute dwelling time for each temperature. The 1500 cycles were performed within 3 days while the 3000 cycles were done within 6 days [19].

**Testing procedure**

The surface roughness samples were tested by the profilometer (Time 3200 / TR200, China) .3 readings for each sample were obtained and the mean value of the 3 readings was reported as roughness value[20].

Shear bond test was done by using universal testing machine (Laryee, China) with 100 Kg load cell capacity and cross head speed of 0.5mm/min. Separation force was calculated and shear bond strength value of each sample was calculated by the following equation:

Shear Bond strength=F (N)/A (mm2).

F= failure force (N).

A= sample cross sectional area (mm2).

After shear bond test samples testing, failure mode at bonded area was then estimated visually. If tearing occurs in soft liner itself named as cohesive failure, while total detachment in the bonded interface between the soft lining and acrylic resin named as adhesive .Mixed failure refers to both failure types[21].

## Results

FTIR results showed that there was no chemical interaction between ethyl acetate and thermoplastic denture base material.

The morphological changes of untreated samples and samples with 180 sec ethyl acetate surface preparation determined by (SEM) are shown in figures (1, 2). SEM found that a dissolved topography

with various pores in the thermoplastic acrylic surface was promoted following ethyl acetate treatment when compared with the control group in which many even parallel scratches have formed following abrasion with silicon carbide paper.

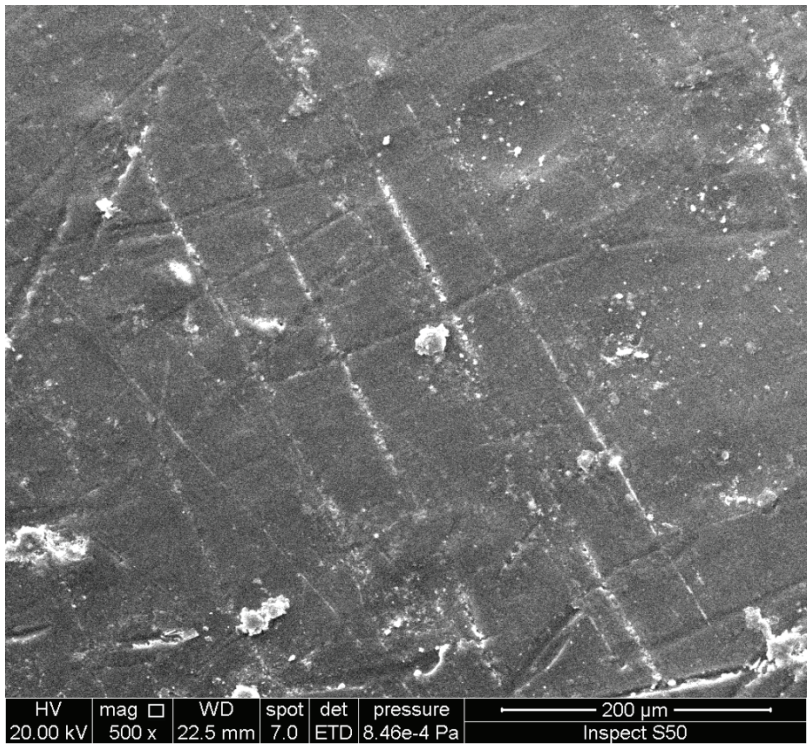


**Figure (1): SEM of the untreated sample surface at a magnification ×500.**

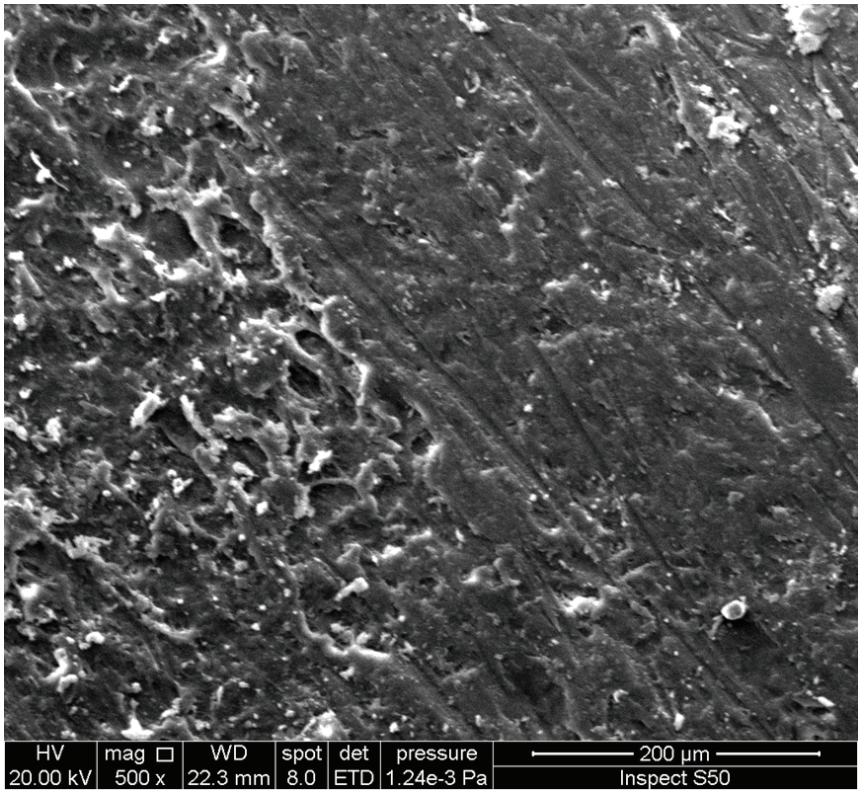


Figure (2): SEM of the 180 sec ethyl acetate surface treated surface at a magnification ×500.

The statistical analysis of the results gained, specified that surface treatment of thermoplastic acrylic with ethyl acetate caused increasing in surface roughness of thermoplastic acrylic material. As shown in table (1) surface roughness mean value of ethyl acetate surface treated groups was significantly higher than that of control group (no surface treatment).

**Table (1): Comparison in surface roughness between study groups (values in µm).**

| Surface roughness | N | Study Group | | Range | Student t-test | P - Value |
|---|---|---|---|---|---|---|
| | | Surface treatment group Mean ± SD | Control group Mean ± SD | | | |
| | 10 | 0.803 ± 0.06 | 0.351 ± 0.03 | 0.308 – 0.916 | 19.581 | 0.001 |

It has also been noticed that mean of shear bond strength test was significantly higher in surface treated group than in control group in cycles 0, 1500, and 3000. Highly significant difference was noted in shear bond strength of surface treated groups as compared to control group as shown in (table2).

**Table (2): Comparisons in shear bond strength test between study groups in cycles 0, 1500, and 3000(values in $N/mm^{2)}$.**

| Shear bond strength test | N | Study Group | | Range | Student t-test | P - Value |
|---|---|---|---|---|---|---|
| | | Surface treatment group Mean ± SD | Control group Mean ± SD | | | |
| 0 Cycle | 10 | 0.845 ± 0.12 | 0.47 ± 0.01 | 0.36 – 1.037 | 7.761 | 0.001 |
| 1500 Cycle | 10 | 0.794 ± 0.04 | 0.382 ± 0.06 | 0.288 – 0.831 | 17.015 | 0.001 |
| 3000 Cycle | 10 | 0.71 ± 0.07 | 0.305 ± 0.05 | 0.18 – 0.812 | 14.009 | 0.001 |

Highly significant difference was also detected in comparison between cycle 0, 1500, and 3000 for each group. Highest significant difference was also detected in mean of shear bond strength in cycle (0) which was higher than that both cycles (3000, 1500) in study groups as shown in table (3).

**Table (3): Comparison in shear bond strength test between cycles for each group. (One-way ANOVA) (Values in N/mm$^{2)}$:**

| Study Group | N | Shear Bond Strength Test | | | F value | P - Value |
|---|---|---|---|---|---|---|
| | | (0) Cycle Mean ± SD | 1500 (Cycle) Mean ± SD | (3000) Cycle Mean ± SD | | |
| **Surface treated group** | **10** | 0.845 ± 0.12 | 0.794 ± 0.04 | 0.71 ± 0.07 | 6.532 | **0.005** |
| **Control** | **10** | 0.47 ± 0.01 | 0.382 ± 0.06 | 0.305 ± 0.05 | 12.708 | **0.001** |

Upon examining failure modes, it appeared that samples which were tested without thermocycling experienced cohesive failure in 80% of samples in control group and 20% had mixed failures with total cohesive failure 100% in samples treated with ethyl acetate.

Thermocycling turned failure mode to a mixed type. Samples that thermocycled 1500 times had mixed failure in which 60% of samples in the control groups failed adhesively while 40% of samples experienced mixed failure for the surface treated group 50% of samples failed cohesively with 50%had mixed failures.

Samples which thermocycled 3000 times had mixed failures total adhesive failure of the control group 100% with 20 % cohesive failure and 80% mixed failure for the surface treated group.

## Discussion

Achievement of high bond strength between a denture base and soft denture liner is a challenging procedure. Several surface preparations have been suggested to improve that bond. Several researchers suggested surface wetting with organic solvents such as ethyl acetate. It was found that the bonding mechanism was based on monomer interdiffusion, swelling and the formation of IPN (interpenetrating network) during polymerization[14].

A significant increase that was noticed in surface roughness values considered that the chemical surface treatment with ethyl acetate had produced pits with a porous surface.

Noticeable improvement in shear bond strength following ethyl acetate surface preparation owed to the solvent ability for denture base surface swelling .Polymer chains infiltration by solvent took place and polymer depolymerizing enhances the formation of IPN and thus improving mechanical interlocking.

Although thermocycling decrease the bonding strength for all tested groups, surface treated groups showed high bonding strength than control groups. Statistical analysis showed that highest shear bonding strength values observed in samples tested without thermocycling while greatest post thermocycling values were observed in samples thermocycled 1500

times while samples which were tested after 3000 cycles had the lowest bonding strength. Decline in bonding strength related to the huge amount of absorbed water during thermocycling leading to swelling and concentration of stresses at the liner/ denture base interface or could be associated with viscoelastic properties changes of the liner [22, 4]. Water ageing, nature of the denture base material and temperature are the main factors affecting bonding between resilient lining and denture base [23, 22]. So declining in bonding strength was due to massive water amount absorbed by the hydrophilic acrylic liner leading to internal polymer damage by water droplets growth and irreversible polymer matrix breakdown and cracks formation by continual droplets growth[24, 4]. The material brittleness will increase and more external load is transferred to the interface. Significantly lower bond strength was detected in all thermocycled groups in comparison to non thermocycled groups. This outcome may be credited to thermal aging and water sorption of material at soft liner/denture base interface[22].Failure was predominantly cohesive in non thermocycled groups and mixed in thermocycled groups. Failure modes after testing suggested that exposing to thermal changes and multidirectional forces in mouth causes denture liner to undergo cohesive or mixed debonding.

## Conclusions

1. Ethyl acetate surface treatment increased the surface roughness of thermoplastic acrylic denture base and improved the shear bond strength of thermoplastic acrylic with acrylic soft liner..

2. Thermocycling had a deleterious effect on bonding strength of lining materials to denture base materials.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

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# A Comparative Study of Immunological and Molecular Techniques to Diagnose Human Cytomegalovirus in renal Failure Patients in Diyala Governor

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## Abstract

**Background**: Cytomegalovirus (CMV)belongsto the herpes virus family, it has the ability to cause systemic infection and serious diseases in immunocompromised patients such as hemodialysis patients. The aim of this study was to investigate the prevalence of CMV infectionamonghemodialysis (HD) patients.. Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate fluid, electrolytes and pH balance of extracellular fluids.Renal failure diesease is a wide dissemination among kidney patients in*Ba'quba City.*

**Aim**: the study was carried out toImmunological andMolecular detection of CMV among renal failure patients whom admitted to IbnSina Center for kidney Dialysis in Baquba Teaching Hospital .

**Patients and Methods:** This study was conducted for the period from 1/12/2019 to 15/6/2020 in Baquba city in Iraq ,The study involved a total of 100 patients (62 males and 38 females) with kidney diseasewith ageand 50 healthy individuals considered as controls.First step includes Human Cytomegalovirus (HCMV) diagnosis in studied groupsbyEnzyme-Linked Immunosorbent Assay technique(ELISA) and second step was detection of Human Cytomegalovirus(HCMV) by Real-time Polymerase Chain Reaction.

**Results**: The obtained results showed that HCMV antibody was detected in renal failure patients by ELISAIgG (100%) while IgM were (15.0%) . Also,Distribution Of Detection of human cytomegalovirus DNA in serum between patients with renal failure and control by using sensitive molecular techniques, The obtainedresults showed that the HCMV DNA was detected in (6 out of 45) or 6 % in patients, while in control group (0 out of 5) or 0.0 %.Also, the HCMV DNA was detected in males 1(16.66) %. while in females was 5(83.34) %, while in control group (0 out of 5) or 0.0 %. with highly precent differences was noticing among both sexes. These results showed the age group 60-70 showed the highest rate of infection among other groups.

**Conclusions:** Real time PCR was rapid, sensitive and useful for diagnosing CMV infection in such patients.The results showed that Cytomegalovirus has relationship with chronic and acute renal failure and can affect the patient's immune status. our results can provide an advanced diagnosis of viral infections among patients in hospitals in Iraq.

***Key Words:*** *CMV, renal failure, ELISA,Real time PCR*

## Introduction

*Renal failure occurs when the kidneys are unable to do their job: to filter wastes from the blood, help regulate blood pressure, and regulate salt and water balances in the body. As blood flows through the kidneys, it is filtered, and wastes are removed and sent to the bladder as urine. If kidney function becomes impaired, acute (rapid) or chronic (gradually developing) renal failure may occur. With acute renal failure, kidney function can return to normal if the underlying cause of the failure is discovered and successfully treated. There are two type of kidney infection: Acute Renal Failure (ARF) and Chronic Renal Failure (CRF).*[1] Human Cytomegalovirus (CMV) is a ubiquitous humanspecific .DNA virus, belonging to the Herpesviridae family. Cytomegalovirus (CMV) is a ubiquitousvirus with high worldwide prevalence ranging from 34%-80% in developed countries to 100% in some parts ofAfrica [2]. Human cytomegalovirus(HCMV) belongs to the herpesviridae family, subfamilyBetaherpesvirinae, genus *Cytomegalovirus* andcharacterized by slow replication and clinically causesasymptomatic infection in immunocompetent individuals[3]. *The virus is the most significant infectious cause of congenital disease, an important opportunist in the immunocompromised hostlike renal failure .Cytomegalovirus (CMV) is a member of the human herpesvirus family*.

*Herpesviruses are enveloped viruses with an icosahedral capsid that encloses a double-stranded DNA genome, CMV is the largest member of the human herpesvirus family, with a genome of 236 kbp and more than 200 open reading frames (ORFs) encoding more than 80 viral proteins, including glycoproteins (e.g., gB), phosphoproteins (e.g., pp65), and other transcription/replication proteins.*[4]*CMV is one of the most successful of human pathogens, since it can be transmitted both vertically and horizontally,* Following primary infection, which is almostalways asymptomatic in people with normal immunity and the virus establishes latency. The spreading of HCMV from person to person is by direct contact such as kissing, sexual contact and getting saliva or urine on your hands and then touching eyes, nose or mouth [5]. The infection can occur through blood transfusion from donors with active or latent infection .*Risk factors for primary CMV infection include blood transfusion (treatment for clotting factors, and etcetera), infected transplants, hemodialysis, and the frequency of dialysis in a week. CMV seroprevalence has been shown to be highest in South America, Africa, and Asia, while it is lowest in Western European countries and the United States.*[6] *Globally, between 60 and 90% of the general population is infected with CMV with generally higher rates in developing countries.*

Diagnosis of active HCMV infection by ELISA for HCMV-specific IgM antibodies has been shown to be superior and practical. Detection of HCMV-specific IgG antibodies in blood is an indicator of previous exposure to HCMV while IgM antibodies are associated with active CMV infection [7]. *and Molecular assays for determining the level of CMV replication such* areal-time PCR assay used in our laboratory targets the conserved region that lies upstream of antigenic the gB gene.Patients with *Renal failure* disease have impaired immune response, which may result in high rates of viral infections, including CMV. Infections in these patients may be due to primary infection or, morecommonly, by reactivation of latent virus or re-infectionwith exogenous virus. [8].

*CMV infection triggers a forceful immune reaction in the human body, including both antibody- and T-cell-mediated responses. Because of its effective immune evasion strategies.*[9] *B-cell immune responses Primary CMV infection elicits a transient IgM response within 1-3 weeks that is followed by the development of persistent IgG antibodies. These*

*antibodies play a minor role in clearing the infection but are believed to play an important role in reducing the severity of CMV disease in adults and protecting the fetus from congenital infection. The antibodies are directed against at least 15 different proteins; the most immunogenic is pp150, against which nearly 100% of the CMV-seropositive individuals have antibodies.* [10] *Another important immunogenic protein is pp65; the antibody response against this protein is very high during the acute phase of the infection. The best characterized protein, however, is glycoprotein B (gB), and up to 50-70% of the host's neutralizing antibody response is accounted for by the response to his protein .* [11]

## Materials and Methods

*Samples collection: A search was performed through The sample* A search was 100 Iraqi patients with renal failure at age range (13-76 years) from IbnSina Center for kidney Dialysis in Baquba Teaching Hospital and 50 healthy controls from Blood donors at the main blood bank in Baqubah at age range (18–45) years during the period from January to March 2020.The patients in this study included 62 males, 38 females , aswellas healthy controls 38 males, and 12 females. First step includes Human Cytomegalovirus (HCMV) diagnosis in studied groups by ELISA *,while study was 45 Iraqi patients with renal failure from IbnSina Center for kidney Dialysis in Baquba Teaching Hospital and 5 healthy controls from Blood donors at the main blood bank in Baqubah during the period from January to March 2020. First step includes* Diagnosis of Human Cytomegalovirus in renal failure patients by ELISA and sconed step Real time PCR *and*

***Collection and Preparation of Samples****: 5 ml of vein blood samples were collected , put in gel tube and left 15 min to allow clotting at room temperature about 20 -25 °C , after that the serum was separated by 3000 rpm for 10 minutes in centrifugation. The serum was collected and distributed in small tubes and stored in -20°C until use to diagnosis HCMV in study groups.*

### *Serological*detection of Human Cytomegalovirus:

*Serological investigation included detection of CMV-IgG antibodies and CMV-IgM antibodies by using enzyme-linked immunosorbent assay (ELISA) (MyBioSource , USA). The procedure was carried out according to the manufacturer's instructions.*

### Molecular detection of Human Cytomegalovirus

#### Extraction of Viral Nucleic Acid

Genomic DNA or RNA was isolated from Serum samples according to the protocol of QIAamp® MinElute® Virus Spin Kit.

#### Diagnosis of Human Cytomegalovirus in renal failure patientsbyReal time PCR:

Fluorion CMV QNP 3.0 Real-Time PCR Kit CMV-FRT was a kit used in PCR test in lab for amplification of nucleic acid. It could be used for detection of DNA of the human cytomegalovirus in suspected sample urine, saliva, urogenital, and blood.

#### Data Analysis

By the end of the thermal protocol, the Fluorion Detection System software automatically sets the baseline cycles and the threshold. The standard curve (Threshold Cycle vs. Log Starting Quantity) is plotted using the data obtained from the defined standards.

## Results and Discussion

*The sample of study was 100 Iraqi patients with renal failure*during the period from January to March 2020This study was conducted to detect the prevalence of cytomegalovirus infection (CMV) among patients

undergoing hemodialysis by using CMV/IgG, CMV/IgM.

The result of infection current research revealed that CMV-IgG was found in 100 out of 100 (100.0%), while CMV- IgM was detected in 15 out of 100 (15.0%) , Chi-Square(8.333) of hemodialysis patients. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection ; reactivation or new infection lead to high percentage of seropositivity, because renal failure patients conceder immunosuppressed individuals. A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life when a person was infected The seroprevalence of CMV varies in differentstudies**.** [12]revealed that CMV-IgG was found in 102 out of 116 (87.9%), while CMV-IgM was detected in 10 out of 116 (8.6%) of hemodialysis patients in Tikrit city. [13]found that the rate of CMV infection among hemodialysis patients (HD) was 98% using CMV IgG and 11% using CMV/IgM. Astudy was carried out in Antakya, Turkey to determine the rate of CMV infections revealed that anti-CMV IgG and IgM was found in 99.6% and 0.4% respectively of the HD patients [14]. The rate of anti-CMV IgG (39%) obtained by the current study was similar to that obtained by **(Firouzjahi etal.,2015)**[15](34%),but differ with [16] (69%) . HCMV IgG antibody levels increased by increasing frequency of exposure and transmission viacrowded and poor living conditions. [17]. These variations in the results may be attributed to several factors including endemicity of infection, study population, the techniques used for diagnosis and immune status of the patients.

**Table 1: Anti CMV IgMAb& Anti CMV IgGAb frequency and percentage in patients' group by ELISA technique**

| Groups | Anti CMV IgMAb | |
|---|---|---|
| | Patient | Control |
| | No. (%) | No. (%) |
| Positive | 15 (15.0) | 0 |
| Negative | 85 (85.0) | 50 |
| Total | 100 (100.0) | 50 |
| Chi-Square | 8.333 | |
| Df | 1 | |
| P value | 0.004 | |
| Positive | 100 | 0 (0.0) |
| Negative | 0 | 50 (100.0) |
| Total | 100 (100.0) | 50 |
| Chi-Square | 150.000 | |
| Df | 1 | |
| P value | 0.000 | |

Moreover of this study results was found that the CMV-IgG was found in 62% in males. while females ,was detected in 38%.The laboratory investigation concerning CMV- IgM among hemodialysis patients revealed that 33.3% of males and 66.7% of females have CMV- IgM antibodies.

**Table 2 : anti-CMV IgG and IgM frequency distribution according to the sex of the patient's group**

| Groups | Patients group | | Control groups | |
|---|---|---|---|---|
| Anti-CMV IgG status | Positive | Negative | Positive | Negative |
| | No. (%) | No. (%) | No. (%) | No. (%) |
| Males | 62 (62) | 0 (0.0) | 0 | 40 |
| Females | 38 (38) | 0 (0.0) | 0 | 10 |
| Total | 100 (100.0) | 0 (0.0) | 0 | 50 |
| **Anti-CMV IgM status** | | | | |
| Males | 5 (33.3) | 57 (67.1) | 0 | 40 |
| Females | 10 (66.7) | 28 (32.9) | 0 | 10 |
| Total | 15 (100.0) | 85 (100.0) | 0 | 50 |

**Table 3: Gender distribution and CMV frequency by real-time PCR in patients' group**

| Gender | CMV frequency by real-time PCR | | control | |
|---|---|---|---|---|
| | Positive | Negative | Positive | Negative |
| | No. (%) | No. (%) | No. (%) | No. (%) |
| Males | 1 (16.66) | 26(66.66) | 0 | 3(60) |
| Females | 5 (83.34) | 13(33.34) | 0 | 2(40) |
| Total | 6 (100.0) | 39(100) | 0 | 5(100) |

Revealed 69 male, CMV-IgG was found in 59 (85.5%) of them. Regarding females, CMV-IgG was detected in 43 out of 47 ( 91.5%). However, that CMV- IgM among hemodialysis patients revealed that 7 out of 69 (10.1%) of males and 3 out of 47 (6.4 %) of females have CMV- IgM antibodies. In Turkey, gender did not contribute independently to the seroepidemiology of CMV($p > 0.01$). [18] Also, [19] reported that there was no difference in CMV prevalence between males (87.9%) and females (96.3%). [16] showed non- significant relation concerning sex status of the CMV-cases. and [13] in U.S. reported that females had higher seroprevalence than males. It is possible that the gender difference in CMV seroprevalence reflects females' exposure to young children. The relationship of child care to CMV infection has been presumed to be attributable to the presence of CMV at high titers in urine and/or saliva. Nevertheless, a previous study suggested that, similar to females, adolescent males are at an increased risk of CMV infection when exposed to young children in the household.

Varying methodologies perhaps may have contributed to the disparities observed. Although immunocompromised patients are at risk for morbidity due to wide variety of pathogens, few, if any of these are capable of producing such widspread disease as CMV. CMV-related morbidity follows a progressive, relentless course in the absence of effective therapeutic intervention. Thus, rapid diagnosis of active CMV infection is of great importance to avoid over treatment with immunosuppressive drugs and to guide antiviral therapy. In recent years, treatment of CMV infection in high-risk patients prior to the onset of clinical disease is preferred.Seroprevalence of CMV in the study groups according to age An increase in the seroprevalence rates was observed with age . Also, a significant association between increase of the age and increment of the seroprevalence confirmed that according to age, a progressive increase in seropositivity was observed in hemodialysis patients.

CMV- IgM was detected at a highest rate in patients within the age group was found among age group 50-70 years. Many investigators observed that 0lder patients were at higher risk of CMV infection . From the previous studies and our study there was an agreement. This may be due to that patients with highest ages will have the low immune response. *The sample of study was 45 Iraqi patients with renal failure*during the period from January to March 2020This study was conducted detection of Human Cytomegalovirus(HCMV) by Real-time Polymerase Chain Reaction,The result of infection current research revealed that These results, the HCMV DNA was detected in (6 out of 45) or 6 % in patients, while in control group (0 out of 5) or 0.0 % as . Also, the HCMV DNA was detected in males 1(16.66) %. while in females was 5(83.34) %, while in control group (0 out of 5) or 0.0 %. with highly precent differences was noticing among both sexes as shown in Table (3).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** None

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# Study the Ability of *Pseudomonas Aeruginosa* Isolated from Different Clinical Cases to Biofilm Formation and Detection of Algd Gene

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## Abstract

98 samples were collected from various clinical sources included ( Burns, wounds, urines, sputums, blood ) From the city of Baghdad , After performing the biochemical and microscopic examination, 52 isolates were obtained for *Pseudomonas aeruginosa* , 17 (32.7 %) isolates from burn infection , 12 ( 23% ) isolates from Wound infection 11 ( 21.2 % ) isolates from urine infection , 7 ( 13.5 % ) isolates of sputum and 5 ( 9.6 %) isolates from blood . Bacteria susceptibility to form biofilm has been detectedby microtiter plate method, The results showed that 80 % of the bacterial isolates were produced the biofilm with different proportions , **alg D** gene ( alginate production ) has been detected by polymerase chain reaction ( PCR ) Which plays an essential role in the formation of the biofilm , The PCR results showed that the percentage of gene presence was( 95.4 % ) .

***Key words :*** *Pseudomonas aeruginosa , biofilm , alg D*

## Introduction

*P. aeruginosa* is considered an important bacterial species due to its parasitism on humans causing many diseases like Bacteremia , Wound and Burn infection , Urinary tract infection , Endocarditis , Meningitis[(1)]It also affects the digestive system, causing gastroenteritis or diarrhea in children[(2)], *P. aeruginosa* can colonize medical devices which increases its prevalence in health care institutions [(3)]

*P. aeruginosa*has many virulence factors including Exotoxin A , Hemolysin , Protease and Biofilm[(4)] , Bacterial cells tend to form clusters that are resistant to unfavorable conditions despite its presence in most of the time freely [(5)] and these clusters are surrounded by a layer of exopolysaccharides as well as protein and DNA, these clusters are characterized by the presence of channels through which nutrients and cellular secretions are transported[(6)] , the biofilm provides protection from many harsh physical and chemical stresses such as high temperature and drought[(7)] , PH and disinfectants[(8)] and fromthe host's body defenses [(9)] , the biofilm is an important feature for the continuation of infection and when it is formed the bacteria are resistant to antibiotics at a high rate that may reach 1000times higher than that of other non-biofilm types, which makes it difficult to treat[(10)].

**algD** gene responsible for the production of the alginate layer for *P. aeruginosa* and it plays an important role in chronic pneumonia[(11)].

The **algD** gene encodes( GDP-mannose 6-dehydrogenase) an enzyme involved in the alginate biosynthesis pathway [(12)], The alginate layer is considered one of the basic elements for the formation of the biofilm,, its a linear unbranched polymer

composed of ( 1-4 ) linked saccharides D-mannuronic Acid (M) and L-guluronic Acid (G)[13] .

The alginit layer contributes to the structural stability and protection of biofilms[14]As well as providing protection from antibiotics[15] , its overproduction is responsible for the mucosal phenotypic growth that protects bacteria from the harsh environment in the lungs of a patient with cystic fibrosisand causes lung lesions by facilitating the attachment of bacteria to the respiratory tract epithelium[16]Interestingly, this layer is produced mainly in patients with cystic fibrosis .

## Materials and Methods

**Collection of bacterial samples :**

98 samples were collected from different clinical sources ( Burns, wounds, urines, sputums and blood )From the Central Child Hospital and Medical City Hospitals in Baghdad, for the period between 30/9/2020 to 1/1/2021, The samples included: 26 from burns, 25 from wounds, 26 from urine, 12 of sputum and 9 samples of blood.

**Isolation and Identification :**

The bacterium was diagnosed by cultivating it on macConkey agar , cetrimide agar and blood agar, Their phenotypic characteristics were studied in terms of colonies' shape, color, and their odor[17] and Examination microscope( Gram stain ) and biochemical tests ( Oxidase , Catalase , Citrate utilization , Gelatin hydrolysis , Hemolysin and Indole test ) .

**Detection of biofilm formation :**

The ability of *P. aeruginosa* to form biofilm was detected using the microtiter plate method[18] ,the bacterial suspension was prepared using sterile saline and compared with the McFarland standard solution ( 0.5 ) , a 180 µL of Tryptone soy broth ( TSB ) supplemented with additional 1 % glucose and a 20 µL of prepared bacterial suspension was added to each well of microtiter plate(Three replications ) negative control represented only the medium , after 24 hours of incubation at $35^5$C the contents of the wells have been discarded and rinsed gently three timeswith 200µL of sterile phosphate buffered saline ( PH 7.2 )and left the room temperature to dry, cells were fixed with 150 µL of Methanol alcohol for 20 minutes , after discarded of methanol, dried and stained with2% Crystal violet ( 150 µL per well ) for 15 minutes then wells were washed with distilled water.after drying , dye bound to biofilm on well released with 96% ethanol alcohol ( 150 µL per well ) for 20 minutes at room temperature , the optical density was measured using a microtiter plate reader at 570 nm .

**DNA extraction :**

The genomic DNA was extractedfrom preserved bacterial isolates ( 22 isolates ) according to company protocol and using DNA kit ( Promega , USA ) .

**Table (1) : The sequence and source of the gene primers used in the study**

| Target Gene | Primer sequence ( 5' – 3') | Size ( bp ) | Annealing Temperature 5C | Reference |
|---|---|---|---|---|
| alg D | F: 5' - ATGCGAATCAGCATCTTTGGT - 3'<br>R: 5' - CTACCAGCAGATGCCCTCGGC - 3' | 1310 | 60 | ( 19 ) |

**Polymerase chain reaction (PCR ) :**

A PCR mixture for detection ofalgD gene was prepared from 1 µL for each forward and reverse primer , 10 µL of Go taq Green master mix Which was processed by the company ( Promega , USA ) , 5 µL of nuclease free water and 3 µL of DNA template and as shown in the table ( 2 ) .

**Table ( 2 ) : Polymerase chain reaction mixture**

| Master mix components | Volume ( µL ) |
|---|---|
| Master mix | 10 |
| Forward primer | 1 |
| Reverse primer | 1 |
| Nuclease free water | 5 |
| DNA | 3 |
| Total | 20 |

**Agarose gel electrophoresis :**

The PCR product reaction was loaded into the wells then pass an electric current with a difference voltage of 100 volts for 60 minutes , the DNA will be transmitted from cathode to plus anodes poles , the Ethidium bromide – stained bands in gel were visualized using gel imaging system .

## Result and Discussion

**Isolation and Identification :**

After performing the biochemical and microscopic examination , 52 isolates were obtained for *P. aeruginosa* , all the isolates were Cram negative, rods-shaped, the colonies were pale in color when grown onmacConkey agar because it does not have the ability to ferment lactose[20]. On sterimide agar, the colonies were bluish and greenish in color due to their water-soluble biocyanin and bioverdin pigments [21] while a transparent aura formed around the colony when it grew on the blood agar due to the hemolysin[22].

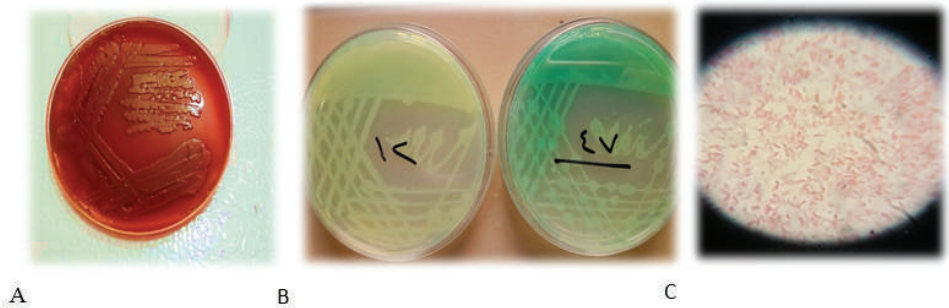


Figure (1): Shape of *P. aeruginosa*bacteria A. blood agar , B. cerimide agar , C. Microscope (oily lens)

**Figure (1): Shape of *P. aeruginosa*bacteria A. blood agar , B. cerimide agar , C. Microscope (oily lens)**

About biochemical tests, all bacterial isolates were positive for the tests : oxidase , catalase , gelatinase , citrate utilization , while were negative for indole , the result of this study were in agreement with[23] .

**The ability to form a biofilm :**

The results of the phenotypic detection showed that 20% of the bacterial isolates were not forming the biofilm while 80% were produced the biofilm in different degrees after comparing it with the negative control.

28.9 % isolates were weakly for Biofilm formation , 24.4 % isolates were moderately and 26.7 % isolates were strong , as shown in Figure (2)

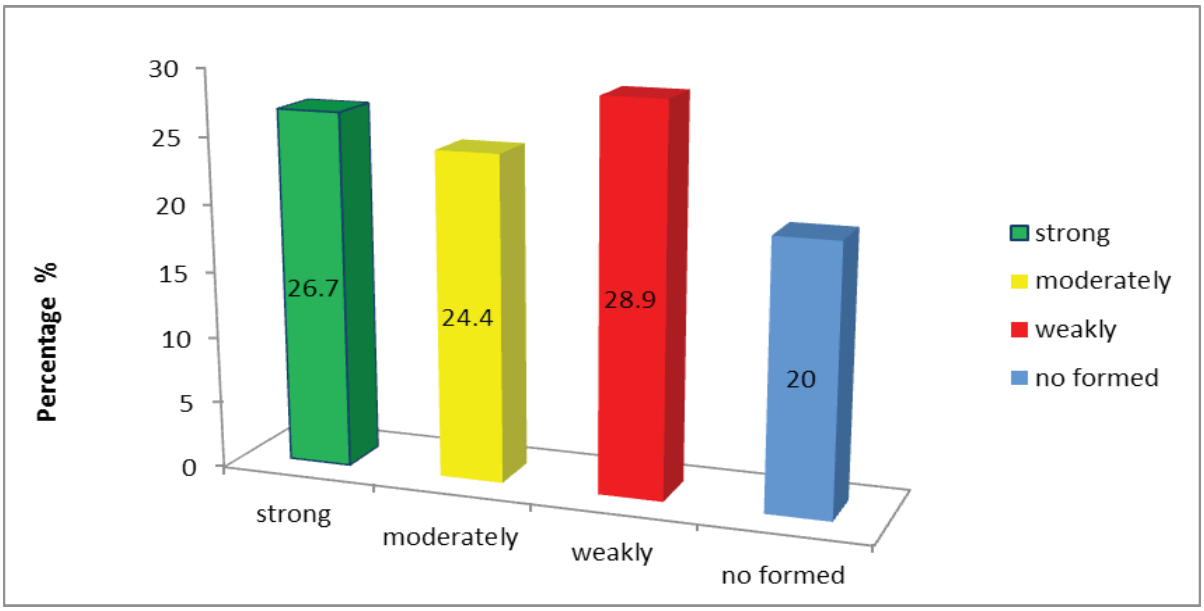


**Figure (2): Percentage of *P. aeruginosa*isolates on biofilm formation.**

The results of this study were in agreement with [24]who found that 14 % not forming the biofilm and 28 % weremoderatelyfor biofilm formation While not in agreement with[25]Who found that 24% of the isolates of *P.aeruginosa* were produced the biofilm .

This difference in the thickness of the biofilm is due to different reasons, perhaps due to the initial number of cells that succeeded in attaching to the wells of the microtiter plate or to the difference in the amount of auto inducers (quorum sensing signal particles ) which is produced from each isolate and play an essential and important role in the formation of biofilms[26] .

**Detection of algD gene :**

The results of the molecular detection of the algD gene that contribute to the production of the alginate layer in *P. aeruginosa*were: all isolates possessed algD gene about ratio 21(95.4 %) and one isolate did not possessed it , shown in figure(3), The results of this study were in agreement with[27] and [28]While not in agreement with[29].

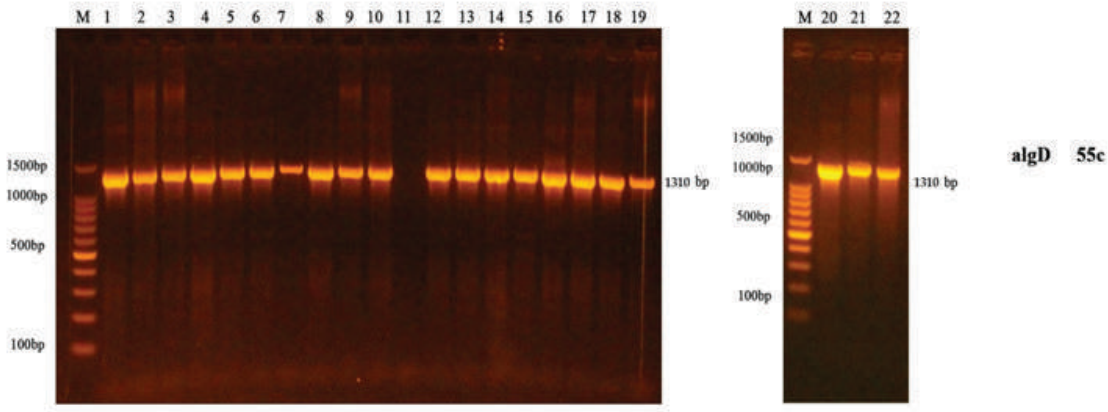


**Figure ( 3 ) : Resuts of algD gene of *P. aeruginosa* on 1,5 % agarose gel electrophoresis , ( M ) DNA ladder 1500 bp**

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of bothMOH and MOHSER in Iraq

**Conflict of Interest:** None

**Funding:** Self-funding

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# Association between Diabetes Mellitus Type-1 and Celiac Disease in Growth Retardation Iraqi Patients

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## Abstract

This study was performed in the ministry of health- Specialized Center for Endocrinology and Diabetes in Baghdad. Measurement of some biochemical parameters in serum of 93 patients with growth retardation divided into two groups: group-1 (G1) control group without diabetes, and group-2 (G2) with diabetes. The results showed that celiac disease reduces weight and consequently body mass index. The Anti-tissue and Anti-Gliadin IgA increase significantly ($p<0.01$) in G2 compared with G1, were the Anti-tissue IgA titer reached 6.88 and 65.30 U/ml and Anti-Gliadin IgA titer reached 7.69 and 72.29 U/ml in G1 and G2 respectively. In addition, the results express positive linear relationship ($p < 0.01$) among glucose level with Anti-tissue and Anti-Gliadin IgA in G2, using the regression equation of Anti-tissue and Anti-Gliadin y=4.327+0.199 (S. glucose) and y=6.027+0.102 (S. glucose) respectively.

***Keywords***: *type-1 diabetes mellitus, celiac disease, anti-tissue IgA and anti-gliadin IgA.*

## Introduction

Diabetes mellitus is one of the most common chronic endocrinology diseases [1]. In type-1 diabetes or insulin-dependent diabetes the pancreas produces little or no insulin [2], and the genetic factor contribute to type-1 diabetes appears usually during childhood or adolescence [3].

Celiac disease or gluten-sensitive enteropathy is an immune reaction in the intestine toward gluten, a protein found in wheat mainly [4], were in some persons this reaction leads to damage of small intestine lining and prevent it from absorbing some nutrients (malabsorption) causing diarrhea, stress, weight loss, and anemia [5].

The association between diabetes mellitus and celiac disease was first reported in 1960s [6]; and the average prevalence of celiac with diabetes mellitus was 4.5% [7]. Type-1 diabetes mellitus and celiac disease have same genetic background related with HLA DQ2 or HLA DQ8 and similar trigger for autoimmune processes [8 and 9]. Positive correlation in the duration was also observed between diabetes type-1 and celiac disease [10].

Anti-gliadin antibodies (IgA and IgG) are produce in response to gliadin (a prolamin in wheat). Gliadin is encoded by three different alleles which can elicit the body to produce different antibodies. Anti-gliadin IgA antibody found in 80% of patients with celiac disease [11]. IgA is useful in determining celiac

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disease because it's produced in the small intestine, where gluten causes irritation and inflammation in the sensitive people [12]. Antibodies to tissue transglutaminase (anti-tTG or anti-TG2) are found in several conditions like celiac disease, inflammatory bowel disease and type-1 diabetes [13]. In celiac disease, these antibodies involve in the destruction of the villus extracellular matrix and target the destruction of intestinal villus epithelial cells by killer cells. The deposits of anti-tTG in the epithelium of intestinal predict the celiac disease [14]. Anti-tissue antibodies (ATA) toward transglutaminases can be classified into 2 different schemes, transglutaminase isoform and immunoglobulin reactivity subclasses (IgA and IgG). The ATA IgA is more frequently found in celiac disease; however, one haplotype, DQ2.5 is found in most celiac disease, has genetic linkage to the IgA-less gene location.

Aim of this study to detect the association between celiac disease and type-1 diabetes mellitus in growth retardation patients.

## Subjects, Materials and Methods

### Subjects and Methods

Ninety-three patients with growth retardation were gathered randomize from the Specialized Center for Endocrinology and Diabetes in Baghdad, after attainment official approval and the verbal consent from participants report the age and gender; measure the body mass index (BMI) using the following equation: **BMI (Kg/m$^2$) = weight / (height)$^2$**. From each patient collect 5 ml of whole blood and after clot at 25 °C centrifuged at 10000 rpm for 5 minutes and stored at -20 °C until used.

Divide the 93 patients into two groups:

Group-1 (G1), number 50 represent control group: growth retardation patients without diabetes.

Group-2 (G2), number 43 growth retardation patients with diabetes**.**

### Materials

· **Glucose measurement (mg/dl)**: glucose concentration was analyzed by an automatic biochemical analyzer.

· **Anti-tissue and Gliadin IgA (U/ml):** Anti-tissue and Gliadin IgA titer in serum was measurement by enzyme-linked immunosorbent assay method and used a commercially obtained ELISA kit (AESKULISA, Germany).

## Statistical Analysis

Statistical package for social sciences program SPSS version 20 for window LEAD Technologies. Inc. USA (2011) were used for the estimation of result's differences between the studied groups consider the significance at P value < 0.05, using t-test for the assay of results and linear regression to find the association between diabetes type-1 and celiac disease.

## Results and Discussion

The basic bibliography of this study in table (1) expressing highly significant differences ($p < 0.01$) in the body mass index between the studied groups, and this is may be due to restriction of some types of food according to the disease state of the patients or the involvement of dietary deficiencies or changes in absorption may play a role in it [15] and this is clear in G2 where the patients underweight. Even though our data showed that BMI analysis of G1 were within the normal range and this is compatible with other study by Pitocco et al [16].

Other results in this table were obviously demonstrate the effect of type-1 diabetes on Anti-tissue IgA and Anti-Gliadin IgA concentration, at which there are highly significant elevation ($p<0.01$)

in the serum level of glucose, Anti-tissue IgA and Anti-Gliadin IgA in G2 compared to G1, also these results were clarified more in figure 1 and 2.

**Table (1): Basic bibliography of the study**

| Parameters | G1<br>Mean ± SE | G2<br>Mean ± SE | P-value |
|---|---|---|---|
| Age | 16.14 ± 6.15 | 16.92 ± 7.21 | > 0.05 |
| BMI | 20.09 ± 5.30 | 15.08 ± 2.14 | < 0.01 |
| S. glucose Mmol/L | 5.59±0.769 | 13.78±2.94 | < 0.01 |
| Anti-tissue IgA U/ml | 6.88±2.94 | 65.30±26.07 | < 0.01 |
| Anti-Gliadin IgA U/ml | 7.69±2.62 | 72.29±25.63 | < 0.01 |

Figure (1) and (2) represent the association among serum glucose with Anti-tissue IgA and Anti-gliadin IgA in G2; at which a positive linear relationship ($p < 0.01$) between serum glucose and Anti-tissue IgA (Fig. 1) assessed by the regression equation y=4.327+0.199 (serum glucose) this mean increasing level of glucose by 4.327 Mmol/L lead to increase the level of Anti-tissue IgA by 0.199 U/ml. These findings confirmed by barker et el that patients with type-1 diabetes carries 33% risk for the presence of transglutaminase autoantibodies also Anti-tissue IgA and Anti-Gliadin IgA titer [17].

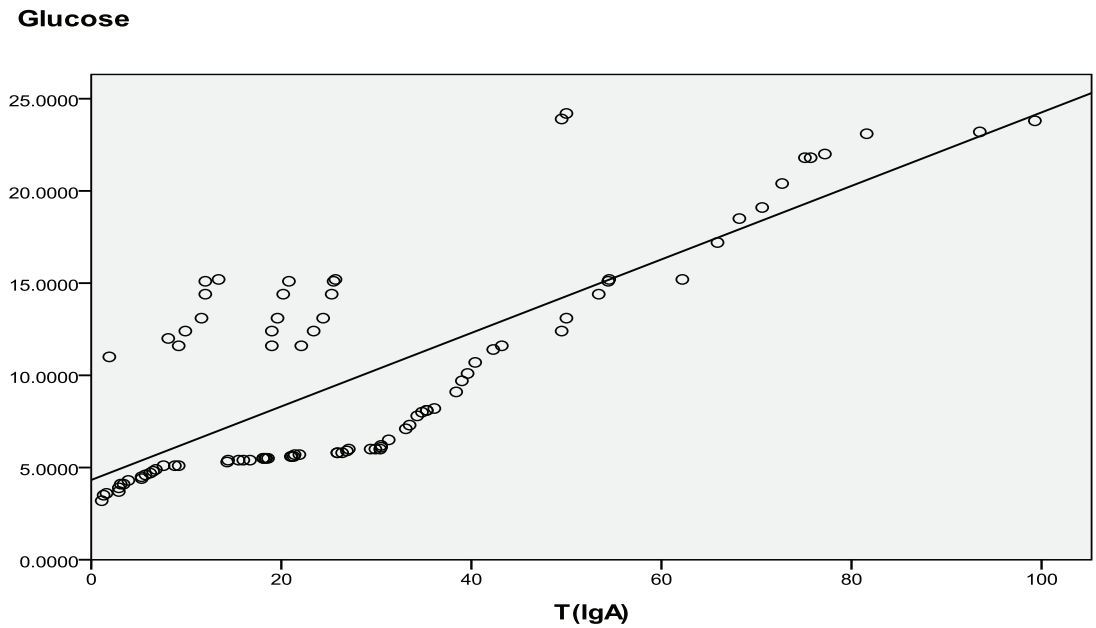


**Figure (1): Association between serum glucose and Anti-tissue IgA in group 2**

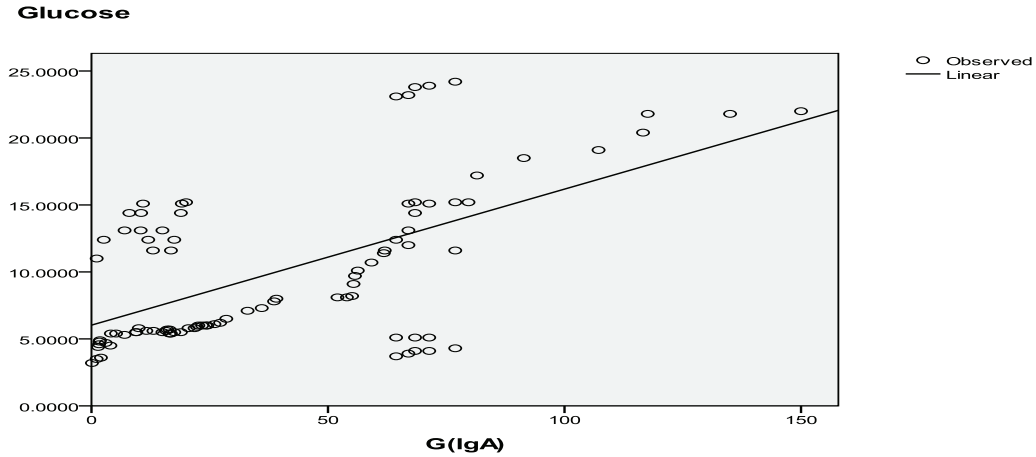


**Figure (2): Association between serum glucose and Anti-gliadin IgA in group 2**

Another positive linear relationship ($p \leq 0.01$) was found between serum glucose and Anti-Gliadin IgA in G2 (Fig. 2) which is explicit by regression equation y=6.027+0.102 (serum glucose).

The positive association between serum glucose and Anti-tissue IgA and Anti-Gliadin IgA is an indicator of the relationship between type-1 diabetes and celiac disease in growth retardation patients and this is consistent with other studies [5,7,8, 9 and 18]. The association between celiac and type-1 diabetes may be due to common genetic predisposition as suggested by increased occurrence of HLA-DR3, DQ2 encoded by the alleles DQA1*501 and DQB1*201, thus providing a common genetic basis for expression of both diseases [8,9 and 19].

## Conclusions

There is a relationship between type-1 diabetes and celiac disease confirmed by the association between serum glucose and Anti-tissue IgA and Anti-Gliadin IgA which can be considered as biomarker or indicator for growth retardation.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: Non

**Funding**: Self-funding

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# Risk Factors of Uncontrolled Hyperglycemia in Children and Adolescents with Type 1 Diabetes Mellitus

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## Abstract

**Background:** In Iraqi children with type 1 diabetes mellitus, glycemic control levels and risk factors for uncontrolled hyperglycemia are unknown. The aim of the study to assess the factors that increase the risk of uncontrolled hyperglycemia in children and adolescents with type 1 diabetes mellitus, and identify levels of glycemic control in children and adolescents with type 1 diabetes mellitus.

**Methods:** A cross-sectional study was carried out from September 21 2020 until the end of May 2021 to identify levels of glycemic control and assess the risk factors of uncontrolled hyperglycemia in children and adolescents with type 1 diabetes mellitus, for the study sample which was 209 type 1 diabetics, selected randomly from the visitors of a Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC). The American Diabetes Association assigned target HbA1c levels to patients based on their age groups. Comparison has been made of well-controlled patients and uncontrolled hyperglycemia patients. To assess each risk factor's role in uncontrolled type 1 diabetes hyperglycemia, the Odds Ratios were calculated.

**Results:** Only 17.2% of children and adolescents with type 1 diabetes mellitus were well-controlled diabetes. Better glycemic control was related to age < 6 years, BMI, and duration of type 1 diabetes <5 years. Glycemic control was not affected by gender, residence, socio-economic status.

**Conclusion:** Type 1 diabetes mellitus glycemic control among children and adolescents in Al-Basra/ southern Iraq varies widely, risking microvascular complications. In well-control type 1 diabetes mellitus patients, females were higher than males and in the age group (1-6) years, higher than the other age groups. While in uncontrolled patients with type 1 diabetes, about (57.2%) had an episode of diabetic ketoacidosis (DKA). The major risk factors for uncontrolled hyperglycemia are excessive sweet intake, fast food, and irregular meals.

***Keywords:*** *Risk factors, Children, Adolescents, Hyperglycemia, Ketoacedosis (KDA)*

## Introduction

Diabetes mellitus is a huge and growing global health problem that demands modern therapy involving greater and earlier use of intensive insulin regimens to achieve better control of blood glucose levels and reduce the long-term risks associated with the condition [(1)]. Well-controlled patients with type 1 diabetes mellitus pediatric are not only important

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to decrease complications and delay progression, but also to normal growth and development [2]. However, in most clinical settings, glycemic control is inadequate. According to one study, Just 37% of diabetes mellitus adults have achieved a 7% HbA1c level [3]. Further, in one pediatric study, the mean level of HbA1c was found to be 8.8% [4], indicating that the majority of children did not meet the recommended target level of HbA1c less than 7.5% [5]. Uncontrolled hyperglycemia may be caused by failure to take medication on time, eat too much and exercise too little. Some episodes of hyperglycemia occur for no apparent cause. Illness may also cause a rise in blood glucose levels. Hyperglycemia can damage the kidneys, nerves, blood vessels, eyes, teeth, and gums over time. Hyperglycemia-related neurocognitive complications have also been reported [6]. During a nationwide Iraq project conducted between 1 January 2012 and 31 December 2016, 818 new cases of type 1 diabetes were found, coinciding with a worldwide rise in the incidence of diabetes. Males made up 417 of these (50%). The annual incidence rate of type 1 diabetes was 7.4 per 100,000, and the prevalence rate of type 1 diabetes in people aged less than 40 was 87 per 100,000 in 2016 [7]. However, few studies in Iraqi children and adolescents with glycemic control scale type 1 diabetes as well as risk factors associated with uncontrolled hyperglycemia have been carried out in Iraq.

## Methods

A cross-sectional study was carried out from September 21 2020 until the end of April 2021 to identify levels of glycemic control and assess the risk factors of uncontrolled hyperglycemia in children and adolescents with type 1 diabetes mellitus, for the study sample which was 209 type 1 diabetics, selected randomly from the visitors of a Faiha Specialized Diabetes, Endocrine, and Metabolism Center (FDEMC). The American Diabetes Association assigned target HbA1c levels to patients based on their age groups. Comparison has been made of well-controlled patients and uncontrolled hyperglycemia patients. To assess each risk factor's role in uncontrolled type 1 diabetes hyperglycemia, the Odds Ratios, was calculated. At the time of the study, we followed the American Diabetes Association (ADA) 2014 Guidelines. The data of all patients were obtained from interview patients and the digital records of (FDEMC) as it has an internal network system and Microsoft Access program for documenting all patients' information and investigations. Statistical analysis: Together, study data are shown as {means, median, and ± standard deviation (SD)} or percentages (%). The variations between the groups of the study were established by the Chi-square test, Mann Whitney test, and the Kruskal Wallis test. Statistical significance is designated by a value of $P. < 0.05$. All statistical investigates were attained by utilizing IBM SPSS (version 25).

## Results

**Table (1) The basic socio-demographic characteristics of the study sample:**

| Parameters | | Glycemic Control No. = 209 | | P. Value |
|---|---|---|---|---|
| | | Uncontrolled HG (n = 173) | Well Control (n = 36) | |
| HbA1c% | N (%) | 173 (82.78%) | 36 (17.22%) | <0.001 |
| | Mean ± SD | 11.39 ± 2.25 | 7.21 ± 0.48 | |
| | Median | 10.90 | 7.00 | |
| | Range | 12.5 (7.8-20.3) | 1.5 (6.5-8) | |
| Age (Mean ± SD (median)) in years | | 12.45 ± 4.05 (13.0) | 10.53 ± 4.20 (10.5) | 0.016 |
| Sex | Male | 91 (52.6%) | 17 (47.2%) | 0.557 |
| | Female | 82 (47.4%) | 19 (52.8%) | |
| BMI In kg/m2 | Very underweight | 2 (1.2%) | 1 (2.8%) | 0.009 |
| | Underweight | 11 (6.4%) | 0 (0.0%) | |
| | Normal weight | 114 (65.9%) | 17 (47.2%) | |
| | Overweight | 34 (19.6%) | 15 (41.7%) | |
| | Obese | 12 (6.9%) | 3 (8.3%) | |
| | (Mean ± SD (Median) | 19.13 ± 4.15 (18.48) | 19.34 ± 4.71 (18.89) | |
| Residence | Urban | 101 (58.4%) | 18 (50.0%) | 0.355 |
| | Rural | 72 (41.6%) | 18 (50.0%) | |
| Socioeconomic status | Low | 72 (41.6%) | 12 (33.3%) | 0.243 |
| | Intermediate | 92 (53.2%) | 19 (52.8%) | |
| | High | 9 (5.2%) | 5 (13.9%) | |
| Age at diagnosis | <= 5 years | 36 (20.8%) | 12 (33.3%) | 0.260 |
| | 6 – 10 years | 82 (47.4%) | 15 (41.7%) | |
| | >10 years | 55 (31.8%) | 9 (25.0%) | |
| | Mean ± SD | 8.20 ± 3.864 | 7.31 ± 4.077 | |
| Duration of T1DM | <= 5 years | 111 (64.2%) | 31 (86.1%) | 0.031 |
| | 6 – 10 years | 48 (27.7%) | 3 (8.3%) | |
| | >10 years | 14 (8.1%) | 2 (5.6%) | |
| | Mean ± SD | 4.26 ± 3.407 | 3.22 ± 3.072 | |

**Table (2) Age grouping distribution according to Glycemic Control:**

| Age Group | Glycemic Control No. = 209 | | P. Value |
|---|---|---|---|
| | Uncontrolled HG n (%) | Well Control n (%) | |
| Total | 173 (82.8%) | 36 (17.2%) | 0.043 |
| 1 – 6 | 17 (65.4%) | 9 (34.6%) | |
| 7 – 12 | 68 (85.0%) | 12 (15.0%) | |
| 13 – 18 | 88 (85.4%) | 15 (14.6%) | |
| Mean ±SD | 12.40 ± 4.05 | 10.53 ± 4.22 | |

**Table (3) Distribution Risk Factors according to Glycemic Control of the sample:**

| Parameter | | Glycemic Control No. = 209 | | OR (95% CI) | P. Value |
|---|---|---|---|---|---|
| | | Uncontrolled (HG) n (%) | Well Control (n = 36) | | |
| Large amount of food intake | Yes | 117 (67.6%) | 16 (44.4%) | 2.61 (1.26-5.42) | 0.009 |
| | No | 56 (32.4%) | 20 (55.6%) | - | |
| Excessive fast foods (Unhealthy foods) | Yes | 123 (71.1%) | 15 (41.7%) | 3.44 (1.64-7.22) | 0.001 |
| | No | 50 (28.9%) | 21 (58.3%) | - | |
| Excessive intake of sweets | Yes | 110 (63.6%) | 10 (27.8%) | 4.54 (2.06-10.03) | <0.001 |
| | No | 63 (36.4%) | 26 (72.2%) | - | |
| Lack of regular times for meals | Yes | 164 (94.8%) | 26 (72.2%) | 7.01 (2.60-18.88) | <0.001 |
| | No | 9 (5.2%) | 10 (27.8%) | - | |
| Lack of Patient compliance with healthy lifestyle | Yes | 162 (93.6%) | 20 (55.6%) | 11.78 (4.80-28.9) | <0.001 |
| | No | 11 (6.4%) | 16 (44.4%) | - | |
| Independent management without parental supervision | Yes | 68 (39.3%) | 4 (11.1%) | 5.18 (1.75-15.31) | 0.001 |
| | No | 105 (60.7%) | 32 (88.9%) | - | |
| Diabetes ketoacidosis (DKA) | Yes | 74 (42.8%) | 1 (2.8%) | 26.16(3.5-195.3) | <0.001 |
| | No | 99 (57.2%) | 35 (97.2%) | - | |
| Have been hospitalized for your diabetes? | Yes | 105 (60.7%) | 15 (41.7%) | 2.16(1.04-4.48) | 0.036 |
| | No | 68 (39.3%) | 21 (58.3%) | - | |
| Recurrent stress and/or infections | Yes | 165 (95.4%) | 28 (77.8%) | 5.89(2.04-16.99) | <0.001 |
| | No | 8 (4.6%) | 8 (22.2%) | - | |
| Frequent unexplained hypoglycemia | Yes | 46 (26.6%) | 1 (2.8%) | 12.7(1.69-95.20) | 0.002 |
| | No | 127 (73.4%) | 35 (97.2%) | - | |
| Do you exercise regularly? | Yes | 13 (7.5%) | 2 (5.6%) | 1.38(0.3-6.4) | 0.679 |
| | No | 160 (92.5%) | 34 (94.4%) | - | |

## Discussion

A total of 209 types 1 diabetic, 36 (17.22%) with well-controlled diabetes, and 173 (82.78%) with uncontrolled hyperglycemia diabetes were already diagnosed as T1DM patients and involved in this study between the ages of (2–18) years. The results show that the patients with well-controlled diabetes in T1DM (median HbA1c = 7.00%) are significantly less than those with uncontrolled hyperglycemia diabetes T1DM (median HbA1c = 10.90%) with a statistically significant difference, was found (P < 0.001). This finding indicates that the percentage of glycemic control of diabetes in uncontrolled hyperglycemia diabetes was higher (about four times higher) than in well-controlled diabetes, which is consistent with other research like this [8] and with a statistically significant difference, was found (P < 0.001). The results show that the patients with uncontrolled hyperglycemia diabetes T1DM (median age = 13 years) are significantly greater than those with well-controlled diabetes in T1DM (median age =10.5 years) with a significant statistical difference between the two diabetic groups (P = 0.016). This study records that in uncontrolled hyperglycemia diabetes patients there were (52.6%) males, while in well-controlled diabetes patients there were (52.8%) females. This result shows that well-controlled diabetes in females was higher than in males, which is in agreement with other studies like these by [1,8–11], with no statistically significant difference, was found (P = 0.557). For body mass index, the median with uncontrolled hyperglycemia diabetes T1DM patients (18.48 kg/m2) was significantly less than that in well-controlled diabetes in T1DM patients (18.89 kg/m2), (P. = 0.009), may be associated with uncontrolled hyperglycemia diabetes and corresponds with the studies by [12,13]. The present study found that among the sampled population with uncontrolled hyperglycemia diabetes T1DM patients, 101 (58.4%) were from urban, 72 (41.6%) were from rural, whereas in well-control diabetes with T1DM patients 18 (50.0%) were from urban, and 18 (50.0%) were from rural, and without a statistically significant difference, was found (P = 0.355), and corresponds with the study by [14]. According to Socioeconomic status in uncontrolled hyperglycemia type 1 diabetes patients, 92 (53.2%) were in an intermediate socioeconomic status, 9 (5.2%) a high socioeconomic status, while in well-control type 1 diabetes, 19 (52.8%) were in an intermediate socioeconomic status, 5 (13.9%) in a high socioeconomic status, and with no, a statistically significant difference, was found (P. = 0.243), and corresponds with the study by (14). This shows that with type 1 diabetes in uncontrolled hyperglycemia patients according to the age at diagnosis, (47.4%) were diagnosed at the age (6 – 10) years old, when the mean of age for all subjects is 8.20 ± 3.864 years old, while in well-controlled with type 1diabetes patients according to the age at diagnosis, (41.7%) were diagnosed at the age (6 – 10) years old, when the mean of age for all subjects is 7.31 ± 4.077 years old. In our group of patients, the age at which they were diagnosed with diabetes did not affect the disease's metabolic control or without a statistically significant difference, was found (P. = 0.260) which is in agreement with this study [15–17]. The duration of type 1 diabetes <= 5 years, in uncontrolled hyperglycemia diabetes patients, was the highest (64.2%), when the mean of age for all subjects is 4.26 ± 3.407 years old, as well in well-controlled T1DM patients <= 5 years was the highest (86.1%) when the mean of age for all subjects is 3.22 ± 3.072 years old, with a statistically significant difference, was found (P. = 0.031) which is in agreement with this study [13].

The study discovered that among the sampled population, 88 (85.4%) of uncontrolled hyperglycemia diabetes T1DM patients were for the age group (13 – 18) years old, with a mean age of (12.40) years, whereas 9 (34.6%) of well-control diabetes with T1DM patients were for the age group (1 – 6) years

old, with a mean age of (10.53) years. This result shows that well-controlled diabetes with T1DM patients in the age group (1 – 6) years was higher than the rest age groups, patients in this group were more likely to be in the good glycemic control community, and with a statistically significant difference, was found (P. = 0.043), which is in an agreement with another study like this by [15].

According to the amount of food intake, the present study found in uncontrolled hyperglycemia diabetes patients, who had 2.61 times (95% CI = 1.26-5.42) higher than odds of good control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P = 0.009). In terms of excessive fast food consumption (Unhealthy foods), Compared with patients with an excessive fast foods intake, had 3.44 times (95% CI = 1.64-7.22) higher odds of uncontrolled hyperglycemia diabetes patients, and good control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P = 0.001) which is in agreement with this study [5]. Regarding the excessive sweets food intake, compared with patients with an excessive intake of sweets, had 4.54 times (95% CI = 2.06-10.03) higher odds of uncontrolled hyperglycemia diabetes patients, and good control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P. < 0.001). However, excess sweets intake, fast food consumption (Unhealthy foods), and a large amount of food intake have been linked to an increased risk of developing uncontrolled hyperglycemia type 1 diabetes with rather than well-controlled hyperglycemia type 1 diabetes. According to the regular times for meals, the present study found in uncontrolled hyperglycemia diabetes patients, compared with patients with a lack of regular times for meals, had 7.01 times (95% CI = 2.60-18.88) higher odds of uncontrolled hyperglycemia diabetes patients, and good control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P. < 0.001) which is in agreement with this study [5]. Regarding patient compliance with a healthy lifestyle, the present study found in uncontrolled hyperglycemia diabetes patients, compared with patient's compliance with a healthy lifestyle, had 11.78 times (95% CI = 4.80-28.9) higher odds of uncontrolled hyperglycemia diabetes patients, and well-control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P. < 0.001) which is in agreement with this study [18]. Excess sweets, unhealthy foods (mainly excessive intake of fast foods), and lack of consistent meal times were the most important factors associated with a significantly higher risk of uncontrolled hyperglycemia in the HG group relative to the good control group, as shown in **Table (3).** The present study found that diabetes ketoacidosis (DKA), in patients with type 1 diabetes with uncontrolled hyperglycemia, had 26.16 times (95% CI = 3.5-195.3) higher than the odds of well-control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P. <0.001), which is in agreement with this study [19]. However, in uncontrolled hyperglycemia diabetes patients, who were hospitalized diabetes through last week, had 2.16 times (95% CI = 1.04-4.48) higher than odds of well-control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference between well-control diabetes and uncontrolled hyperglycemia diabetes was found (P. = 0.036) which is in agreement with this study [20]. According to recurrent stress and/or infections, in uncontrolled hyperglycemia diabetes patients, who had 5.89 times (95% CI = 2.04-16.99) higher than odds of well-control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia

diabetes. with a statistically significant difference, was found (P. < 0.001) which is in confirm this study [20]. Regarding the frequent unexplained hypoglycemia, in uncontrolled hyperglycemia diabetes patients, had 12.7 times (95% CI = 1.69-95.20) higher than odds good control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with a statistically significant difference, was found (P = 0.002) which is in agreement with this study [1,21]. According to regular exercise, in uncontrolled hyperglycemia diabetes patients, who had 1.38 times (95% CI = 0.3-6.4) higher than odds well-control diabetes patients, an indicator of high risk for uncontrolled hyperglycemia diabetes, with no statistically significant difference, was found (P. = 0.679) which is in agreement with this study [22]. For people with type 1 diabetes, daily exercise has many health benefits (e.g., improved cardiovascular fitness, muscle strength, insulin resistance, etc.)

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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# Evaluating the Effect of Different Mouthwashes on the Titanium and Nickel Ions Released from Ordinary and Blue NiTi Archwires (An In-vitro Study)

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## Abstract

**Objectives:** This studywas carried out to evaluate the effect of alcohol present in mouthwash on the Ni and Ti ions release from ordinary andblue NiTiarchwire.

**Materials and Methods:** Sixty specimens of 2cm length from 0.017×0.025 inch maxillary Flexy and Blue Flexy NiTi archwire (30 per each archwires) were immersed in 15 ml. distilled water, alcoholic and alcohol-free Corsodyl mouthwashes (10 per immersing media for each archwire) for one and half hour. After that, Ni and Ti ions released in mouthwashes and distilled waterwere measured using atomic absorption spectrophotometer. Unpaired sample t-test and one way ANOVA test were used for comparison between the archwire types and among different immersion media.

**Results:** Regarding Ni ion, the release of this ion washigher significantly in distilled water followed by alcohol-free Chlorhexidine while the least amount released in alcoholic Chlorhexidine. For Ti ion, the higher amount of Ti ion was released significantly from alcoholic Chlorhexidine followed by alcohol-free Chlorhexidine and the least amount was released in distilled waterin both types of archwires.Both ions were released significantly more from conventional NiTi archwire in all mouthwashes.

**Conclusions:** Ions released from the tested archwires appear to be low with blue NiTi archwire. Ni ion was released more with alcohol-free mouthwash and just the opposite forTiion.

***Key words:*** *NiTi archwire, mouthwash, ion release, orthodontics.*

## Introduction

In early 1970s George AndreasenintroducedNickel Titanium orthodontic archwires [1].Unitek Corporation developed the NiTi alloy for clinical use with the trade name Nitinol® in 1972.The composition of this alloy was 55% of nickel and 45% titanium in an equiatomic structure[2].

Due to their excellent mechanical properties having shape memory, high spring back, wide working range, applying light continuous force and most importantly is super-elasticity, so these alloys are widely used in early stages of orthodontic treatment i.e. leveling and alignment[3,4]also being strong and resilient make these alloys beneficial in reducing number of orthodontic arch wires during treatment and thus the frequency of orthodontic appointments[5].

In an attempt to reduce the release of nickel ion and improve the corrosion resistance, recentlyOrthometric Company from Brazil manufactured the Flexy Blue-NiTi archwire. This archwire was treated by oxidation under high temperature to enhance aforementioned properties and produce by smooth

and more homogeneous surface in comparison with the conventional NiTi archwire. The surface was treated by the oxidation that change the color of the wire to blue hence the name is given.

One of the most important aspects in successful orthodontic treatment is maintaining good oral health[6], yet suboptimal levels of oral hygiene will lead to plaque accumulation that results in demineralization, white spot lesions and dental caries[7]. Orthodontists prescribe mouthwashes as prophylactic agents to prevent plaque accumulation during treatment but they may have certain adverse effects due to their chemical compositionssuch as corrosion and discoloration of stainless steel and Nickel Titanium alloys [8].

Due to their prolonged broad spectrum antibacterial activity and plaque inhibitory potential, Chlorhexidine mouthwashes is considered as the "gold standard" antibacterial mouth agents [9]. Many researches studiesthe effect of Chlorhexidinemouthwash on orthodontic wires [9-12]. Up to the author's knowledge, there is no study assessing the concentration of Ni and Ti ions released from blue NiTi archwires after immersion in alcoholic and alcohol-free Chlorhexidine mouthwash, so this study as carried out.

## Materials and Methods

Two types of NiTiarchwires namely; FlexyNiTi and Flexy BlueNiTi (Orthometric Company, Marília, Brazil) with a gauge of 0.017×0.025 inch were chosen to be immersed in two types of mouthwash (Alcoholic and non-alcoholic Corsodyl from GlaskoSimthKline Co., Brentford, U.K.) in addition to the distilled water as a control media.

A 2-cm length piece from the straight posterior portion of the maxillary archwire was cut using archwire cutter. Thirty specimens from each type of archwire were then immersed in glass tube containing15 ml of one of the immersing media(ten for each media) and incubated at 37°C for 1.5 h.Before commencing the experiment, the pH of each mouthwash and the distilled water was measured using pH meter(pH meter, Sartorius 2010, pp 25, Switzerland).

After incubation,the immersion solution was measured with atomic absorption spectrophotometer (Shimadzu AA-7000, Japan)to determine the concentrations ofNickel and Titanium ions in part per million.

## Statistical Analyses

Data were analyzed using SPSS program version 25. Descriptive statistics included means, standard deviations, minimum and maximum values while the inferential statistics included independent sample t-testand one way ANOVA then Post-hoc Tukey's test. Probability value was set at 5%.

## Results

Descriptive statistics and effect of different mouthwashes on the Ni and Ti ions release from both types NiTi archwires were presented in tables 1 and 2 respectively. The general pattern of Ni ion released appeared to be higher significantly in distilled water followed by non-alcoholic Chlorhexidine while the least amount released in alcoholic Chlorhexidine. This can be applied for both conventional and Blue NiTi archwires.

Regarding Ti ions the scenario as somewhat different. The higher amount of Ni ion was released significantly from alcoholic Chlorhexidine followed by non-alcoholic Chlorhexidine and the least amount was released in distilled water. Again this pattern was applied in both archwires

Comparing Ni and Ti ions released from the two types of NiTi archwires in different mouth washes were presented in Table 3. Generally, both ions were released significantly more from conventional

NiTi archwire in all mouthwashes except for Ti ions released in distilled water which showed non-significant difference.

**Table 1: Descriptive statistics and effect of different mouthwashes on the Ni and Ti ions released from different NiTi archwires**

| Ions | Archwires | Media | Descriptive statistics | | | | Media difference | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | S.D. | Min. | Max. | F-test | p-value |
| Ni | Flexy NiTi | D.W. | 0.414 | 0.004 | 0.410 | 0.420 | 2095.717 | 0.000 |
| | | Alcoholic CHX | 0.289 | 0.005 | 0.281 | 0.300 | | |
| | | Non-alcoholic CHX | 0.328 | 0.004 | 0.320 | 0.331 | | |
| | Blue Flexy NiTi | D.W. | 0.359 | 0.013 | 0.343 | 0.377 | 529.218 | 0.000 |
| | | Alcoholic CHX | 0.207 | 0.011 | 0.198 | 0.232 | | |
| | | Non-alcoholic CHX | 0.311 | 0.009 | 0.300 | 0.324 | | |
| Ti | Flexy NiTi | D.W. | 0.210 | 0.006 | 0.201 | 0.220 | 4113.258 | 0.000 |
| | | Alcoholic CHX | 0.395 | 0.005 | 0.389 | 0.401 | | |
| | | Non-alcoholic CHX | 0.290 | 0.002 | 0.288 | 0.294 | | |
| | Blue Flexy NiTi | D.W. | 0.207 | 0.005 | 0.200 | 0.212 | 1709.863 | 0.000 |
| | | Alcoholic CHX | 0.378 | 0.009 | 0.356 | 0.388 | | |
| | | Non-alcoholic CHX | 0.261 | 0.005 | 0.254 | 0.268 | | |

**Table 2: Post hoc multiple comparisons among different mouthwashes**

| Ions | Archwires | Media | | Mean difference | p-value |
|---|---|---|---|---|---|
| Ni | Flexy NiTi | D.W. | Alcoholic CHX | 0.125 | 0.000 |
| | | | Non-alcoholic CHX | 0.086 | 0.000 |
| | | Alcoholic CHX | Non-alcoholic CHX | -0.039 | 0.000 |
| | Blue Flexy NiTi | D.W. | Alcoholic CHX | 0.152 | 0.000 |
| | | | Non-alcoholic CHX | 0.048 | 0.000 |
| | | Alcoholic CHX | Non-alcoholic CHX | -0.105 | 0.000 |
| Ti | Flexy NiTi | D.W. | Alcoholic CHX | -0.185 | 0.000 |
| | | | Non-alcoholic CHX | -0.080 | 0.000 |
| | | Alcoholic CHX | Non-alcoholic CHX | 0.105 | 0.000 |
| | Blue Flexy NiTi | D.W. | Alcoholic CHX | -0.170 | 0.000 |
| | | | Non-alcoholic CHX | -0.054 | 0.000 |
| | | Alcoholic CHX | Non-alcoholic CHX | 0.117 | 0.000 |

**Table 3: Mean values, standard deviations and comparison of Ni and Ti ions released from different NiTi archwires immersed in various mouthwashes**

| Ions | Media | Archwires | Descriptive statistics | | Wire difference | |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | t-test | p-value |
| Ni | D.W. | Flexy NiTi | 0.414 | 0.004 | 13.034 | 0.000 |
| | | Blue Flexy NiTi | 0.359 | 0.013 | | |
| | Alcoholic CHX | Flexy NiTi | 0.289 | 0.005 | 22.279 | 0.000 |
| | | Blue Flexy NiTi | 0.207 | 0.011 | | |
| | Non-alcoholic CHX | Flexy NiTi | 0.328 | 0.004 | 5.614 | 0.000 |
| | | Blue Flexy NiTi | 0.311 | 0.009 | | |
| Ti | D.W. | Flexy NiTi | 0.210 | 0.006 | 1.334 | 0.199 |
| | | Blue Flexy NiTi | 0.207 | 0.005 | | |
| | Alcoholic CHX | Flexy NiTi | 0.395 | 0.005 | 5.295 | 0.000 |
| | | Blue Flexy NiTi | 0.378 | 0.009 | | |
| | Non-alcoholic CHX | Flexy NiTi | 0.290 | 0.002 | 17.707 | 0.000 |
| | | Blue Flexy NiTi | 0.261 | 0.005 | | |

## Discussion

After bonding fixed orthodontic appliance, orthodontists demonstrated the correct method of teeth brushing in the presence of the appliance with prescription of appropriate anti-microbial mouthwash to maintain good level of oral hygiene hence decreasing the risk of plaque accumulation and dental caries development [11].

Mouthwash containing Chlorhexidine Digluconate is considered as the gold standard that had wide range anti-microbial activity. Basically, it comes in two forms either alcohol-free or alcoholic form. The latter differs from the first one by containing 7% ethanol. Ethanol causes initial burning sensation, bringing an unpleasant taste and dryness of the mouthby damaging almost all types of bacteria in mouth – both the bad and good bacteria. One the other hand, alcohol-free mouthwash may not completely clear mouth from bacteria, but selectively targeting more bad bacteria than good creating a desirable balance to avoid further side effects or bad breath[13].

Generally, orthodontic metal alloys are at a risk of corrosion and ions release when subjected in oral environment. This can be determined mainly by the chemical and mechanical factors. NiTi alloys characterized by good corrosion stability provided by the passive protective layer of titanium oxide ($TiO_2$) and a small amount of nickel oxide (NiO)[12,14].

One of the methods used to improve the properties of NiTi alloy is oxidation under high temperature to produce light (sky) blue color form.At higher oxidation temperatures, oxygen will diffuse into the surface layer of the alloy then titanium will migrate from the NiTi alloy bulk toward the outersurface easily; consequently, the oxide layer on the alloys' surface becomesthicker and increases with increasing oxidation temperature and time, so this gives the blue NiTi archwire additional protective layer against the corrosion attack[14].

This is the first study in the world that addresses the effect of different mouthwashes (alcoholic and alcohol-free types) on the Ni and Ti ions released from blue NiTi archwire. Archwires were immersed in the selected media for one and half hours at attempt tosimulate the accumulative three monthsuse of chlorhexidine mouthwash for one minute daily [9].

Reviewing tables 1 and 2, the amount of released Ni ion appeared to be higher significantly in distilled water followed by non-alcoholic Chlorhexidine and the least amount was released in alcoholic Chlorhexidine. The pattern is applied for both conventional and Blue NiTi archwires. This could be explained by the absence of ions in the composition of distilled water making it a violent solvent. The corrosion of metals in water is influenced by the oxygen content, the level of pH, water temperature, and immersion time. The pH of distilled water was 7, so the acidity is not the matter that leads to corrosion. Yet the of lack of minerals in its contents is the major issue, so distilled water acts like a magnet that absorb ions from NiTi alloys and this comes in agreement with previous findings[15-18].

Both types of mouthwashes had almost the same pH (alcoholic=5.30, alcohol-free= 5.36) which is considered as acidic environment that attacks the protective titanium oxide leading to Ni leaks out that appears significantly more with non-alcoholic mouthwash[19].Moreover, alcohol (ethanol) with chemical formula $CH_3$-$CH_2$-OH contained in mouthwash may bind to the $TiO_2$ layer leading to leak out of Ti more with alcoholic mouthwash. Yamaguchi et al. [20] found that alcoholic solvents reduced the thickness of the protective oxide layer and affecting its structure.

Comparing ions released from conventional and blue NiTi revealed that Ni and Ti ions were released (leak out) significantly more from conventional NiTi

archwire and in all immersion media even with the presenceof the protective oxide layer (Table 3). Blue NiTi archwire had thicker oxide layer that reduced the amount of ions released, so the amount of ions release were less.

## Conclusions

Ions released from the tested archwires appear to be low with blue NiTi archwire. Ni ion was released more with alcohol-free mouthwash and just the opposite for Ni ion

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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# Assessment of Soluble PD-1 and PD-L1 in Iraqi Women Patients with Breast Cancer with Toxoplasmosis

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## Abstract

*Toxoplasma gondii* is an obligate apicomplexan intracellular protozoan parasite and considered the most common global parasite which infects a wide range of warm-blooded animals and is the etiological agent of one of the most common parasitic infections in humans. Breast cancer is the most common cancer in women worldwide; nearly 1.7 million new cases were diagnosed in 2012, making it the second most common type of cancer. The main objective of the present study was to investigate the sero-prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi breast cancer patients and to clarify the role of soluble programmed death-1 (sPD-1) and (sPD-L1) in Iraqi Iraqi breast cancer patients with toxoplasmosis. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect anti- *T. gondii* IgG antibodies in the sera of 108 patients with breast cancer and 50 apparently healthy controls. The results showed that 26(26%) samples of sera patients have been founded breast cancer with toxoplasmosis, 80(74%) samples have breast cancer, 10(20%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 40 (80%) cases samples were considered as a control group without any infections. Sera (sPD-1 and sPDL-1) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed that levels of sPD-1 and sPDL-1 levels were significantly higher in patients group than healthy subjects (P<0.01).

***Keywords:*** *Toxoplasma gondii, Toxoplasmosis, Breast cancer, sPD-1 and sPDL-1*

## Introduction

*Toxoplasma gondii* is an obligate intracellular coccidian parasite distributed widely throughout the world. The most common form of infection in humans is asymptomatic, but it causes opportunistic infections in immune compromised individuals. Symptomatic infection is usually characterized by lymphadenopathy and reticular cell hyperplasia [(1)].

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Cancer constitutes an enormous burden on society affecting both developing and developed countries and based on GLOBOCAN estimates [(2)], about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide. The occurrence of cancer is increasing because of the growth and aging of the population, as well as an increasing prevalence of established risk factors such as smoking, overweight, and physical inactivity [(3)].

Breast cancer is abnormal growth of cells and ducts lining the breast branches. The growth of these cells is random and uncontrollable and has the ability to spread to tissues, cells and other organs

of the body. Both men and women can have breast cancer. Studies have shown that breast cancer is rare in men. Breast cancer is the most common cancer in women worldwide; nearly 1.7 million new cases were diagnosed in 2012, making it the second most common type of cancer[4].

Toxoplasmosis is also considered to have a role in cancer induction. For instance, a study reported that the risk of brain cancer in human's increases in patients with *T. gondii* infection, and another study showed that the mortality rates positively correlated with the seroprevalence of *T. gondii* [5]. The latter study suggested that *T. gondii* should be further investigated as a possible oncogenic pathogen to humans [5].However, there are several studies indicating that the seroprevalence of toxoplasmosis is significantly higher in patients with cancer than non-cancer patients, including breast cancer [6-7].

*Toxoplasma gondii* is seen in some malignancies like lymphoma, acute and chronic myeloma. Recent studies show that *Toxoplasma gondii* antibodies can be seen in women with breast and ovarian cancer. Although the mechanism behind this is unknown, but there is no doubt that Toxoplasmosis paves the way for tumor development [8].

In Iraq, a recent study [9] showed that the proportion of breast cancer in females was 33.81%. In comparison with the other Arabic countries, the rate reported by Al-Hashimi and Wang [9] was very similar to that reported in Lebanon but lower than that observed in several Arab neighboring countries such as Turkey and Iran [10].

PD-1 is a trans membrane glycoprotein type I with 50~55 kDa molecular weight and composed of 288 amino acids [11]. Human PD-1 proteins have 60% homology with murine PD-1 (mPD-1) [12]. This cell surface monomer protein is an inhibitory receptor [13] and belongs to the Ig superfamily [14], specifically the CD28 cytotoxic T lymphocyte antigen-4 (CTLA-4) family [15].

Human PD-L1 belongs to the B7 family; the PD-L1 has an important role in immune evasion by tumor cells and can enhance tumor cell growth by promoting apoptosis among antigen-specific and tumor-reactive T cells [16]. PD-L1 also is necessary in maintaining immune homeostasis in normal physiological condition. This ligand down regulates cytotoxic T cell activity when it binds to specific receptors on T cells and protects normal cells from collateral damage [17]. Thus, tumor cells expressing PDL1 can hinder activation of new T cells [18]. The main objective of the present study was to investigate the sero- prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi women patients with breast cancer with Toxoplasmosis and to clarify the role of soluble programmed death-1 (sPD-1) and (sPD-L1) Iraqi women patients with breast cancer with Toxoplasmosis.

## Materials and Methods

### Subjects and Samples

This study was included 108 samples of patients with breast cancer attending to Babylon Cancer Center affiliated to Marjan Teaching Hospital in Babil Governorate, Iraq. During the period from November 2019 to June 2019. Out of this sample, a group of 50 healthy subjects were considered as control group. The age of all patients and healthy subjects were ranged from 20 – 79 year. Five ml of venous blood were collected from each subjects (patients and control) and placed in gel tube, the serum was separated and divided in ependorff tubes then stored at -20C° until it is used.

### Serological tests

**1- ELISA *T. gondi* – IgG :** The sera of all samples (Patients and control) were tested with the presence

of specific IgG antibodies of *Toxoplasma gondii*, via ELISA kits which had supported by (Bioactiva Company, Germany) and applied the test according to the manufacturer's instructions.

**2-Serum Level of PD-1:** Serum levels of **PD-1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (R&D Company, USA), according to the manufactures protocol.

**3-Serum Level of PD-L1:** Serum levels of **PD-L1** was measured by using specific enzyme-linked immunosorbent assay) ELISA) kit (R&D Company, USA), according to the manufactures protocol.

## Statistical Analysis

The Statistical analyses were done by Statistical Package for the Social Sciences for Science (SPSS) version 2010. The statistical tests was included Descriptive statistical tables, Mean, Standard Deviation, under $P>0.05$ and $P<0.01$ to considered statistically significant.

## Results

In the summarize examine results, the study samples showed that 28(26%) samples of sera patients have been founded breast cancer with toxoplasmosis , 80(74%) samples have breast cancer, 10(20%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 40 (80%) cases samples were considered as a control group without any infections (Table1).

The cut– off value of positive IgG (1 IU/ml) in all studied groups. The results recorded in the table 1 were shown higher results of levels of IgG in breast cancer with toxoplasmosis group as ±1.66 0.55 IU/ml, followed by positive control group 1.64 ± 0.35 IU/ml, and negative control group with value 0.48 ±0.24 IU/ml, while breast cancer group presented low results of this antibody 0.33± 0.25 IU/ml.

**Table 1: Levels of IgG antibodies (IU/ml) for all study groups.**

| Groups | No. of Samples | % | Mean ± SD. | Lower value | Upper value |
|---|---|---|---|---|---|
| Breast cancer with toxoplasmosis | 28/108 | 26 | ±1.66 0.55 | 1.12 | 2.61 |
| Breast cancer | 80/108 | 74 | 0.25 ± 0.33 | 0.12 | 0.72 |
| Positive control | 10/50 | 20 | 0.35 1.64 ± | 1.31 | 1.73 |
| Negative control | 40/50 | 80 | 0.48 ±0.24 | 0.15 | 0.82 |

Table (2) shows the comparisons in the means of the IgG among all studied groups, highly significant differences ($P < 0.01$) were registered when comparing the values of IgG for the patient's breast cancer with toxoplasmosis and breast cancer only, and negative control .

**Table 2: Multiple comparisons of the IgG concentrations ( IU/ml) for potential couples between studied groups.**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| IgG | Breast cancer with toxoplasmosis | Breast cancer | 0.000 | HS |
| | | Positive control | 0.748 | NS |
| | | Negative control | 0.000 | HS |
| | Breast cancer | Positive control | 0.000 | HS |
| | | Negative control | 0.657 | NS |
| | Positive control | Negative control | 0.000 | HS |
| HS: Highly Significant at P< 0.01 ,; NS: No Significant at P> 0.05 | | | | |

Table (3) showed the mean values of sPD-1 in all the groups, breast cancer patients has registered the highest value 111.39±27.36 pg/ml then breast cancer with toxoplasmosis patients 107.04±26.13 pg/ml, finally, positive and negative control groups has 106.46±10.82 pg/ml , 99.4±20.35 pg/ml respectively, also the table was referred to the highest and lowest response of sPD-1 levels.

**Table 3 : Levels of sPD-1 (pg/ml) for all study groups.**

| Groups | No. of Samples | Mean± SD | Lower value | Upper value |
|---|---|---|---|---|
| Breast cancer with toxoplasmosis | 28 | 107.04±26.13 | 85.41 | 141.54 |
| Breast cancer | 80 | 111.39±27.36 | 73.37 | 126.83 |
| Positive control | 10 | 106.46±10.82 | 81.42 | 122.31 |
| Negative control | 40 | 99.4±20.35 | 76.84 | 125.43 |

Table (4) referred to the differences of the means for sPD-1 among all studied groups, the results didn't record significant difference when comparing the level of sPD-1 in breast cancer patients with toxoplasmosis and the groups of breast cancer, positive and negative control respectively, while significant differences at probability of P<0.05 were recorded when comparing the breast cancer group with negative control.

**Table 4: Multiple comparisons of the sPD-1 concentrations (pg/ml) for potential couples**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| sPD-1 | Breast cancer with toxoplasmosis | Breast cancer | 0.43 | NS |
| | | Positive control | 0.95 | NS |
| | | Negative control | 0.22 | NS |
| | Breast cancer | Positive control | 0.55 | NS |
| | | Negative control | 0.02 | S |
| | Positive control | Negative control | 0.42 | NS |
| HS: Highly Significant at P< 0.01; S: Significant at P< 0.05 ; NS: No Significant at P> 0.05 | | | | |

between studied groups.

Table (5) showed high level of sPDL-1 in the positive control group 73.04 ±12.5 pg/ml compared to breast cancer group 71.48 ±13.32 pg/ml, negative control group 70.92 ±11.4 pg/ml and breast cancer with toxoplasmosis group 67.43±15.38 pg/ml respectively.

**Table 5: Levels of sPDL-1 (pg/ml) for all study groups.**

| Groups | No. of Samples | Mean± SD | Lower value | Upper value |
|---|---|---|---|---|
| Breast cancer with toxoplasmosis | 28 | 67.43 ±15.38 | 44.93 | 95.37 |
| Breast cancer | 80 | 71.48 ±13.32 | 45.37 | 105.23 |
| Positive control | 10 | 73.04 ±12.5 | 42.83 | 98.37 |
| Negative control | 40 | 70.92 ±11.4 | 41.28 | 108.63 |

Table (6) illustrates the differences between studied groups that found no significant differences (P>0.05) appear between a group of breast cancer with toxoplasmosis and breast cancer, positive, negative control.

**Table 6: Multiple comparisons of the sPDL-1 concentrations (pg/ml) for potential couples**

| Parameter | Group(1) | Group(j) | P-value | Sig. |
|---|---|---|---|---|
| sPDL-1 | Breast cancer with toxoplasmosis | Breast cancer | 0.17 | NS |
| | | Positive control | 0.25 | NS |
| | | Negative control | 0.3 | NS |
| | Breast cancer | Positive control | 0.73 | NS |
| | | Negative control | 0.83 | NS |
| | Positive control | Negative control | 0.65 | NS |
| NS: No Significant at P> 0.05 | | | | |

between studied groups.

## Discussion

In people with normal immune system, the infection is usually self-limited, but the parasites can survive for years in the host body in the form of tissue cysts. During this phase, tissue cysts are controlled by humoral and cellular immune system, including T lymphocytes and macrophages [19]. People with immune compromised systems, especially those with higher chronic infection risk due to cellular immune deficiency, as well as patients with cancer, collagen tissue diseases, transplant recipients treated with immune suppressive drugs, or immune-deficient hemodialysis patients with chronic renal failure, are more susceptible to be infected with *T. gondii* [20].

The susceptibility to the infection with toxoplasmosis in immune compromised could be due to many reasons such as the geographical variation, customs, habits, difference in genetic susceptibility and the acquisition method of *Toxoplasma* infection [21, 22]. Persistent infections may promote cancer because long-term host defensive responses induce inflammation, which increases mutation rates [23].

IgG antibodies indicate chronic infection and an increased titer of IgG antibodies might show reactivation [24]. These chronic infections probably persist throughout the life and may remain undiagnosed until or unless it is reactivated as a result of severe immune suppression [25].

The parasitic infection with *T. gondii* considers the most frequent protozoan causing opportunistic infections in immunocompromised individuals. However, little is known about the epidemiology of *T. gondii* infection in patients who are immunocompromised that having immunosuppressive therapy [26] . Chronic inflammation commonly stimulates carcinogenesis and may prompt an individual to cancer [27]. The present study displayed that (26 %) of breast cancer patient and (10 %) of the control group were confirmed to be positive for *Toxoplasma* IgG antibodies.

Recently, Yuan et al. [28] conducted study to determine *T. gondii* antibodies in 42 Chinese breast cancer patients by using ELISA and they found (9.53%) positivity rates of *T. gondii* IgG in cancer

patients than the control individuals. Cong et al. [29] collected blood from 67 Chinese breast cancer patients to detect anti-*T. gondii* antibodies by ELISA and reported that the prevalence of anti-*T. gondii* IgG in breast cancer patients (35.8%) was significantly higher than that in controls (17.4%). Molan and Rasheed [26] conducted study to determine *T. gondii* antibodies in 106 Iraqi breast cancer patients by using ELISA and they found (56.6%) positivity rates of *T. gondii* IgG in cancer. Ahmed and Saheb [30] detected a study to determine the serum levels of *T. gondii* IgG antibodies in 80 Iraqi breast cancer and their results showed that the overall sero positivity rate was 77.5%. Assim and Saheb [31] detected a study to determine the serum levels of *T. gondii* IgG antibodies in 90 Iraqi breast cancer and their results showed that the overall sero positivity rate was 72.22%.

T cell exhaustion is a state of cellular hyporesponsiveness that occurs in response to continued antigen stimulation or inflammation, wherein T cells produce fewer cytokines and cytotoxic molecules, lower expression levels of activating receptors, and increased expression levels of inhibitory receptors [32]. T cell exhaustion was first characterized in chronic viral infection models but is now widely studied in cancer [33], bacterial infection [34], and parasitic infection models [35, 36] and is in part programmed response to limit immune pathology in these settings.

PD-1/PD-L1 pathway is found to play a key role in escape of cancer from immune surveillance,

with PD-1 expression seen on effector T-cells and exhausted T-cells in tumor microenvironment (TME)

and PD-L1 expression seen on cell surface in several types of cancers including bladder, lung, colon, breast, kidney, ovary, cervix, melanoma, glioblastoma, multiple myeloma and T-cell lymphoma [37].

Furthermore, no study has reported the detection of serum PD-1 and PDL-1 in breast cancer patients with toxoplasmosis at less in Iraq. In this study, it was found that soluble PD-1 and PDL-1 levels was increased in both the breast cancer with toxoplasmosis patients and breast cancer compared to healthy controls, suggesting that both the breast cancer with toxoplasmosis and breast cancer patients had immune suppression.

In this respect, there are no available literatures about the role of sPD-1 and sPDl-1 in toxoplasmosis. However, some studies have evaluated the serum level of sPD-1 during other infectious disease. These studies observed significantly higher levels of sPD-1 among patients with chronic HCV [38], *Echinococcus granulosus* ([39], pulmonary tuberculosis and those whom having active pulmonary TB with co-incidental *Strongyloides stercoralis* [40] infection than control subjects free from these diseases.

High level of sPD-1 among toxoplasmosis patients may be explained based on the mechanism of soluble sPD- 1 formation by proteolytic cleavage of the membrane-bound form and nature of *Toxoplasma gondii* as intracellular pathogen that inhibition of host-cell apoptosis is one of its survival strategies [41]. Soluble PD-l inhibits the PD-1/PD-L signaling pathway primarily via interacting with the cell surface molecules, PD-1/PD-L1 binding creating a negative signal and lead to inhibit the activation and proliferation of T cells. Thus, increase in sPD-1 level inhibits the PD-1/PD-L1 signaling pathway in T cells through negative feedback. Consequently, it reduces the inhibition of T cell activation and increases the activity of the immune system for managing CL. This supports the findings of Wang *et al*. [42] who suggested that sPD-1 blocks the membrane PD-1 binding site on activated T-cells, thereby attenuating the PD-1 signaling pathway and increasing the immune response.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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# Risk Factors' estimation of Non Communicable Diseases in Al-Basrah Province/ Iraq During 2020-2021

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## Abstract

**Background:** Non communicable diseases such as cardiovascular disease, cancers, diabetes, and respiratory diseases are the leading causes of morbidity and mortality worldwide. They share risk factors such as unhealthy diet, physical inactivity, smoking, and harmful alcohol use. The share of these risk factors raises the probability of developing non-communicable diseases. The aim of study to assess non communicable diseases risk factors in Al-Basrah province during 2020-2021.

**Methods:** A cross sectional study was implemented among 250 respondents aged from 18 years and above according to random sampling method. Data was collected according to face -face interviews with those attended to different primary health care centers in Al-Basrah province from September 2020 to February 2021.

**Results:** The study found 134(53.6%) respondents suffering from at least one of selected non communicable diseases due to higher increase in behavioral risk factors. The proportion of participants with unhealthy diet was 211(84.4%) while for overall salt intake was 207 (82.8%). the prevalence of a currently smoker was 60 (24%) among participants and higher in males than females while for a currently alcohol consumption the prevalence was 1 (0. 4%).the prevalence of physical inactivity was 176 (70.4%) and this percentage higher among females. The prevalence of overweight and obesity (BMI>25 kg/$m^{2)}$ was 35.6% and 38% and this proportion was higher among females than males.

**Conclusion:** Non communicable diseases risk factors were alarming increases among population of Al-Basrah and this increase due to social transition and eating habits without any affective programs for prevention of these risk factors and control of non-communicable diseases.

***Keywords:*** *Non communicable diseases, risk factors, Basrah.*

## Introduction

Non-communicable diseases (NCDs) are diseases or medical conditions that is not infectious and cannot be passed from person to person. Currently (NCDs) are the major cause of mortality and disease burden worldwide, the four major types of (NCDs) include: cardiovascular disease, cancer, diabetes, and respiratory disease. [(1)] The causes of NCDs are multifactorial; these diseases may arise from

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a combination of underlying, non-modifiable and modifiable risk factors. [2] Non-modifiable factors are those over which a person has no influence, such as age, gender, race, and genetic, Modifiable indicators were those that can be modified by individuals, such as behavior, socioeconomic, and cultural influences ,the four major NCDs are linked to preventable lifestyle risk factors such as an unhealthy diet, lack of physical activity or poor physical exercise, obesity, and overweight, smoking, and unsafe alcohol use, all of which require a plan to reduce [3,4]In Iraq, lifestyle and eating habits have led to an increase in the number of non-communicable diseases over the decades, Rapid increase in the prevalence of some non-communicable diseases such as cancer, diabetes, respiratory diseases, and cardiovascular diseases collectively cause half of the deaths. About a third of these die before reaching the age of 70 regardless of the gaps in medical care and the population's knowledge of non-communicable diseases [5]

**Objective**s of this study was to estimate the prevalence of risk factors of non-communicable in Basrah / Iraq during 2020-2021.

## Material and Methods

The Study design was a cross sectional study was conducted in Basrah province during the period from September 2020 to February 2021.

### Ethical consideration

Legal permission was obtained from Southern Technical University, the Ministry of Health, and the Public Health Department in Basra to conduct the research.

### Study sample

The study included 250 attendants to Basrah Primary Health Care Centers. Study sampling was a random sample method. The total sample was conducted according to the equation of the minimum size of the sample collected randomly to cover all parts of Basrah province.

$$N= P (1-P) (Z/E)^2 \quad (6)$$

### Data Collection

Data collected from interviews of people who attended the (PHCCs) by Questionnaire was prepared according to the WHO stepwise survey for chronic disease, Questionnaire included three-step to provide information from participants. Step1 include socio-demographic characteristic such as age group, gender, material state, education level, residence and employment state. Step 2 includes the history of chronic disease (cardiovascular disease, cancer, diabetes mellitus, hypertension, asthma, and epilepsy). Step 3 include most risk factor contributing with chronic disease (obesity, salt intake, unhealthy diet, physical inactivity, and stress) from participant different parts of Basrah.

### Limitation of study

The study was implemented during the COVID-19 pandemic that makes face-face interviews more difficult. missing in patients with cancer diseases because fair from infection.

## Statistical Analysis

Data of the study were collected and analyzed by Microsoft Excel 2016, and SPSS version 23to estimate (mean, ± standard deviation SD, and median) with frequency and percentage. The coloration between a variable of data by chi-square test with p.value<0.05.

## Results

The founding of the study found the respondent rate (96%) from 260 respondents, the percentage of female was138 (55%) and male was112 (45%), according to age the highest percentage 59 (23.6%) was found to be among the age group of (50-59) while lowest percentage 35 (14%), regarding to

marital status the highest percentage was 195 (78%) in currently married while lowest found to be among Divorce was 8(3.2%), however, for education level the highest percentage was 76 (30.4%)found in not read or write while lowest in postgraduate was 1(0.4%),according to employment status the highest percentage was found in non-employee 169(67.7%) while lowest percentage in student 1(0.4%), Over 189 (76%) of the sample was from Basra's city center. as shown in Table (1).

**Table 1: - The socio-demographic characteristics of the study sample.**

| N | Parameter | | Frequency(N) N=250 | Percent(%) |
|---|---|---|---|---|
| 1 | Age (Mean ±SD with (Median)) in years | | 44.944±14.82(45) | |
| 2 | Age group | 18-28 | 35 | 14 |
| | | 29-39 | 57 | 22.8 |
| | | 40-49 | 56 | 22.4 |
| | | 50-59 | 59 | 23.6 |
| | | 60-69 | 26 | 10.4 |
| | | >70 | 17 | 6.8 |
| 3 | gender (Mean±SD) | Male(47.67±14.484 ) | 112 | 44.8 |
| | | Female(42.73±14.783) | 138 | 55.2 |
| 4 | Marital status | Single | 21 | 8.4 |
| | | Married | 195 | 78 |
| | | Divorce | 8 | 3.2 |
| | | Widower | 26 | 10.4 |
| 5 | Education level | Not read or write | 76 | 30.4 |
| | | Primary certificate | 58 | 23.2 |
| | | Intermediate certificate | 37 | 14.8 |
| | | Secondary certificate | 27 | 10.8 |
| | | Diploma degree | 28 | 11.2 |
| | | Bachelor's degree | 23 | 9.2 |
| | | Post graduate | 1 | 0.4 |

***Cont...*** **Table 1: - The socio-demographic characteristics of the study sample.**

| | | | | |
|---|---|---|---|---|
| 6 | Employment status | Employee | 58 | 23.2 |
| | | Non-employee | 169 | 67.6 |
| | | Other | 22 | 8.8 |
| | | Student | 1 | 0.4 |
| 7 | Residence | Center of Basrah | 189 | 75.6 |
| | | Shatt Al-Arab | 25 | 10.0 |
| | | Al Qurna | 1 | 0.4 |
| | | Al zubair | 35 | 14.0 |

The Prevalence of obesity and overweight 95 (38%) and 89 (35.6 %) was more than twice higher in the study population when compared to overweight 65 (26%) BMI was significantly higher in a female with a mean of BMI 29.91± 6.04 than the male with a mean of BMI 28.05± 4.66 as shown in table (2)

**Table (2) Distribution of overweight and obesity in study sample according to BMI.**

| N | BMI (kg\m2) | Male | | Female | | Total | P.value |
|---|---|---|---|---|---|---|---|
| | | N | % | N | % | | |
| 1 | Underweight | 0 | 0.0% | 1 | 100% | 1<br>0.4% | 0.016 |
| 2 | Normal weight | 34 | 52.3% | 31 | 47.7% | 65<br>26% | |
| 3 | Over weight | 48 | 53.9% | 41 | 46.1% | 89<br>35.6% | |
| 4 | Obesity | 19 | 32.2% | 40 | 67.8% | 59<br>23.6% | |
| 5 | Obesity II | 9 | 36.0% | 16 | 64.0% | 25<br>10% | |
| 6 | Obesity III | 2 | 18.2% | 9 | 81.8% | 11<br>4.4% | |
| 7 | Mean±SD | 28.05± 4.66 | | 29.91± 6.04 | | 29.07± 5.53 | |

Pearson Chi-Square test, df=5, n=250

The prevalence of behavioral risk factor was highly increased in both female and male in the population of Basrah with a higher percentage of unhealthy diet 211 (84.4%) and the prevalence of salt intake was higher than recommended by who (10 grams per day) was 207 (82.8%) of study sample while fruit and vegetable more than 175 (70% )with weekly frequent (less than five per week) according to fat and sugar intake highly prevalence of eating food with high fat and sugar equal to 203 (81.2% )on the other hand decline in fast food and soft drink to 125(50%) with the quarantine because of COVID 19 pandemic. The prevalence of a

current smoke was 60(24%) with significantly higher in males than females. While alcohol consumption only 1(0.4%) of the sample study. Regarding stress more than one to third of the sample with 184(73.2%) suffering from stress issues as presented in table (3).

**Table (3) Distribution of Behavioral risk factor in the study sample**

| N | Parameter | | Frequency | Percentage |
|---|---|---|---|---|
| 1 | Diet | Healthy | 39 | 15.6% |
| | | Unhealthy | 211 | 84.4% |
| 2 | Salt intake | Yes | 207 | 82.8% |
| | | No | 43 | 17.2% |
| 3 | Frequency of salt intake | I don't eat | 43 | 17.2% |
| | | Moderate | 157 | 62.8% |
| | | Always | 50 | 20. % |
| 4 | Fruit& vegetable intake | Yes | 221 | 88.4% |
| | | No | 29 | 11.6% |
| 5 | Frequency Fruit& vegetable intake | I don't eat | 28 | 11.2% |
| | | Daily | 47 | 18.8. % |
| | | Weekly | 175 | 70% |
| 6 | Fat & sugar intake | Yes | 203 | 81.2% |
| | | No | 47 | 18.8% |
| 7 | Fast food intake | Yes | 123 | 49.2% |
| | | No | 125 | 50 % |
| | | I don't know | 2 | .8% |
| 8 | Soft drink consumption | Yes | 132 | 52.8% |
| | | No | 118 | 47.2% |
| 9 | Smoker status | Yes | 60 | 24. % |
| | | No | 190 | 76. % |
| 10 | Alcohol consumption | Yes | 1 | .4% |
| | | No | 249 | 99.6% |
| 11 | Physical activity status | Yes | 74 | 29.6% |
| | | No | 176 | 70.4% |
| 12 | Type physical activity | Low | 2 | 2.7% |
| | | Moderate | 63 | 85.1% |
| | | High | 9 | 12.2% |
| 13 | Suffering from Stress | Yes | 184 | 73.2% |
| | | No | 42 | 16.8% |
| | | I don't know | 25 | 10 % |
| 14 | Type of Stress | Nervous | 170 | 84 % |
| | | Quite | 40 | 16 % |

Our study found a highly significant relationship between high salt intake, fat and sugar intake, and junk food intake with non-communicable diseases. While they are not statistically significant in terms of other variables available (diet, low fruit and vegetable intake, smoke status, physical inactivity, and stress) as shown in Table (4).

**Table (4): - Relationship between non-communicable diseases and their risk factors in the study sample.**

| N | Subject | | Presences of Non-communicable diseases | | | | Total | Df | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | | | Yes | | No | | | | |
| | | | n | % | n | % | | | |
| 2 | Diet | Healthy | 25 | 10.0% | 14 | 5.6% | 39 | 1 | 0.152 |
| | | Unhealthy | 109 | 43.6% | 102 | 40.8% | 211 | | |
| 3 | salt intake | Yes | 101 | 40.4% | 106 | 42.4% | 207 | 1 | 0.001 |
| | | No | 33 | 13.2% | 10 | 4.0% | 43 | | |
| 4 | Fruit& vegetable intake | Yes | 116 | 46.4% | 105 | 42.0% | 221 | 1 | 0.331 |
| | | No | 18 | 7.2% | 11 | 4.4% | 29 | | |
| 5 | Fat & sugar intake | Yes | 104 | 41.6% | 107 | 42.8% | 211 | 1 | 0.001 |
| | | No | 30 | 12.0% | 9 | 3.6% | 39 | | |
| 6 | Fast food intake | Yes | 56 | 22.4% | 67 | 26.8% | 123 | 2 | 0.036 |
| | | No | 77 | 30.8% | 48 | 19.2% | 125 | | |
| | | I don't know | 1 | .4% | 1 | .4% | 2 | | |
| 7 | Soft drink consumption | Yes | 66 | 26.4% | 66 | 26.4% | 132 | 1 | 0.220 |
| | | No | 68 | 27.2% | 50 | 20.0% | 118 | | |
| 8 | Smoke status | Yes | 35 | 14.0% | 25 | 10.0% | 60 | 1 | 0.390 |
| | | No | 99 | 39.6% | 91 | 36.4% | 190 | | |
| 9 | Alcohol consumption | Yes | 1 | .4% | 0 | 0.0% | 1 | 1 | 0.350 |
| | | No | 133 | 53.2% | 116 | 46.4% | 249 | | |
| 10 | Physical activity status | Yes | 37 | 14.8% | 37 | 14.8% | 74 | 1 | 0.450 |
| | | No | 97 | 38.8% | 79 | 31.6% | 176 | | |
| 11 | Suffering from Stress | Yes | 101 | 40.4% | 82 | 32.8% | 183 | 2 | 0.504 |
| | | No | 19 | 7.6% | 23 | 9.2% | 42 | | |
| | | I don't know | 14 | 5.6% | 11 | 4.4% | 25 | | |

N=250

## Discussion

This study was reported a high prevalence of self-reported non-communicable diseases among the population of Basrah and this increase due to highly increase in risk factors associated with NCDs, a better understanding of the socio-demographic as shown in table (1) determinants of disease risk factors in communities would enable us to identify at-risk populations that assist in controlling the epidemic of non-communicable diseases. [7] In most recent studies, there was a significant association between urbanization and most marine risk factors such as obesity, lack of physical activity, and decreased fruit and vegetable intake, Prevalence of non-communicable diseases, especially cardiovascular disease, type 2 diabetes, and hypertension. [8] According to the prevalence of overweight and obesity our founding higher than a survey of non-communicable diseases risk factor in 2015[9] and comparable with previous local surveys in Erbil region and Bagdad [10] and more than in Iran 59.3 % in 2016 [11] and Morocco (35.5 % overweight \ 20.6 % obesity) but less than the prevalence in Kuwait (37 % overweight \40.3 %obesity) [12] and in Jordan (>75 % overweight \obesity [13], according to physical activity, a recent study demonstrated that those who was physically inactive are nearly twice as likely to die as those who exercise. For the time being, there were worldwide plans to get the physical inactivity level down to 10 % by the year 2025. [14] the prevalence of physical inactivity among respondents higher than in Lebanon was 61.0% and Egypt was 32.1%, Palestine 46.5%. [15,16,17] Because there was no major program promoting physical activity in Basrah, more emphasis should be placed on promoting it. Policies aimed at improving health, Regrinding to smoke one from four respondents were a currently smoker and higher than WHO step survey 2015 in Iraq was 21%[18] and Palestine 20.2%and less than the prevalence in Lebanon was 38.5% and in similarity with Egypt 24.4%. [15,16,17] for Alcohol consumption in similarity with national step survey 2015 was0.6% because of agriculture of Arab word and Islamic religion that Promot to avoid Alcohol consumption [19] While the prevalence of low fruit and vegetable intake was less than recommended by those who were five Servings per day. According to studies, eating fruits and vegetables in abundance has been shown to reduce the risk of coronary heart disease, obesity, and possibly some types of cancer and heart diseases [20] so it makes sense to eat the recommended amount daily, Affordability and accessibility were crucial in low-and middle-income countries. [21]

## Conclusions

The study found that the percentage of non-communicable diseases was high among the population of Basrah especially in the elderly, this increase is attributed to an increase in risk factors due to social transformation and eating habits without any effective programs to prevent these risk factors and control non-communicable diseases.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: None

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# Eradication of Biofilm Produced by *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Wound Infection by Using Proteinase K Enzyme

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## Abstract

This research is aimed to eradicate the biofilm formed by bacteria causing wound infection through using proteinase K enzyme. For this purpose six different concentrations of proteinase K were used for the degradation of biofilm produced by Staphylococcus aureus and Pseudomonas areuginosa. These two species are the most common abundant bacteria causing infection by biofilm. Each of the concentrations was kept in contact with the pathogenic bacteria for 1, 2 and three hours. After 3 hours of incubation period the concentration (4 µg /ml) resulted in the highest eradication ability against S. aureus biofilm, while the same concentration was no significant in the eradication of Pseudomonas aeruginosa biofilm.

***Keywords:*** *Biofilms, Eradication, Proteinase K, Wound Infection, Pseudomonas aeruginosa, Staphylococcus aureus.*

## Introduction

There are different causative-agents of chronic wound infection caused by bacteria, biofilm one of the most agent that is difficult to eradicate by treatment with antibiotics. Regardless of living-style of bacteria as free-living (planktonic) or as biofilms for causing infection to wounds, presence of biofilms in wound cause chronic infection due to delaying in healing of wounds, biofilms are more than 1000 times difficult to treat by antibiotics than free-living bacterial cells, so biofilm need to new and special ways in order to eradicate biofilm [(1)(2)]. The difficulty of eradication by using antibiotics due to resistance, for example, Staphylococcus aureus are microorganisms which acquire resistance rapidly, on the other hand Streptococcus pyogenes are less resistance. Prevalence of bacteria that resist antibiotics is a big trouble in antibiotics-therapy [(3)]. Degradation of biofilms by enzymes is consider effectively way for eradication, by degradation of biofilm contents, which consider potential way to degrade biofilm, for example, the degradation of extracellular polymeric substances (EPS) one of biofilm contents. The effects of antibiotics on cells which embedded in the biofilm enhanced by protease-treatment, lead to disruption of biofilm-matrix, due to releasing of components and planktonic-cells which then cleared by the immune system [(4)]. Proteases are a large-class of enzymatic-molecules that catalyze the cleavage of peptide bonds, all living organisms have these enzymes, proteases enzymes have important physiological functions ranging from generalized-protein-degradation to very specific-regulatory-activity. In nature, proteases divided to intracellular and extracellular. Although, substrate-recognition of extracellular enzymes is little and cleave in equal efficiency both of self- and non-

self-molecules, extracellular enzymes are essential enzymes and consider as zymogens, or in their inactive form, to prevent premature of proteolytic activity which hurt the producer cell itself [5].

According to researches findings, it is believed that biofilm matrix consists of polysaccharides, in addition to surface and secreted proteins and extracellular-DNA (eDNA) are also important factors in the formation, the stability and the regulation of biofilm. Interestingly, according to recent researches, proteases role becomes very clear, that the application of different proteases on the bacterial-cultures result in reduction of formation of biofilm and in the dispersal of established biofilms [6].

The aim of the research was eradication and degradation of biofilm produced by wound infection bacteria using different concentrations of proteinase K enzyme.

## Materials and Methods

### Specimen's Collection

Swabs samples were taken from 90 surgical wounds of patients attending Medical City Hospital in Baghdad for the period from July to September 2018. The samples were taken by the attending physician of the hospital through using sterile applicator stick with cotton swabs moistened in test tubes were used to collect them.

### Isolation and Identification of Staphylococcus aureus and Pseudomonas areuginosa

All collected-swabs were transported to the laboratory in an appropriate medium. Bacteria were isolated by using the routine laboratory techniques. Each specimen was streaking on the surface of nutrient Agar, mannitol salt agar & blood agar. Thereafter, all plates incubated for 24 hours at 37°C. The isolates were identified depending on the microscopic characterization, colony morphological features, Gram staining, the biochemical tests including the catalase test, coagulase test, and others were perform according to Bergey 's Manual [7].

### Detection of the bacterial ability to produce slime layer and Biofilm formation

The tissue culture plate assay described by Mathur and et al. [8] is the most widely used and was considered as standard test for the detection of biofilm formation. This method was applied on isolates of Staphylococcus aureus and Pseudomonas aeruginosa the bacterial cells were grown in Nutrient broth overnight at 37°C under aerobic conditions. A suspension of bacterial isolate that equivalent to the McFarland No.0.5 turbidity standard were inoculated in nutrient broth and incubated for 18- 24 hours at 37°C in individual wells of sterile, polystyrene, 96-well, flat bottomed tissue culture plate stationary condition. Nutrient broth supplemented with 1% glucose. After that, 200 µl of the inoculum were transferred to the assay wells of a sterile 96-well assay plate, which corresponds to an inoculum of approximately $5 \times 10^6$ cells/well. Each plate was covered with the lid supplied by the manufacturer. Subsequently, inoculated assay plates were transferred to an incubator set at 37°C for 18–24 hrs. without shaking. Negative control wells contained sterile Nutrient broth only; while, positive control wells contained nutrient broth with bacterial cells without glucose. After incubation, assay plates were uncovered. The optical density (OD) was measured at 630 nm of each well using a multi-well plate reader to quantify overall growth of biofilm. This step is achieved to identify strains that are defective in overall growth or conditions that inhibit overall growth, resulting in decreased biofilm growth. Liquid culture was removed from each well, and non-adherent bacteria were removed by washing each well 3-4 times with phosphate-buffered saline (PBS; pH 7.4). To staining fix adherent cells prior, washed plates were incubated at 60ºC for at least 60

min. This step reduces variability caused by loss of biofilm during the staining process. Biofilms can be detected and quantified using various stains. Biofilms were stained by adding 200 µl of 0.1% crystal violet dissolved in distilled water to each well and allow at least 15 minutes for staining. After the staining reaction has been completed, excess stain was removed by repeated washing (3-4 washes) with phosphate-buffered saline (PBS). As described above. The wash solution should be clear after final last washing step and dried. Afterwards, 200 µl of 95% ethanol was added to each well for 10 minutes. All assays were done in triplicates. The amount of crystal violet extracted by the ethanol in each well can be directly quantified spectrophotometrically by measuring the OD 630 using an appropriate microplate reader [9]. As shown in (table 1) Classification based on OD values obtained for individual strains of Staphylococcus spp. were used for the purpose of data simplification and calculation.

**(Table 1): Classification of bacterial adherence by tissue culture plate method**

| Mean OD 630 nm | Biofilm Construction Adherence |
|---|---|
| OD ≤ ODc | Non–adherent |
| ODc < OD & OD ≤ 2ODc | Weakly |
| 2ODc < OD & OD ≤ 4ODc | Moderately |
| 4 ODc < OD | Strongly |

**Biofilm dispersal by different concentrations of proteinase K 4**

Biofilm stability was investigated against proteinase K treatment was tested as described by Rohde et al. [10], with the following modifications. Bacteria were grown at 37°C overnight in nutrient broth supplemented with 1 % (w/v) glucose in 96-well microtitre plates as descripted in Tissue culture plate method. Supernatants were carefully removed and each well was washed with 200µl phosphate-buffered saline (PBS). Biofilms were treated with different concentrations of proteinase K (2, 4, 8, 16, 32, 64µg/ml) in distilled water Afterward, plates were incubated at 37°C for 1, 2, 3hrs.the control wells contained media with bacteria only. Following incubation, each well was carefully washed with 200 µl (PBS), and dried for 30 second at 60°C prior to staining with 0.1% (w/v) crystal violet solution. The A630 of the adhered, stained biofilms were measured using ELISA reader. Each strain was tested at least three times and means results are presented. After staining, the plates were again washed three times with PBS. After that, the micro titer plate was thoroughly air dried at room temperature, the dye bound to the cells should be re-solubilized, i.e. eluted from attached cells with 200 µl of 95% ethanol per well. Ethanol should be gently added and thereafter the microtiter plate covered with the lid (to minimize evaporation) should be left at room temperature for at least 10 min without shaking and measured as above [11].

## Results and Discussion

Bacterial isolation and identification

From a total of 90 samples were collected, only 30

isolates (33%) had the ability to grow on the Mannitol salt agar which considered selective and differential media for genus Staphylococcus [12]. On culture media, appearance of colonies was round, smooth, raised, mucoid and glistening. Consequently, these isolates were belonging to the genus Staphylococcus. Colonies of bacteria on mannitol salt agar appeared large golden colonies with luxuriant growth and medium-color turned from pink to yellow, because some isolates able to ferment mannitol [7][13] reported that the carotenoid pigment (Staphyloxanthin) is responsible for S. aureus characteristic golden color and plays a role in the environmental fitness of S. aureus. Golden-pigment is a trait of human-pathogen S. aureus, which protect the bacterial cell from clearance by oxidation [14]. Microscopic examination was applied to all 30 isolates after staining by Gram stain to detect their response to stain, the cells appeared as Gram positive cocci mostly arranged in grape-like irregular clusters. All 30 isolates gave negative result to the oxidase test, which preformed to differentiate Staphylococcus from genus Micrococcus that usually gives positive result. Moreover, all isolates gave positive catalase test, which was done in order to differentiate Staphylococcus species from Streptococcus species that normally gave a negative result [15]. Noticeably, all mannitol fermenters were coagulase and DNase positive; Moreover, all MRSA isolates were able to give positive results for DNase and developed beta hemolysis behavior on blood agar as shown in table 2.

**(Table2): The characterization of Staphylococcus aureus**

| Test | *Staphylococcus aureus* |
|---|---|
| Gram stain | Gram-positive |
| Blood agar medium | β-hemolysis |
| Mannitol salt agar | Yellow colony |
| Catalase | Positive |
| Oxidase | Negative |
| Coagulase | Positive |
| Bacitracin susceptibility | Resistance |
| Deoxyribonuclease (DNase agar) | Positive |

A total of 25 isolates were able to grow on cetrimide agar plates, which may be suspected to be Pseudomonas sp., where further, identified according to morphological characteristics and biochemical tests. Colonies of each isolate were plate on nutrient agar showed different morphological characteristics of Pseudomonas sp. Such as mucoidal growth, smooth in shape with flat edges and elevated center, whitish or creamy in color and has fruity odor and most of them were produce pyocyanin. While colonies of Pseudomonas areuginosa grown on MacConky agar medium appeared pale in color, with irregular edge, oval and large. On blood agar Pseudomonas areuginosa was able to hemolyse blood agar completely, these

results are reasonable with the results demonstrated by Collee et al., [16]. Microscopical examination of Pseudomonas sp. showed that the cells were gram negative, bacilli, appeared single, pairs or short chain and non-spore forming. These results are comparable to the reported morphological characteristics of Pseudomonas aeruginosa. These results were agreed with Holt et al., [17] who certify the identification. Biochemical tests for Pseudomonas sp. were made also. Results indicated in (table 3) showed that these isolates gave a positive result for oxidase and catalase which indicate that these isolate belongs to Pseudomonas aeruginosa.

**(Table 3): Morphological and biochemical characteristics of the isolated Pseudomonas aeruginosa.**

| Test | Result |
|---|---|
| Colony color | Green |
| Cell shape | Rod or Bacilli |
| Gram stain | Negative |
| Catalase | Positive |
| Oxidase | Positive |
| Growth on King A | Positive |
| Growth on King B | Positive |
| Growth on cetrimide | Positive |
| Citrate utilization | Positive |
| Growth at 4ºC | Negative |
| Growth at 42ºC | Positive |

**Detection of the bacterial ability to produce slime layer and Biofilm formation**

For detection formation of biofilm, microtiter palte assay (MtP) is the most common way and consider as standard test for detection [18]. As reported, MtP-method is most sensitive, accurate and reproducible screening method in the determination of biofilm-production by clinical isolates of Staphylococci and Pseudomonas in addition to MtP advantage as a quantitative tool which used in comparing the adherence of different strains [8]. Using MtP-method for the detection of biofilm formation by S. aureus and P. aeruginosa isolates. When grown in nutrient broth without any supplementation, 100% S. aureus isolates were able to form weak biofilm. In the presence 1% glucose lead to enhanced biofilm forming capacity in for 66% of S. aureus isolates moderate producers. While P. aeruginosa isolates were able to form moderate biofilm in presence1% glucose.

**Eradication of biofilm by Proteinase K enzyme**

To investigate the factors that contribute to biofilm degradation, we performed an enzyme treatment on the formed biofilm. Enzymatic solutions with concentrations (2, 4, 8, 16, 32,64µg/ml) of

proteinase K. Proteinase K treatment hampered the biofilm development of S. aureus isolates. All isolates, (weak biofilm-producing strain), showed significant inhibition in biofilm growth when treated with 2 µg/ml proteinase K after 2hr. as shown in (Fig. 1). Proteinase K enhances dispersal in S. aureus biofilms: To investigate the biofilm dispersal activity of proteinase K against S. aureus biofilms, proteinase K treatment was given to 1, 2, 3, hrs. Proteinase K treatment of S. aureus biofilms caused a significant disruption of all S. aureus biofilm. The proteinase K can be used in biofilm dispersion. Interestingly, significant; but not 100%; removal of biofilm was achieved by treatment with the proteinase K which has a wide specificity as other proteases enzymes in biofilm degradation [19]. The proteinase K have frequently been used as efficient biofilm removal agents that hinder bacterial adherence and biofilm formation in S. aureus presumably through degradation of surface structures, also reported that proteinaceous-biofilms formed by S. aureus with the help of Bap proteins were susceptible to proteinase K mediated detachment and dispersal. Our findings showed that (4 µg/ml) the best concentration of enzyme have a degrading effect against S. aureus biofilm. Among various surface proteins in S. aureus, has been reported to have a major role in early adhesion, as well as in the biofilm development [20]. After proteinase K treatment, a significant decrease in the protein and extracellular DNA (eDNA) but not in the carbohydrate content in extracellular polymeric substance (EPS). Extracellular DNA (eDNA) is also known to play very important role in S. aureus biofilm stability [21]. This enzyme targeting peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids [22]. It is proved that proteinase K degrade effectively the EPS of P. aeruginosa biofilms, by binding and hydrolysis of the protein molecules due to converting them into smaller units and been transported to through cell membranes, the metabolized. In most cases proteins seem to be the main constituents of the biofilms EPS and are found mostly at the outer layer of the P. aeruginosa biofilms [23]. So that, breaking of EPS in addition to proteins of outer layer by proteinase K, will make proteinase K better than other enzymes like amylase which only degrade the proteins of EPS, these enzymes are less efficient than proteinase K in degradation of P. aeruginosa biofilms [24].

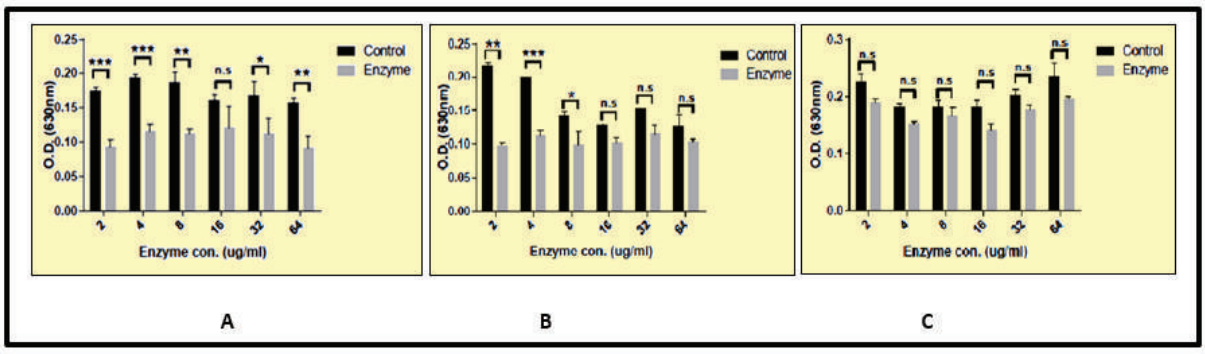


**Figure (1): Effect of different concentration of proteinase K on the formation of Staphylococcus aureus biofilms after 1hour of treatment.**

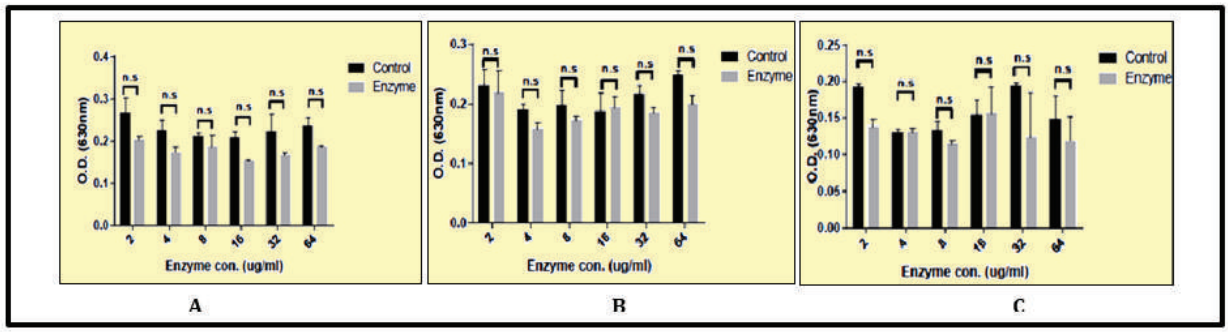


**Figure (2): Effect of different concentration of proteinase K on the formation of Staphylococcus aureus biofilms after 2hours of treatment.**

Figure (1): Effect of different concentration of proteinase K on the formation of *Staphylococcus aureus* biofilms, (A) show degradation activity of proteinase K after one hour of treatment (B and C) are the influence of proteinase K after two and three hours of treatment respectively.

Figure (2): Effect of different concentration of proteinase K on the formation of Pseudomonas aeruginosa biofilms, (A) show degradation activity of proteinase K after one hour of treatment (B and C) are the influence of proteinase K after two and three hours of treatment respectively.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: None

**Funding**: Self-funding

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# Study Some New Metallic Coordination Complexes and their Antibacterial Activity Against Methicillin- Resistant *Staphylococcus Aureus*

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## Abstract

The ligands were prepared by condensing aldehydes with aromatic amines, then they were held together with Ruthenium element Ru (III) and gold Au (III). Spectroscopic studies were conducted on them and demonstrated that the gold complex was a good conductor opposite to the ruthenium complex depending on its molar conductivity value**.** The complex was described by measuring the spectrum of FT. IR (C. MASS JV-VISIBLE and C.H.N). From spectroscopic data, the octahedral geometry of the ruthenium and Square Berner complex has been proposed for the gold ligands nodes. Furthermore, ligands and complexes have been selected for the activity of the methicillin-resistant golden cluster bacterium and the study of gene expression, i.e., the impact of ingested substances on RNA bacteria (MAR).

***Keywords*** *: Complex , schiff base , MARS , ligand , coordination , staphylococcus , ruthenium .*

## Introduction

Schiff's Base are of great chemical importance because they possess highly efficient ligands towards many ions, transition, and non-transition elements, and they are huge groups of metal-organic compounds. Besides, raw materials for the manufacture of heterogeneous ring compounds. [(1)] the Schiff Base Complex for Transition Metals contributes to multiple applications and in various fields, such as pharmaceutical medicine, industrial applications, organic creation, and biologic stimulation, as inhibitors against several types of bacteria and fungi. In the pharmaceutical field, it has been used as an anti-inflammatory. [(2)] anti-cancer, anti-cramping, tuberculosis, and low blood pressure .[(3, 4)] In the industrial area, they are used as anti-corrosion materials, polymerization catalysts, ink dyeing, and printing [(5.6).] The Schiff Base (imin compounds) consist of a condensate reaction of primary amines with aldehydes first prepared by the German scientist Schiff in 1864 and is distinguished by its containment of the effective isomethane group (C = N) and its formula [(7)] .

The efficacy and biological activity of isomethane complex are due to the correlation or hydrogen bonding between them and the transition elements, where the biological activity of the complex is enhanced and the cytotoxicity of each complex element is reduced [(8).]

### Expermantal part

Chemicals, solvents, and salts of elements have been used according to the companies producing

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them. All materials used are high purity:

3,4-Di chloro benzal dehyde (Aldrich )

4-Formyl benzo nitrile ( Aldrich )

4-Nitro –O- phenylene diamine ( Aldrich )

3,4- Diamino toluene ( Aldrich )

$Rucl_3$, $HAucl_4$. $3H_2o$ ( Aldrich )

Enthanol , methanol ( Scharlau )

**Instrument**

FT.IR was measured within the range of 400-4000cm-1schimadzu (KBr Disc), UV-Vis, lc-Mass, and C.H.N measured and melting point measured.

**Working methods**

- L1, L2 and its equipment N1.N2.N3.N4.N5

1.75 mg of 3,4-Di chloro benzal dehyde was dissolved in 25 mL of absolute ethanol in a 100 mL baker and heated to melting and dissolving 0.766mg of 4-Nitro-O-phenylene diamine in 50 ml of absolute ethanol in a baker's capacity of 100 ml and heated until melting and then mixed the contents and raised the mixture for 3h until the solution precipitated and cooled with ice and filtered and collected the sediment and was recrystallized in ethanol [9.10].

The ratio of 2 ligands was then dissolved in a mixture of (ethanol and methanol) and 1 ml of ruthenium chloride or gold chloride dissolved in ethanol [11, 12], then rose for 4h, filtered the solution, and washed with water and ethanol.

**(Table-1) Physical property and analysized for Azomethine compounds and its Metal complexes**

| Comp code | Molecular formula | M.wt | M.P. (oC) | Colour | Yield (%) | C% | H% | N% | CL % | M% |
|---|---|---|---|---|---|---|---|---|---|---|
| L1 | C20H1202N3CL4 | 467.13 | 205-207 | Yellow | 68% | 52.04<br>51.42 | 2.98<br>2.37 | 9.51<br>9.00 | 30.68<br>30.36 | -- |
| L2 | C23H16N4 | 348.41 | 240-242 | Orangish white | 81% | 79.85<br>79.29 | 4.01<br>4.63 | 17.23<br>16.08 | | -- |
| N1 | C40H22N6O4AuCl9 | 1166.68 | 300 < | Green | 83% | 41.44<br>41.18 | 2.40<br>1.90 | 8.07<br>7.20 | 27.99<br>27.35 | 17.08<br>16.88 |

**Anti bacterial activity ( MARS )**

The bacterial activity of ligands and the complexes prepared in the laboratory were tested to inhibit the growth of bacteria by the method of diffusion by drilling at a concentration of 15 mg/ml by dissolving the ligands and the complexes with a Dmso solvent [13].

- Gene expression (molecular) was studied during molecular diagnosis of bacterial isolation:

a. Isolation of DNA methylation.

b. Diagnosis with PCR technology.

c. Electrophoresis of acarose gel.

After confirmation of isolation and proof that it was a positive, methicillin-resistant Staphylococcus aureus, RNA was extracted and transferred to CDNA

and the gene expression was determined by PCR examination.

## Results and Discussion

ligands were prepared by reacting aromatic aromatic aldehydes and aromatic amines by dissolving them in ethanol and then preparing their complexes with two elements of ruthenium and gold in a ratio of 2: 1 through the consistency between the nitrogen atom in (HC = N) and the elements. It was found that all the compounds dissolve in DMSO and DMF, and most of them dissolve in the rest of the solvents in varying ways, such as chloroform, methanol, and ethylene glycol. The prepared compounds were diagnosed by C.HN, FT.IR, UV.VIS and LCMASS spectroscopy and the results were identical to the suggested formulas.

### Infrared spectrum

The infrared spectra of the gold and ruthenium complexes were determined and compared with the spectra of ligands, where they showed clear and strong signals at 1610-1661 belonging to the C = N group of azomethine (14), and the mixture appeared to ruthenium at lower frequencies where exposure occurred in the C-N beam due to the difference of the ligands [15]. When the complexes are formed, the absorption sites of the azomethine group are shifted to either a high or low frequency, and this shift is a clear indication of symmetry between the nitrogen atom belonging to the group (C = N) and the gold ions and ruthenium [16]

### Magnetic studies

The sensitivity measurements for the Ru (III) and AU (III) ruthenium complexes are characterized by the magnetic DIAA properties indicating that the hybridization is d2sp3 and the shape of the Au (III) octahedral complexes (17).

Ultraviolet radiation spectrum UV.Vis

Fixed UV. vis spectra of Ru (III) and Au (III) complexes in DMSO solution within the range of 200-800 nm as in the table:

**(Table-2) Electronic spectrum analysis for ligand and Its Metal Complexes , magnetic moments ,proposed structure and metal complexes**

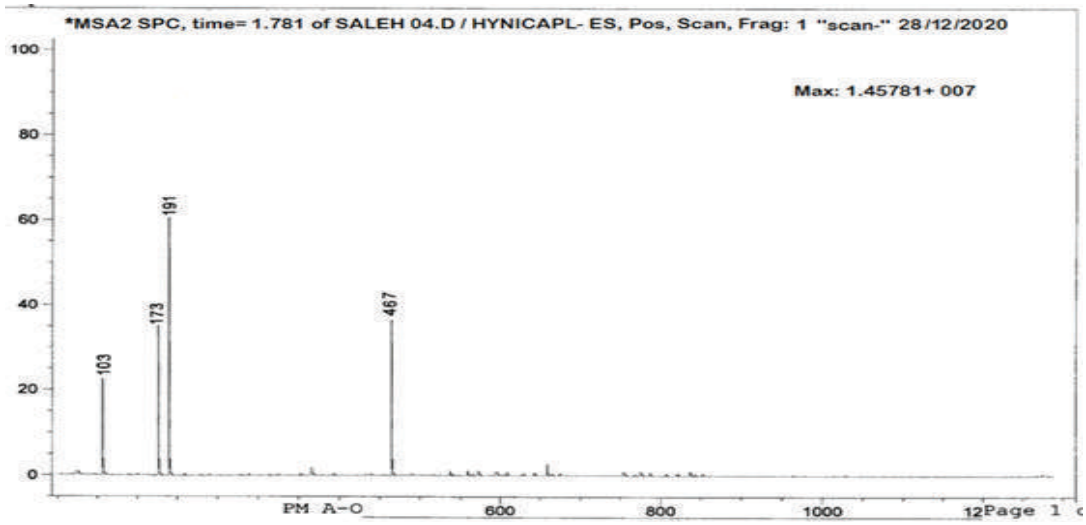
| Compounds | λmax | ύcm -1 | Transition |
|---|---|---|---|
| L1 | 322 | 31055 | *π-π |
| | 364 | 27472 | n-π* |
| L2 | 313 | 31948 | *π-π |
| | 354 | 28248 | n-π* |
| [Au (L1)2]Cl | 278 | 35971 | Ligand field |
| | 444 | 22522 | C.T |
| | 618 | 16181 | 1A1g 1B1g |
| | 677 | 14771 | 1A1g 1A2g |
| [Au (L2)2]Cl | 335 | 29850 | Ligand field |
| | 447 | 22371 | C.T |
| | 616 | 16233 | 1A1g 1B1g |
| | 679 | 14727 | 1A1g 1A2g |
| [Ru(L1)2Cl2] | 265 | 37735 | Ligand field |
| | 320 | 31250 | C.T |
| | 542 | 18450 | 1A1g 1T2g |
| [Ru(L4)2Cl2] | 263 | 38022 | Ligand field |
| | 327 | 30581 | C.T |
| | 542 | 18450 | 1A1g 1T2g |
| [Ru(L1)(L4)]Cl3 | 254 | 41322 | Ligand field |
| | 343 | 29154 | C.T |
| | 541 | 18484 | 1A1g 1T2g |

The absorption spectra of the azomethene group of 280 - 242 nm, which belong to the Likandian domain, which did not change at complexity (18), and 343-320 nm signals attributed to the charge transfer, appeared due to the electron transfer from ligands to the metal's orbit [19].

Also, transfers appeared at 542 back to transfers of ruthenium complex, that is, d-d-transfer, it was suggested that the octahedral figure [20], as for the gold complexes, the gold complex showed transfers to the ligand domain within the range of 335 - 218, the cargo transfers within 459-320, and the gold complex d-transfer within 679-574. All this confirms that the shape is a square level [21].

- Mass Spectra

The mass spectrum explains the basic critical LC Mass (M.), which is the partial ion package that validates the proposed formula equivalent to a mass that appeared for ruthenium at 869 and Mix Ru at 987.51 m/z [22].




**(Figure 1 mass spectrum of ruthenium at 869**

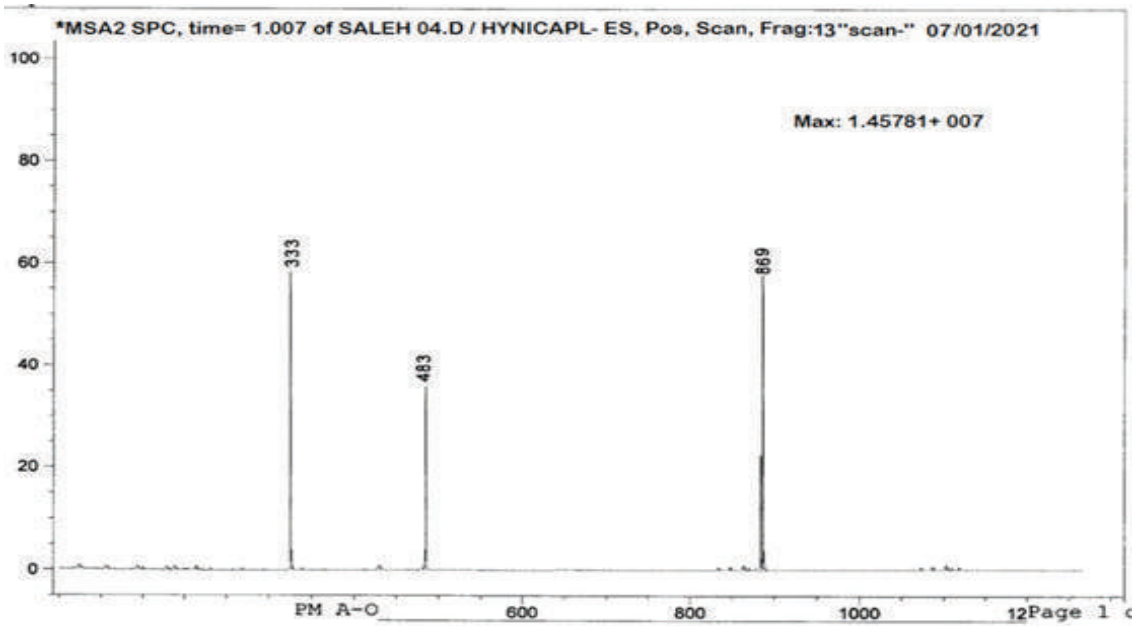



**Figure 2 mass spectrum of ruthenium at 987.51 m/z**

**Figure 2 mass spectrum of ruthenium at 987.51 m/z**

Bioactive

The effect of the bioactivity of ligands and complexes prepared with Au (III) and Ru (III) ions on pathogenic methicillin-resistant staphylococci has been studied, with inhibition values showing good standards for each other and with little *( 23 )* concentration.

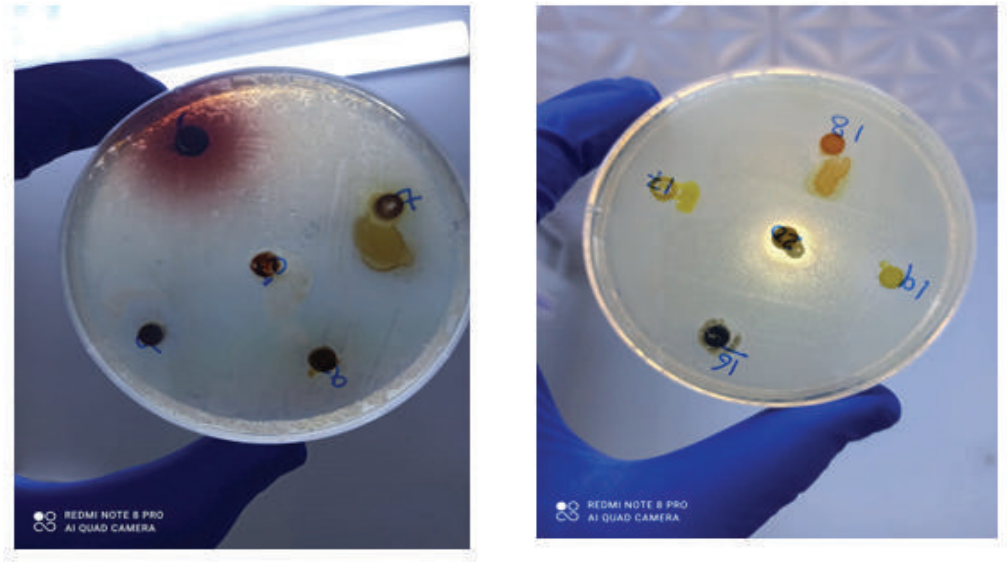



**Figure 3 The effect of the bioactivity of ligands and complexes prepared with Au (III) and Ru (III) ions on pathogenic methicillin-resistant staphylococci**

**Gene expression and molecular diagnosis**

DNA (S-aureus) was studied and recovered. After confirming that isolation was required in the study, RNA was recovered for samples treated with chemicals with the sample of (Control)**.** Besides, the extent of gene expression was then measured and determined by the PCR examination, where the ligands and complex were very efficient in reducing the amount of gene expression of the NORAs up to Max gene and had a clear and strong effect in reducing the value of gene expression (24) as in table(3 ) .

**Table 3: The amount of gene expression of the NORAs up to Max gene and had a clear and strong effect in reducing the value of gene expression (24)**

| Sample | 2(-ΔΔct) | Fold of expression | Conclusion |
|---|---|---|---|
| L1 | 0.002 | 500 | Down- regulation |
| L2 | 0.0003 | 333.33 | Down- regulation |
| N1 | 0.325 | 3.07 | Down- regulation |
| N2 | 46.85 | 45.85 | Down- regulation |
| N3 | 0.031 | 32.25 | Down- regulation |
| N4 | 0.146 | 6.84 | Down- regulation |
| N5 | 0.389 | 2.57 | Down- regulation |

## Conclusion

Based on the diagnostic spectroscopic results of the ligands and the complexes under study, the carbonyl group C = O of aldehyde was established to be associated with the amine group in amines and formed the ligands and was then held with the transition elements Au and Ru and formed two-clawed complexes at a ratio of 2: 1. The shape of the gold complexes was Flat Square and ruthenium complexes for octahedral surfaces. The prepared complexes and ligands showed good impact and activity against bacteria used and had clear and strong gene expression in reducing bacterial resistance by decreasing the specific virulence factor NOR A of MARS bacteria.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: None

**Funding**: Self-funding

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# Load Deflection Properties of Small Diameter Titanium-Niobium-Tantalum-Zirconium Archwire(An In Vitro Study)

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## Abstract

**Objectives:** This study aimed to evaluate the load deflection properties of new niobium-based beta titanium archwire (Gummetal) in comparison with superelasticnickel titanium (SE-NiTi) and copper NiTinickel titanium (Cu-NiTi) archwires.

**Methods and Material:** Gummetal, superelasticNiTiand copper NiTiarchwire segments of 0.014-inch diameter were examined by three point bending test, using Instron testing machine with 10 Newton (N) load cell. Wire segments were tested at 2 and 4 mmdeflections, and at a temperature of 37±1°C. One-way analysis of variancewas used to compare the means of the groups at a significance of $p < 0.05$.

**Results:** At 2 mm deflection, the maximum force values and unloading forces of Gummetalwere significantly higher than those of the control (SE-NiTi and Cu-NiTi) archwires. At 4 mm deflection, there was no significant difference between the maximum force values of Gummetal and SE-NiTiarchwires, however they were significantly higher than those of Cu-NiTiarchwire. Unloading forces of Gummetalarchwire at 4 mmdeflection were initially significantly higher, then became significantly lower than the control wires at 1 mm unloading deflection point.

**Conclusions:** The present study showed that Gummetalarchwirewas less efficient in providing continuous forcesin comparison with SE-NiTi and Cu-NiTiarchwires.Gummetalarchwire cannot be considered superelastic, and its use in the alignment phase may better be limited to mild crowding cases.

***Key words**: Titanium-niobium-tantalum-zirconium; Gummetal;load deflection properties; three-point bending test.*

## Introduction

Aligning archwires that are used at the beginning of the orthodontic treatment are intended to relieve dental crowding and rotations with light continuous forces; therefore these wires should ideally havegood springback, formability, joining ability, biocompatibility, low friction and a low cost [1,2]. Nickel-titanium (NiTi) alloy wires are most commonly used during the leveling and alignment stage of orthodontic treatment owing to their superelasticity and excellent springback properties[3].Despite these advantages, NiTi wires have drawbacks, such as their poor formability, which prevents adding detailing bends to compensate for incorrect bracket positioning at the beginning of orthodontic treatment;they are also not shape-formable and may cause expansion or contraction in the canine and/or molar region when an ideal arch form is not available;in addition, they may not be suitable for patients with nickel allergy due to their high nickel content [4].

A new niobium-based beta titanium alloy wire has recently been developed. Its chemical composition in mole percentage is Ti 23, Nb 0.7, Ta 2, Zr 1.2, along with oxygen [5]. The new titanium-niobium-tantalum-zirconium (TiNbTaZr) archwire is manufactured by Rocky Mountain Morita Corporation in Japan and is marketed under the trade name Gummetal®. According to the manufacturer, the wire is nickel-free, superelastic, highly formable, has a low coefficient of friction and a high springback effect without hysteresis. These properties can make Gummetalarchwire a good alternative to NiTiarchwires in the alignment phase of orthodontic treatment when a formable and nickel-free wire is needed.Some of the mechanical properties of Gummetalarchwirewere studied, including its bending stiffness, torque moment, cyclic fatigue behaviour, and frictional forces [3, 6-8]. In addition, the effectiveness of Gummetal as initial archwire in aligning teeth was compared clinically with that of superelastic-NiTiin a double-blind randomized clinical trial, and the study showed no significant difference in performance between these two types of wires, thus it was suggested that a laboratory study would be more appropriate to determine precise differences between Gummetal and NiTiarchwires[4].

Since limited information are available in the literature regarding the load deflection behavior of TiNbTaZr (Gummetal) archwire, the aim of the present study was to evaluate the load-deflection properties of small diameter TiNbTaZr (Gummetal) archwirein comparison with superelastic-NiTi and copper-NiTiarchwires, at 2 mm and 4 mm deflections, by using three-point bending test.

## Methods and Material

In this study, three different types of titanium archwires, with a diameter of 0.014-inch were tested, as shown in table 1.

**Table 1 Orthodontic wires tested.**

| Type of the wire | Manufacturer | Dimension | code |
|---|---|---|---|
| Titanium-Niobium-Tantalum-Zirconium | JM Ortho Corporation, Tokyo, Japan | 0.014-inch | TiNbTaZr (Gummetal) |
| Superelastic- Nickel Titanium | Dentaurum, Ispringen, Germany | 0.014-inch | SE-NiTi |
| Copper-Nickel Titanium 27° | Ormco Corporation, Glendora, Italy | 0.014-inch | Cu-NiTi |

The specimens were prepared by cutting the two straight end sections from each type of wire; the sections were 30 mm in length. A custom acrylic block with two fulcrums was madefor the three-point bending test. A central incisor and a canine bracket (Dentaurum, Ispringen, Germany) with a slot size of 0.022 × 0.030 inches were bonded to the fulcrums with an inter-bracket span of 14 mm, which is equivalent to the distance between the central incisor and canine brackets [9].To eliminate the effect of tip and torque, a 21×25 stainless steel wire was ligated into the brackets before bonding them.

Because a heat-activated wire (Cu-NiTi 27°C) was used as a control in this study, all wire specimens were tested inside a digital water bath (HH-S, Zhejiang, China) that was placed at the base of the

testing machine.

The acrylic block was fixed to the base of the water bath. The water inside the bath was kept at a temperature of 37±1°C. Before starting the testing procedure, each wire specimen was ligated into the brackets on the block using elastomeric ligatures and left submerged in the waterfor at least 60 seconds to achieve thermal equilibrium[10]. The test was then carried out by using universal testing machine (Instron H50KT Tinius Olsen testing machine, England) with a 10 N load cell. The force was applied by chisel-edge blade attached to the moving part of the Instron machine in a vertical (occluso-gingival) direction toward the middle portion of the wire at a crosshead speed of 2 mm/min for both loading and unloading as shown in figure 1. The wire specimens were deflected by 2 and 4 mm in separate load deflection tests. The test was repeated five times for each type of wire in both deflections, thus a total of 30 wire sections were tested. Load values were gathered from the unloading (deactivation) curve, as these represent the forces that the wire exerts to move the teeth when it elastically springs back after being placed [11,12], in addition to the maximum force value. Load values were reported at 1, 2, 3 and 4 mm when the wire specimen was deflected by 4 mm, and at 1, 1.5and 2 mm when the wire specimen was deflected by 2 mm [9].

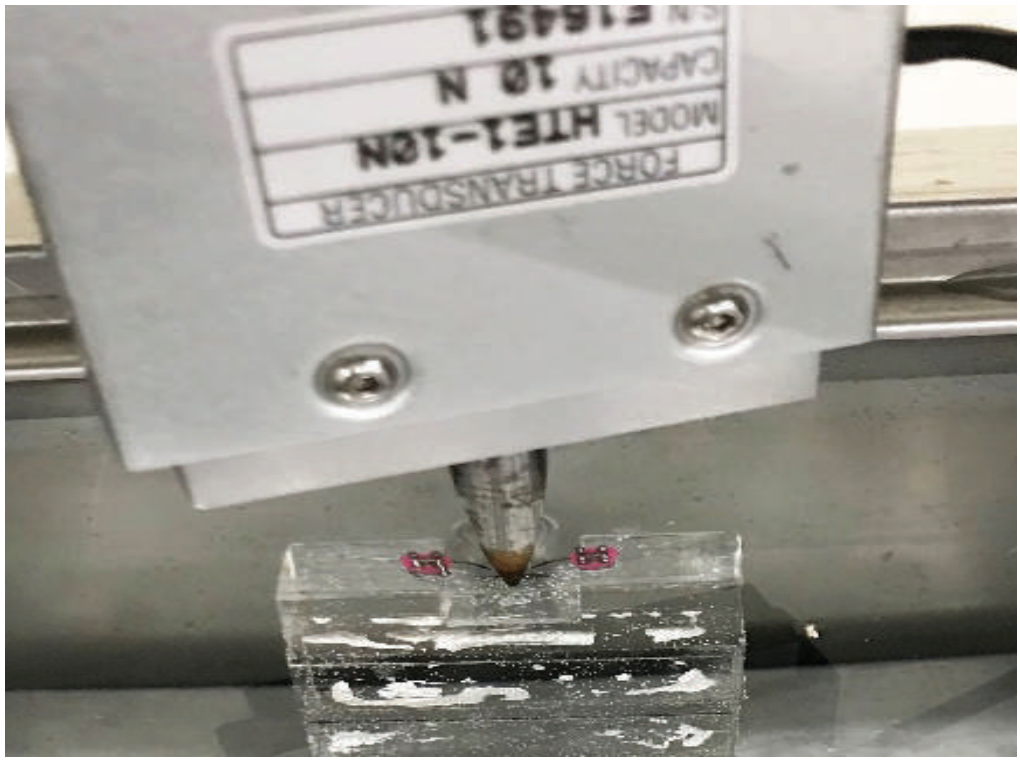


**Figure 1: Wire segment during testing**

## Statistical Analysis

The data were analysed using SPSS software (SPSS Inc., Chicago, Illinois, USA) version26 for Windows. The Shapiro–Wilk test was used to assess the normality of variance between groups and showed normally distributed data. Thus, one-way analysis of variance (ANOVA) was used to compare the means of the groups. A p-value of $< 0.05$ denoted statistical significance.

## Results

Figures2 and 3 show the load deflection behavior of the TiNbTaZr (Gummetal) archwire compared with the control (SE-NiTi and Cu-NiTi) archwires at 2 and 4 mm deflections, respectively. In figure 2, Gummetalarchwireexhibited higher loading and unloading curves as compared to SE-NiTi and Cu-NiTiarchwires. While, in figure 3 the loading curve of Gummetal was, to some extent, similar to that of SE-NiTi. They generated almost the same amount of force at maximum deflection; however,the forces weremarkedly higher than in the Cu-NiTiloading curve. It can also be shown in figure 3 that the unloading curve of Gummetalwas higher, steeper and reached zero force faster than that of the SE-NiTi and Cu-NiTiarchwires. But it is worth to note that at both deflections,narrower vertical distance between the loading and the unloading curves was observed forGummetal as compared with the control archwires.

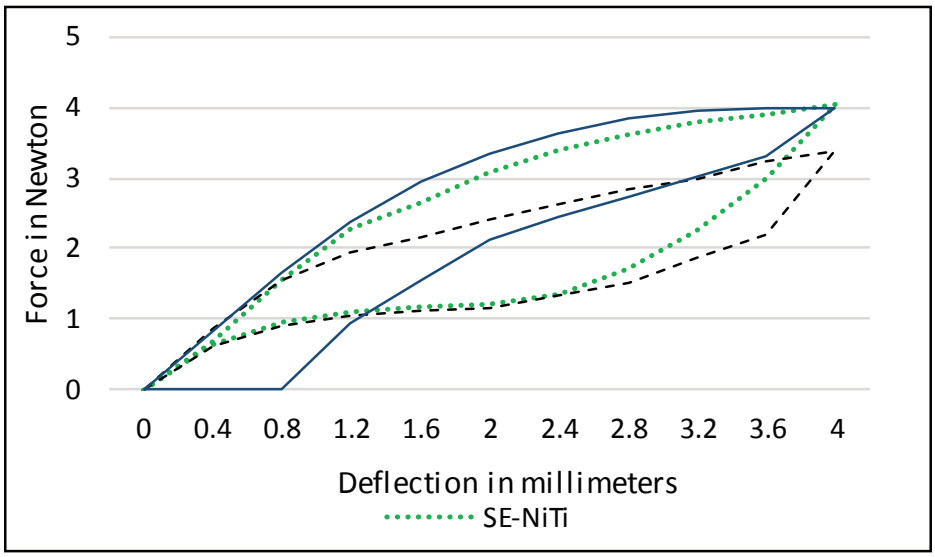


**Figure2 Average load deflection behavior of TiNbTaZr (Gummetal), SE-NiTi and Cu-NiTi at 2 mm deflection**

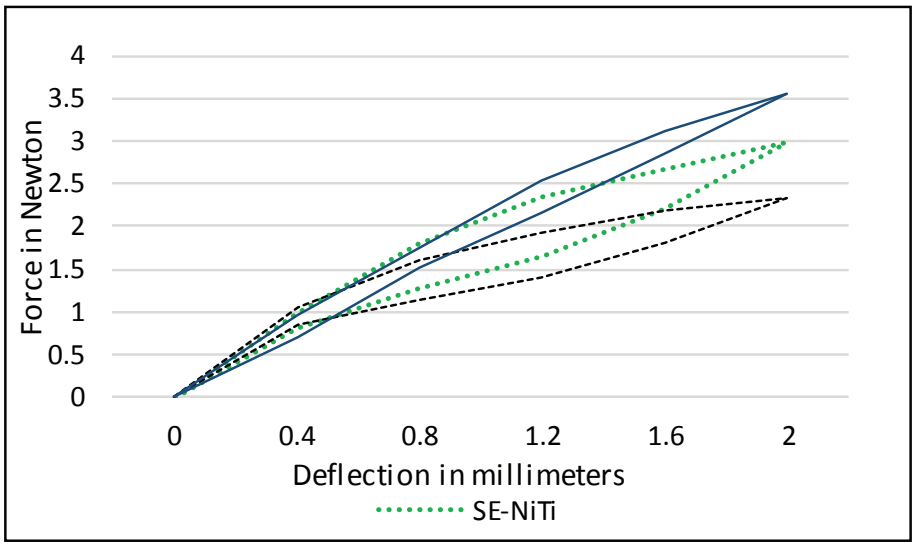


**Figure 3 Average load deflection behavior of TiNbTaZr (Gummetal), SE-NiTi and Cu-NiTi at 4 mm deflection**

In table 2, it can be observed that the mean force values produced by Gummetalwere significantly higher than those produced by SE-NiTi and Cu-NiTi at maximum deflection and at 1.5 and 1 mm unloading deflection points.

**Table 2 Descriptive statistics and results of ANOVA (F-test) in N at maximum deflection (2 mm) and at 1.5 and 1 mm unloading deflection when the tested wires were deflected by 2 mm.**

| Deflection | Archwire | Mean | Min. | Max. | SD | F-test | P-value |
|---|---|---|---|---|---|---|---|
| 2 mm (Max. deflection) | Gummetal | 3.554 | 3.500 | 3.580 | 0.033 | 200.658 | 0.000 |
| | SE-NiTi | 2.981 | 2.876 | 3.068 | 0.078 | | |
| | Cu-NiTi | 2.332 | 2.237 | 2.584 | 0.144 | | |
| 1.5 mm | Gummetal | 2.674 | 2.604 | 2.767 | 0.070 | 185.105 | 0.000 |
| | SE-NiTi | 2.049 | 2.00 | 2.115 | 0.054 | | |
| | Cu-NiTi | 1.689 | 1.614 | 1.885 | 0.111 | | |
| 1 mm | Gummetal | 1.858 | 1.700 | 1.965 | 0.111 | 61.357 | 0.000 |
| | SE-NiTi | 1.445 | 1.413 | 1.527 | 0.047 | | |
| | Cu-NiTi | 1.264 | 1.194 | 1.420 | 0.090 | | |

Table 3 shows thatat maximum deflection (4 mm), TiNbTaZr (Gummetal) and SE-NiTi generated a comparable mean force values, which were significantly higher than those of Cu-NiTi. At the 3 and 2 mm unloading deflection points, Gummetal exerted significantly higher forces than either SE-NiTi and Cu-NiTi. However, at 1 mm unloading deflection point, the mean force values of Gummetal were significantly the lowest.

**Table 3Descriptive statistics and results of ANOVA (F-test) in N at maximum deflection (4 mm) and at 3, 2 and 1 mm unloading deflection when the wires were deflected by 4 mm.**

| Deflection | Archwire | Mean | SD | Min. | Max. | F-test | P-value |
|---|---|---|---|---|---|---|---|
| 4mm (Max. deflection) | Gummetal | 4.008 | 0.264 | 3.71 | 4.31 | 8.796 | 0.004 |
| | SE-NiTi | 4.053 | 0.089 | 3.936 | 4.15 | | |
| | Cu-NiTi | 3.398 | 0.388 | 2.725 | 3.716 | | |
| 3 mm | Gummetal | 2.876 | 0.097 | 2.78 | 3 | 98.019 | 0.000 |
| | SE-NiTi | 1.944 | 0.032 | 1.906 | 1.986 | | |
| | Cu-NiTi | 1.751 | 0.212 | 1.377 | 1.888 | | |
| 2 mm | Gummetal | 2.111 | 0.128 | 1.95 | 2.251 | 132.616 | 0.000 |
| | SE-NiTi | 1.207 | 0.021 | 1.187 | 1.232 | | |
| | Cu-NiTi | 1.162 | 0.125 | 0.95 | 1.275 | | |
| 1 mm | Gummetal | 0.644 | 0.041 | 0.585 | 0.695 | 32.164 | 0.000 |
| | SE-NiTi | 1.034 | 0.058 | 0.97 | 1.11 | | |
| | Cu-NiTi | 0.978 | 0.125 | 0.77 | 1.1 | | |

## Discussion

When a new wire material is introduced toorthodontics, orthodontists should understand its properties in order to take full advantage of it during clinical practice, especially if the material considerably differs from the conventional one [13]. A new beta-titaniumarchwire, named Gummetal, has been recently introduced to the field. Its characteristics include being highly elastic, formable and nickel-free [14].The goal of this study was to evaluate the load deflection properties of small-diameter TiNbTaZr (Gummetal) archwire in comparison with superelastic-

NiTi and copper-NiTiarchwires in an attempt to assess the mechanical efficiency of Gummetalas aligning archwire.

Load deflection properties of orthodontic wiresare considered the most important parameters in determining the biologic nature of orthodontic tooth movement[15]. They are better assessed by three-point bending test, since it closely simulates the clinical situation to which orthodontic wires are being subjected and also has the ability to recognize wires with superelastic properties [12].In the principle of physics, it is recommended not to deflect a wire more than 5 per cent of its length, in order to ensure that the wire is being tested within the range of its metallurgical properties. However, in the present study, wire specimens were deflected by 2 and 4 mm, as such deflections are considered normal in the oral environment[16,17].

At 2 mm deflection Gummetalarchwireexhibited a higher loading curve than the SE-NiTi and Cu-NiTiarchwires, indicating that Gummetalarchwire requires higher forces to be engaged into the bracket slot. While at 4 mm deflection, Gummetal and SE-NiTiarchwires produced almost the same maximum force values.This could be related to deflection of the Gummetal wire beyond its elastic limit and that a plastic deformation had occurred in the wire.The vertical distance between the loading and unloading curves was narrower for Gummetal than for SE-NiTi and Cu-NiTiarchwires, both at 2 and 4 mm deflections. According to Segner and Ibesuch vertical distance represents the combined effect of hysteresis and friction [11]. This can be true for NiTiarchwires, but for Gummetalthis distance might reflect friction and plastic deformation rather than hysteresis, becauseFuruta et al reported that Gummetal alloy exhibits no hysteresis [18].

Regarding the unloading (deactivation) force, which represents the force delivered to teeth during orthodontic treatment, a rank order of Gummetal > SE-NiTi > Cu-NiTi was found for the tested wires, both at 2 and 4 mm deflections. However, Gummetalarchwire exhibited a rapid decreasein force in comparison with the control wires, especially when it was deflected by 4 mm, in which unloading forceof Gummetal dropped to zero at 0.8 mm unloading deflection point, whereas SE-NiTi and Cu-NiTiarchwires were still giving minimal amount of force. This rapid decrease in the Gummetal unloading forcegives an indication that the wire has a lower springback effect and hence a reduced ability to produce continuous forces compared with the SE-NiTi and Cu-NiTiarchwires.

Despite the significantlyhigher forces produced by TiNbTaZr (Gummetal) compared to the control archwires, these forces are considered normal from a clinical perspective, as suggested by Rock and Wilson who considered forces of approximately 4 N to be appropriate for use with fixed orthodontic appliances [19]. Additionally, a systematic literature review by Ren et al stated that the term “optimal force” is controversial, and forces that are considered to be high in a situation may be ideal in another [20]. Nevertheless, it may not be suitable to consider Gummetalas superelastic wire, becausesuperelastic materials should exhibit a reversible change upon

stress[21], and in this experiment Gummetalshowed permanent deformation during deflection.

According to these findings, the use of TiNbTaZr (Gummetal) archwire in the alignment stage of orthodontic treatment may be limited to mild crowding cases that do not require excessive deflection of the archwire, otherwise bending loops will be necessary to increase the flexibility and range of the wire.

**Limitations:**

Although laboratory studies can provide basis for comparing the behavior of different orthodontic wires, they cannot reflect clinical reality. In addition,in the present study only 0.014-inch diameter wires were tested and only in combination with one type of brackets (conventional metal brackets). Results might be different if another wire dimension (e.g., 0.016 or 0.018 inches) and/or another type of bracket, such as self-ligating or esthetic brackets were used.

## Conclusions

1. The study showed significant differences between the load-deflection behavior of TiNbTaZr (Gummetal) archwire and that of the NiTiarchwires.

2. Gummetal generated higher unloading forces in comparison with SE-NiTi and Cu-NiTi, at both 2 mm and 4 mm deflections.

3. Gummetal was less efficient than SE-NiTi and Cu-NiTi in providing continuous forces.

4. Gummetal cannot be considered superelastic.

5. The use of Gummetalarchwire in the alignment phase may better be limited to mild crowding cases.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# Influence of Tannin Extracts on Hematological and Production Properties of Male Rabbits Fed Mycotoxin Diets

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## Abstract

Tannins, as secondary metabolism of plant products, have several beneficial properties, such as antioxidant, antibacterial, and antifungal capabilities. These features have been used by the industry to improve animal performance.

Twenty-eight healthy local male rabbits were weighted (average 1382.7 g) the animals were divided regularly and equally into four groups, which contained control (fed basal diet), Mycotoxin group (fed contaminated diet), the third group was fed mycotoxinsdiets plus giving orally tannin extract (125 mg/ml), and the fourth treatment was given mycotoxinsdiets plus giving orally tannin extract (250 mg/ml).

The results demonstrated that tannin extracts, both concentrations of either 125 or 250 mg/ml, caused considerable variations ($p \leq 0.01$) in weight growth when compared to the control or the mycotoxin group. The body weight of the groups, however, was unaffected by these concentrations. Furthermore, using these extracts on mycotoxin diets caused a change in the feed conversion ratio and feed intake among rabbit groups, with the tannin groups showing lower values than the others.On the other hand, when compared both groups, the mycotoxin and low dose of the tannin, with a high concentration of tannin extract, 250 mg/ml, revealed significant values in liver enzymes (ALT and AST). Additionally, the same treatment (250 mg/ml) significantly reduced creatinine and urea levels as compared to other groups.

In conclusion, a high concentration of tannin extract, from black tea, showed beneficial effects on the productive trials, the biochemical properties in male rabbits that fed contaminated mycotoxin diets.

***Keywords:*** *Tannins, biochemical blood tests, production properties, male rabbits, mycotoxin*

## Introductions

Plant containing-tannins have a high molecular weight of natural and medicinal plants, which range between 3000 and up to 20,000 Da. They include significant amounts of animal diets. These plants showed varying molecular weights that exist as water-soluble polyphenols. Therefore, the industry has applied these compounds as a feed supplement to enhance animal performance[1].Tannins of the plants have very useful qualities from them are antioxidant, antibacterial, antifungal, antitumor[2], as well as an important parameter determining by the number of hydroxyl radicals and aromatic rings, so the polymerization and molecular weight of tannins play an important role for antioxidant activity because the connection between tannins and protein through hydrogen bonding is very strong, for this reason, it cannot be broken down by digestive enzymes or the attack of microorganisms[3].

Currently, tannin has been introduced and utilized in many areas including medicine, food, beverage, manufacture of ink and adhesives, dye and tanning industry, plastic resins, water purification, and surface coatings. This depends on the concentration of tannin, as a complexing agent or a precipitating agent[4]. Therefore, it is necessary to clarify the effects of tanninson mycotoxin that productive from a different type of fungus, includingOchratoxin-A (OTA), Aflatoxins, T-2 toxin, Fumonisin[5-8].

Black tea contains a large number of condensed tannins. It has broad-spectrum and specific medicinal effects in antioxidant, anti-inflammatory and anti-cancer and antimicrobial[9].Black tea has antioxidant characteristics, which help to promote heart health, lower LDL cholesterol, enhance gut health, lower blood sugar levels, and lower cancer risk[10]. Thus, the present study focuses mainly on the effect of tannin extract from black tea on rabbit diet that could contain some Mycotoxins, which secreted by fungi such as ochratoxin, aflatoxin, t-2 toxin and fumonisin.

## Materials and Methods

### The experimental design

Twenty-eight healthy local male rabbits were bought at age of about 5 –6 months; with average body weight (1382.7 g) animals were kept in cages of the Animal-house of the Veterinary College, University of Baghdad. The animals were divided regularly and equally into four groups contain (7 rabbits each); the body weight was considered and kept in cages specialized for rabbits and closed tightly.

The first group was daily fed freely on diet as a control group (C). This group has offered the ad libitum of water and the animal dietThe second group was daily fed on diet Mycotoxins; this group is called (Treat - X).The third group was daily fed on the diet, which contains (X) plus giving oral administered of tannin extracts orally, (125 mg/ ml Tannins), each rabbit. This treat is named (T1)(diet Mycotoxins + orally tannin extract).The fourth group was daily fed on a diet that similar to (T1 diet) but the animals administered the tannin extracts orally, which is concentrated to 250 mg/ ml Tannins for each rabbit; this treatment is marked as (T2)(diet Mycotoxins + orally tannin extract).

### Tannin extraction

Theapplication methodof the analysis on insoluble tannin is the Butanol-HCL reaction to insoluble plant materials were used in the tannin assays as described by [11]. The colourimetric reaction was used to an acid-catalyzed oxidative de-polymerization of tannins to produce the colour ofanthocyanidins.The acid-butanol assay remains the most commonly used method for determining tannins in plant tissues, despite the limitations outlined above[12].

### Body weight and weight gain

The animals of this experiment were weighted average every two weeks to determine the change in body weight and to find out the total gain during the experimental period on the following equation:

Mean body weight gain = body weight at the end of two weeks – body weight of preceding weight[13].

### Feed intake

Feed intake was measured daily for each rabbit in a group up to the end of the experiment**.** In the firstmonth,200g of concentrated diet put for each rabbit but the second month increased the concentrated diet per rabbit to 300g and after 24 hours is calculated the remaining and thrown from the consumer[13].

### Blood samples

The blood samples were taken twice every month, which was taken in the morning before treatments were given. The blood samples were withdrawing from the heart after sterilization at the site of blood drawn by

using disposable syringes sterilized**.** the blood samples were kept in sterilized tubes (10ml) including a gel and clot activator; then separated by centrifuge (3000 rpm) for 5 minutes**,** divided sera used to evaluate the tests mentioned earlier. Most biochemical tests were done on the same day and tested at the Laboratory of Animal Husbandry/ Department of Veterinary Public Health/ College of the Veterinary Medicine/ University of Baghdad.

## Results and Discussion

### Feed intake (g)

Table (1)showed in the 8$^{th}$week, the mycotoxin group and low tannin concentrated groups recorded significantly the highest values (192.2g and 192.6 respectively) compared to the control and high concentrated groups, which demonstrated (184.2g and 185.40, respectively).

**Table (1): Effect of tannin extracts with different concentrations on mycotoxin diet in feed intake (g)**

| Times / Groups | W1 | W2 | W3 | W4 | W5 | W6 | W7 | W8 |
|---|---|---|---|---|---|---|---|---|
| C | 126.80 ± 0.37 b | 126.40 ± 1.46 c | 134.80 ± 0.37 c | 139.80 ± 0.66 b | 144.20 ± 0.20 b | 162.00 ± 0.83 | 174.00 ± 0.44 b | 184.20 ± 0.37 b |
| X | 128.40 ± 0.24 a | 135.80 ± 0.66 a | 138.40 ± 0.40 a | 149.60 ± 1.83 a | 154.40 ± 1.36 a | 163.60 ± 3.12 | 180.80 ± 1.74 a | 192.20 ± 1.15 a |
| T1 | 126.80 ± 0.20 b | 129.80 ± 0.48 b | 137.80 ± 0.58 ab | 150.60 ± 0.40 a | 154.40 ± 0.40 a | 166.60 ± 0.67 | 182.80 ± 1.01 a | 192.60 ± 1.02 a |
| T2 | 126.20 ± 0.20 b | 127.20 ± 0.21 c | 136.80 ± 0.66 b | 149.00 ± 0.31 a | 152.00 ± 0.83 a | 166.40 ± 0.87 | 180.80 ± 2.28 a | 185.40 ± 0.67 b |
| LSD | 0.793 * | 2.54 * | 1.56 * | 3.02 * | 2.49 * | 5.12 NS | 4.62 * | 2.59 * |

*** The small letters in the same column indicate significantly different (*p≤0.05*); (Mean ±SEM)**

The feed intake was higher in mycotoxin and low tannin concentrated groups compared with the highly concentrated groups and the control groups (*p≤0.01*). This could happen due to the protein digestibility, which reduced in the mycotoxin diets and no improving effect of tannins especially with low concentrated groups, as well as this could be associated with the effect of the tannin concentration. The higher feed intake was observed in mycotoxin and the low concentrate of tannin groups could also be explained by the effect of tannins in the small intestine. It is suspected that proteins are bound with tannins in the range of intestinal pH and might therefore be less available for digestive processes; these results agreed with those reported by [14].

However, the high concentrations of tannins (250 mg/ml) may have significant effects by reducing the impact of Mycotoxins; while the low concentrations (125mg/ml) usually do not affect. The influence of tannins could depend on tannin contents that are administered or the chemical structures plus the molecular weight, which is not only on the concentration. Moreover, the condensed tannins that

exist in the black tea have a critical effect compared with the other types of tannins (hydrolysable tannin) that include in other types of nutrition[15; 16].

The present findings also indicated that not only the feed intake influenced but also the growth performances challenge some of the generalizations made in the past years on the effects of tannins in rabbit diets[17] (Mueller-Harvey et al., 2006). In particular, it has been suggested that high levels of tannins administered orally (250 mg/kg) may exert detrimental effects, whereas low levels (125mg/kg) could result in neutral or even positive effects[18]. These mechanisms were linked to the reduction of the voluntary feed intake often observed when animals are given high doses of tannins. Some authors suggested that reducing the intake of tannin-rich feeds is a defence mechanism for the animal, particularly against the hydrolysable tannins [19]. Lastly, tannins were also proposed to impair growth performances due to a reduced digestibility of the diet, with major effects on protein availability[20], although this study did not examine the diet digestibility.

**Body weight and body gain**

The rabbits in all groups responded well to all dietary equally; however, no significant differences observed at the end of the experimental time. The findings of weight gain demonstrated increased at the end of the experiment, while the body weight demonstrated that the body weight was closed in the values among the groups, but no significant changes were illustrated in Table (2).

**Table (2): Effect of tannin extracts with different concentrationson mycotoxin diet on body weight and weight gain (g); (Mean ±SEM)**

| Times / Groups | W1 | W2 | W3 | W4 | W5 | Weight gain |
|---|---|---|---|---|---|---|
| **C** | 1533.20 ±59.61 | 1635.80 ±57.89 | 1695.80 ±75.37 | 1701.40 ±81.95 | 1713.40 ±75.71 | 180.20 ±58.47 c |
| **X** | 1520.60 ±102.94 | 1595.20 ±129.48 | 1621.00 ±125.15 | 1720.80 ±121.43 | 1692.40 ±120.22 | 171.80 ±119.70 c |
| **T1** | 1390.00 ±70.42 | 1490.80 ±76.81 | 1540.60 ±81.54 | 1613.80 ±68.04 | 1611.80 ±56.69 | 221.80 ±70.64 b |
| **T2** | 1370.40 ±36.61 | 1509.00 ±48.14 | 1563.60 ±43.29 | 1660.60 ±45.12 | 1708.60 ±34.95 | 338.20 ±41.58 a |
| **LSD** | 214.38 NS | 252.33 NS | 259.07 NS | 251.40 NS | 235.22 NS | 40.13* |

The small letters refer to significant differences between groups (*P≤0.05)*; NS indicates no significant differences.

As current data showed that no significant differences were found in average daily body weight, while showed that increases were found in body gain at the end of the experiment. Indeed, the rabbit administered orally high concentrated tannins showed similar performance to those of rabbits administered orally low concentrated tannins. Furthermore, at low concentration seems that tannins played a role as a protective factor of the intestinal mucosa and as a control of peristaltic activity in presence of digestive disorders[21].

The current results showed that no significant differences were observed for all groups of rabbits, which showed the lowest values in the mycotoxin and low concentrated tannins groups. This might be because the rabbits in control and high concentrated tannins showed a lower feed intake than the mycotoxin and low concentrated tannins groups which could determine a lower development of the gastrointestinal tract, even if this difference did not influence significantly the growth performances. Therefore, these results related to the tannin effect as anti-nutritive substances; on the other hand, [22]reported that dietary tannins affected negatively the body weight, and this matches with the present data of this study. However, tannins may not affect nutrient availability and not affect appetite by binding to nutrients or enzymes in the gastrointestinal tract, as the current data of feed intake were recorded.

**Biochemical tests**

Aspartate aminotransferase level (AST)

The level of aspartate aminotransferase illustrated in Table 3, which showed the group of tannin extracts that administered orally 250 mg/ml was recorded the less value and roughly equal to the control group, so it demonstrated no significant difference (*P≤0.05*) at the end of the experiment; however, in mycotoxin group and the group of tannin extracts that administered orally 125 mg/ml recorded the highest value in the 30$^{th}$ day. As a result, on the 60$^{th}$ day of the experiment,

**Table (3): Effect of tannin extracts with different concentrations on mycotoxin diet onAspartate aminotransferase level (AST; U/ L) (Means ±SEM)**

| Groups / Times | Control | X | T1 | T2 | LSD |
|---|---|---|---|---|---|
| 0 day | 33.18 ±0.9 | 31.78 ±0.8 | 33.16 ±0.8 | 32.46 ±0.7 | 1.52 NS |
| 30th day | 27.14 ±1.6 c | 49.20 ±2.2 a | 45.12 ±2.7 a | 34.70 ±1.9 b | 5.15* |
| 60th day | 25.48 ±2.8 b | 48.46 ±2.9 a | 43.70 ±2.2 a | 27.74±2.5 b | 5.31* |

* The small letters refer to significant differences between groups (*P≤0.05)*; NS indicates no significant differences.

In this study, the valueswere increasingly seen in the level AST in the mycotoxin group and the administrated tannin extraction (125 mg/kg) orally. The highest values in liver enzymes indicate the damage produced in hepatocytes, which consequently causes the release of intracellular enzymes in the blood. Therefore, increased plasma levels of AST suggested damage of both hepatic cellular and mitochondrial membranes[23]. These findings are similar to [24] results that indicated a rise in serum ALT, AST, and ALP actions in young rats.Furthermore, the AST enzyme is an indicator of tissue necrosis and in the case of liver damage, its serum levels can be increased as in the mycotoxin and T1 group. Rabbit diets included in mycotoxin and T1 groups presented higher concentrations of AST compared to those in T2 and control groups. The decreased in serum AST concentrations could be the effect of 250 mg/kg tannin extraction on the liver. Changes in the AST levels may be due to conditions not involving the liver. However, they are considered to be a sensitive biomarker of liver cell injury in bovines[25]. The current findings could suggest initial hepatocellular damage caused by the mycotoxin, as already reported in rabbits[26]. In our opinion, given an oral dose of 250 mg/kg of tannin extraction orally, could be a safe procedure for the rabbit to reduce or reduction of mycotoxin infection in the diet.

**Alanine aminotransferase level (ALT)**

Table (4) demonstrated that the level of alanine aminotransferase highly fluctuated among these groups. At the end of the experiment, both mycotoxin and low tannin groups showed a huge increase compared to high tannin and control, which were significantly go-up at level (*P≤0.05*).

**Table (4): Effect of tannin extracts with different concentrationson mycotoxin diet onAlanine aminotransferase level (ALT;IU/ L) (Means ±SEM)**

| Groups / Times | Control | X | T1 | T2 | LSD |
|---|---|---|---|---|---|
| 0 day | 27.68 ±0.23 | 28.10 ±0.07 | 27.70 ±0.28 | 27.80 ±0.11 | 0.65 NS |
| 30th day | 26.40 ±0.26 c | 39.24 ±0.14 a | 31.18 ±0.07 b | 28.90 ±0.16 bc | 3.10* |
| 60th day | 29.02 ±0.18 b | 40.38 ±0.25 a | 37.50 ±0.15 a | 31.34 ±0.22 b | 2.97* |

* The small letters refer to significant differences between groups (*P≤0.05)*; NS indicates no significant differences.

When the liver is injured or damaged as a result of diseases, such as mycotoxin in this study, the permeability of hepatocyte cell membranes is impaired resulting in a release of AST, ALT. Liver injury may lead to impairment to conjugate and excrete bilirubin in bile resulting in elevation of total bilirubin in the blood [27]. In this study, administration of tannin extraction in a dose of 125 mg/kg induced significant hepatic damage within the 30$^{th}$ day manifested by elevation of liver enzyme activities, and note that it

is similar to mycotoxin group increased in ALT and AST.

Furthermore, administration of 250 mg/kg of tannin extraction orally increased the generation of free oxygen radicals and decreased the endogenous antioxidant potential. This resulted in increased oxidative stress evidenced by the elevation of ALT. In a dose of 250 mg/kg of tannin extraction, the root extract significantly reduced the elevated ALT level [28].

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: None

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# Tobacco Smoking as A Risk Factor in DNA Methylation of Repair Gene (MLH1) Using Cytobbrush from Lateral Border of the Tongue

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## Abstract

**Objective:** The aim of this study was to evaluate the epigenetic effect in the process of oral carcinogenesis by screening the methylation of repair gene in chronic tobacco smokers.

**Material and Method:** Study design: One hundred male volunteers, divided into two groups: the 1st group consisted of 58 smokers, each consumed 20 cigarettes/day for at least 10 years; and the 2nd group consisted of 48non-smokers who were consider as a control group.

The samples were taking from lateral border of the tongue by exfoliative cytology, and the extracted DNA was treated with phenol-chloroform gDNA,Screening of methylation was done by Methyl Specific PCR (MSP).

**Results** :- The results of this studyshowed significant effect of tobacco smoking in methylation of MLH1 gene in site 1in comparison to non-smoker group,($P > 0.05$), with Odds ratio = 4.957 CI ().

***Keywords:****This study concluded that smoking considered as a risk factor predisposed to process of oral carcinogenesis.*

## Introduction

The DNA methylation mechanism has a significant regulatory role in gene expression. The epigenetic modification induced by the hyper methylation act to suppress anti-oncogene expression by inhibition of transcription of its promotor region [(1)]. In addition, the direct effect of DNA methylation on DNA repair gene which inhibits transcription of its promotor region that induce cancer. Methylation of DNA is reversible so, the early detection as early as will help correct the error and avoid the serious hazardous consequences[(1,2)].

**Oral mucosa mutagenesis** takes place by multiple factors over several steps induced by hereditary and environmental factors affected the genetic material in which, the smoking considered as one of the most important risk factors [(3)].

Cellular genomic stability rely on the efficiency of DNA repair accordingly, the modification in DNA repair genes can cause cancer [(4)].

**MutL Homolog 1** (MLH1) gene act as a DNA repair gene that can correct of error of base pairing along with other repair genes as MSH2-gene. The hyper methylation of these genes cause repress of their expression and predispose oral carcinogenesis [(5)].

**Smoking** considered one of the main hazardous risk habits that is associated with many diseases especially that are related with oral mucosa, smoking

can act initiator and/or inducer of squamous cell carcinoma as a result of DNA damage [6,7,8].

## Materials and Methods

Cross-sectional study performed on 100 males (Age 20-40 years) who were divided into two group's (smokers) and control group (nonsmokers). The study done in Babylon city (The blood bank), Ethical approval for the study and informed patients consent obtained, and each patient will filled a case sheet questionnaire:

Including age, name, systemic diseases ……..

All the cases that included in this study had to fitful the exclusion criteria which

1. History of malignant neoplasia, vesicobullous and ulceration.

2. Absence of any visibleoral tissue changes alterations in the normal oralmucosa.

3. Alcohol drinking current and X.

Inclusion criteria include male with age between 20-40 years and heavy smoker (20 cigarette/ day ) at least and duration ≥ 10 years.

Thecytobrush samples were collected from the lateral border of the tongue from the deep layer using a Rovers® Orcellex® Brush Soft Oral Cell Samplex (Rovers Medical Devices, NL, Netherlands). As such a procedure is only minimally painful, it is not necessary to use local anesthesia when performing it,All participants were refrained from drinking or eating for about 30 minutes, and then they asked to rinse their mouth with water before sample collection.

DNA extraction from whole tissues. The protocol begins with phenol-chloroform gDNAextraction **(10).**

**Screening of MethylationbyMethyl Specific PCR (MSP):**

The DNA was bisulfite-treated conversion was used and cleaned up by**EZ DNA Methylation™ Kit Catalog No.D5002** from (**ZYMO RESEARCH**, **USA**) MSP was performed primers as ;.

**First site** which employ the following primer according to[11].

M3f TATATCGTTCGTAGTATTCGTGT

M3r TCCGACCCGAATAAACCCAA 154bp

U 3 f TTTTGATGTAGATGTTTTATTAGGGTTGT

U3r ACCACCTCATCATAACTACCCACA 128 bp

**Second site employ as primer site** according to [12]

M2f gatagcgatttttaacgc 93bp

M2r tctataaattactaaatctcttcg

U2f agagtggatagtgatttttaatgt 100bp

U2r actctataaattactaaatctcttca

The PCR(Biometra TRIO Thermocycler)were conducted for two sites in **promoter region of MLH1 gen** .by employ the following added 8 µ**l** of (2.5X) master mix (Cyntol, Russian), 1 µ**l**of ( 10 PM) of each primer and the total volume completed to 20 µ**l** grade water and PCR conditions according to table 1.

Product then refer by **Agros 2**□ PCR product resolved by 2 prestained (ethmoidepromide ) the presence of corresponding band with dedicate molecular weight assumed as positive result[13][14].

**Table 1: Thermocyclingcondition for PCR**

| Stage | Steps | Tempreature | Time | No. of Cycling |
|---|---|---|---|---|
| 1 | Intial denaturation | 94 | 5 min | 1 |
| 2 | DNA DENATURATION | 94 | 30 sec | 35 |
| | Primer annealing | 60 | 30 sec | |
| | Extension | 72 | 50 sec | |
| 3 | Final extention | 72 | 5 min | 1 |

## Statistical Analysis

All statistical calculation were performed using SPSS Version 21.0. USA) and Microsoft Excel (2010, Microsoft Corp. USA). All the results were expressed A $p < 0.05$ was considered statistically significant.. Chi-Square Test, and odds ratio were employed to evaluate the association between smoking and MLH1 gene methylation.

## Results

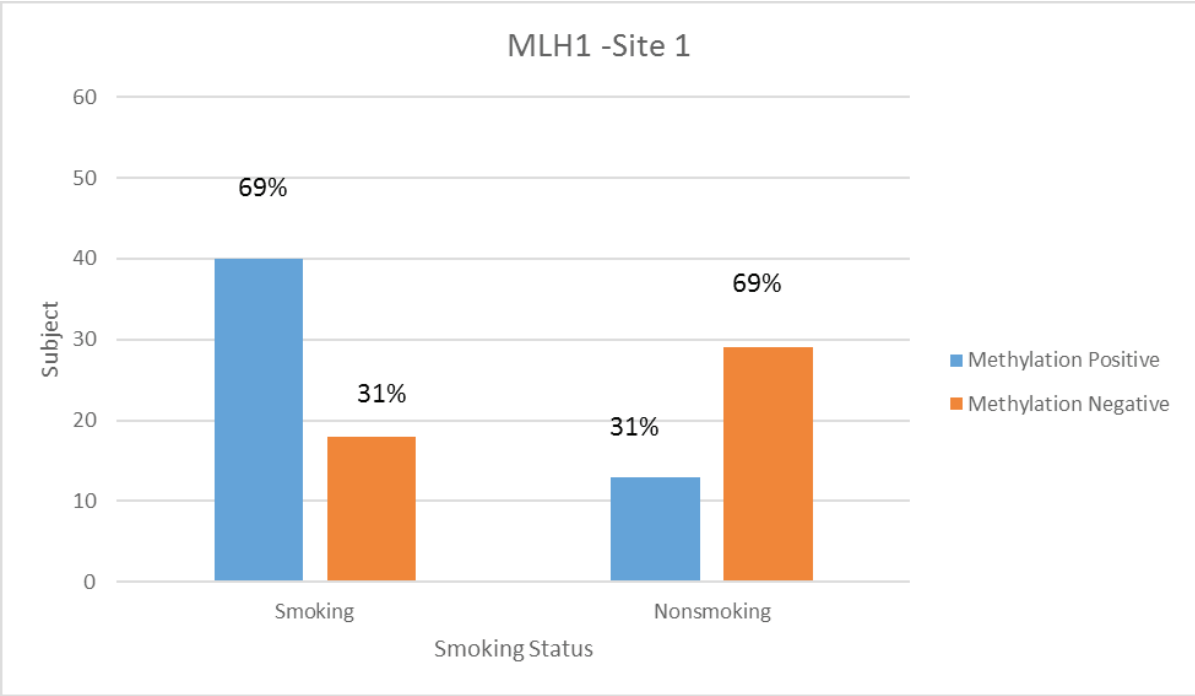
Smoking significantly hypermethylate MLH1 gene site- 1 with ( $P > 0.05$) and non-significant relation on site -2 with ( P.0.160) Table.2.

**Table 2:The association between smoking and MLH1-gene promoter site 1and 2methylation.**

| Subject | | MLH1 site 1 | | | | MLH1 site 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Negative | Positive | Chi-Sequare | P-value | negative | positive | Chi-Square | P-value |
| | Nonsmoking | 29 | 13 | 14.131 | 0.000 | 36 | 6 | 2.470 | 0.160 |
| | Smoking | 18 | 40 | | | 55 | 3 | | |
| Total | | 47 | 53 | | | 91 | 9 | | |

Figure.1&2 shows that 69% of smoker have MLH1 gene methylation in the site 1, and 31% have not affected with methylation in site 1, considering non-smokers only 31% of them have methylation in nonsmokers group.

In the site 2, there is only 5% of smokerswere found with methylation and 95% have not affected.




**Figure(1):The effect of smoking as a risk exposure factor on the methylation of repair**

Gene site 1 MLH1.

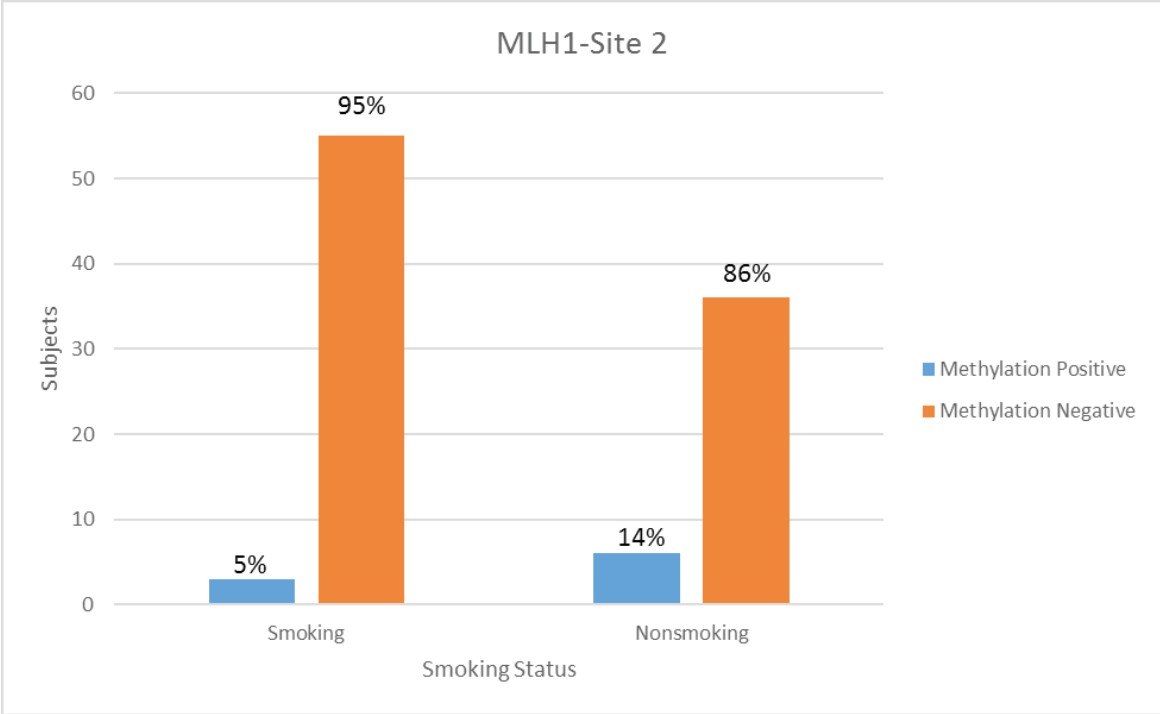



**Figure (2): The effect of smoking as a risk exposure factor on the methylation of repair gene MLH1 site 2.**

The hypermethylation of MLH1 gene induced by smoking can cause repressing of gene expression and inhibit transcription causing gene silencing and so impair MLH1 gene repair function. The odds ratio of this association was 4.957 with C/I (2.1-11.7), that illustrated the risk exposure to smoking.

## Discussion

Exposure to tobacco smoke is considered to be the most important etiological factors for the development of squamous cell carcinoma of the head and neck (SCCHN)**.**

THE MLH1 gene is plays critical role in different types of cancer. Epigenetic silencing of MLH1 promoter methylation can cause mismatch repair (MMR) deficiency. Which may cause insertion or deletion mutations in repeated sequences[15].

The MLH1 promoter methylation has been reported as a well-established biomarker in several types of cancer, such as esophageal cancer, colorectal cancer, non-small cell lung cancer, gastric cancer, papillary thyroid cancer, and bladder cancer [16].

Carcinogens and activated procarcinogens in tobacco smoke may react with the DNA of exposed human tissues, such as the epithelial cells of the upper aero digestive tract. This can lead to the formation of DNA adducts and subsequently to mutations in crucial genes such as oncogenes and tumor suppressor genes, ultimately resulting in the development of cancer [17].

An important epigenetic mechanism of gene inactivation during carcinogenesis is gene silencing caused by hypermethylation of the promoter region[18,17].

The present study was showed that chronic smoking has direct effect on oral mucosa leads to the methylation of repair gene (MLH1), this result provide evidence that smoking causes methylation repair gene. The table (1) was showed that there was a significant effect of smoking on methylation of MLH1 gene compare to nonsmoking group $P > 0.001$. This result agree with[8,19,20].

It concludes that smoking present risk factor with an Odds ratio of(4.957)(confidenceintervals) as shown in figure (1.1).

difference function of DNA methylation comprises, other than the regulation of gene expression, the protection of the integrity of the genome .

**Conflict of Interest:** No

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**Ethical Clearance:** Not Required

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# Amelogenin Localization in Periodontium Healing of Glucocorticosteriod-Induced Osteoporosis in Rabbit

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## Abstract

Several recent studies had focused on impact of the changes in osteoporosis and its relation to the periodontium healing process. The aim of our present study is to evaluate the effect of osteoporosis on the periodontium healing immunohistochemicaly and on amelogenin. A total of twenty female New Zealand rabbits were assigned randomly into two groups, a ten healthy (control), and an other ten were subjected to a glucocorticoid induce osteoporosis. ligatures were haphazardly placed for the 1st mandibular molars in order to induce periodontitis, then animals had been sacrificed after 4 weeks and the specimens were processed routinely for a serial decalcified sections for the histological and the immunohistochemical study on "amelogenin". The histological results of our present study had revealed a delay in the process of healing of periodontium of the experimental group as compared to the control group. The immunohistochemical findings had shown a higher immunoreactivity of the periodontium cells of the osteoporotic groups than the control groups. This study found that the osteoporotic state induced by glucocorticoids may cause a delay in the healing by inducing periodontitis with a mild to moderate immunoreactivity to amelogenin as compared to the control group.

***Key words:*** *Periodontium, Amelogenin, Osteoporosis*

## Introduction

The regeneration of the mineralized tissues which is affected by a chronic disease comprises a major clinical and scientific challenge. Periodontitis, which is a prevalent disease, involves destruction of the alveolar bone, supporting tissues of the tooth, cementum and periodontal-ligament, which will often lead to loss of the tooth [(1)].

The amelogenin had been thought to be an enamel (epithelial origin) protein. However, the amelogenin also has been detected in the odontoblasts and dentin matrix, during the cementogenesis in the remnants of the Hertwig's root sheath and in cells of PDL [(2)]. Also amelogenin expression had been described in bone cells; osteoblasts, osteoclasts and osteocytes and in the chondrocytes of the cartilage and differentially in the growth plate cells. The Amelogenin expression had been also identified in the non-mineralizing tissues cells like haematopoetic cells and brain cells [(3)]. The large number of amelogenin ,the spliced mRNA translated polypeptides, and the fact that the amelogenin had been found to be expressed in different tissues (soft tissues and calcifying) and with different embryonic origin, can possibly reflects a different functions of the amelogenin[(4)].

The osteoporosis that is Glucocorticoid-induced is one of the most common causes of the secondary osteoporosis, the Glucocorticoids can cause a rapid loss of the bone in a few months of use, but one of the most important effects of the drug is its suppression

of the bone formation. The risk of a fracture at the hip and spine is usually related to the doses, but even with a small dose it can increase the risk. Glucocorticoid therapy can lead to the loss of the trabecular bone more than the cortical one [5] .

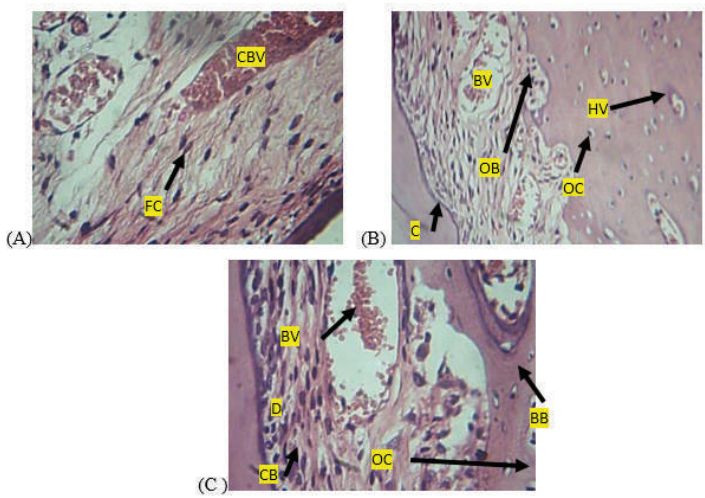
## Marerials and Methods

Twenty *healthy adult female rabbits with an average weight of 1.25-1.75 Kg and aged (8-12 months) were used in our study. All of the animals were kept in a separate cages and with a standard ventilation conditions, housing and feeding, and they were given a standard diet (barseem and pellet) and with an easy access to a tap water. All of the animals were kept in the animal house of the College of Veterinary Medicin with the approval of a research committee issue by the faculty of college of dentistry/University of Baghdad,number198. These animals were randomly divided into two groups,* 10 rabbits had received (10 mg/kg body weight) of hydrocortisone daily by intramuscular injection for 8 weeks to induce osteoporotic –like condition [6,5]. Ligatures were randomly placed around the first mandibular molars to induce periodontitis for all animals in both groups [7,8]. The animals were sacrificed after 4 weeks. All tissue specimens were fixed in 10% neutral formalin and processed in a routine paraffin blocks after complete decalcification of bone. Each paraffin-embedded block from all the studied samples were prepared in a 4µm thickness serial sections and mounted on clean glass slides for routine H&E staining. Other 4µm thickness sections were mounted on a positively charged slides for immunohistochemical localization of amelogenin. The procedure of the IHC assay was carried out in accordance with the manufacturer instructions of Rabbit polyclonal antibody to AMELX (ab59705) Abcam UK and mouse specific HRP/DAB detection Kits System, Abcam UK .

## Result

### Histological findings:

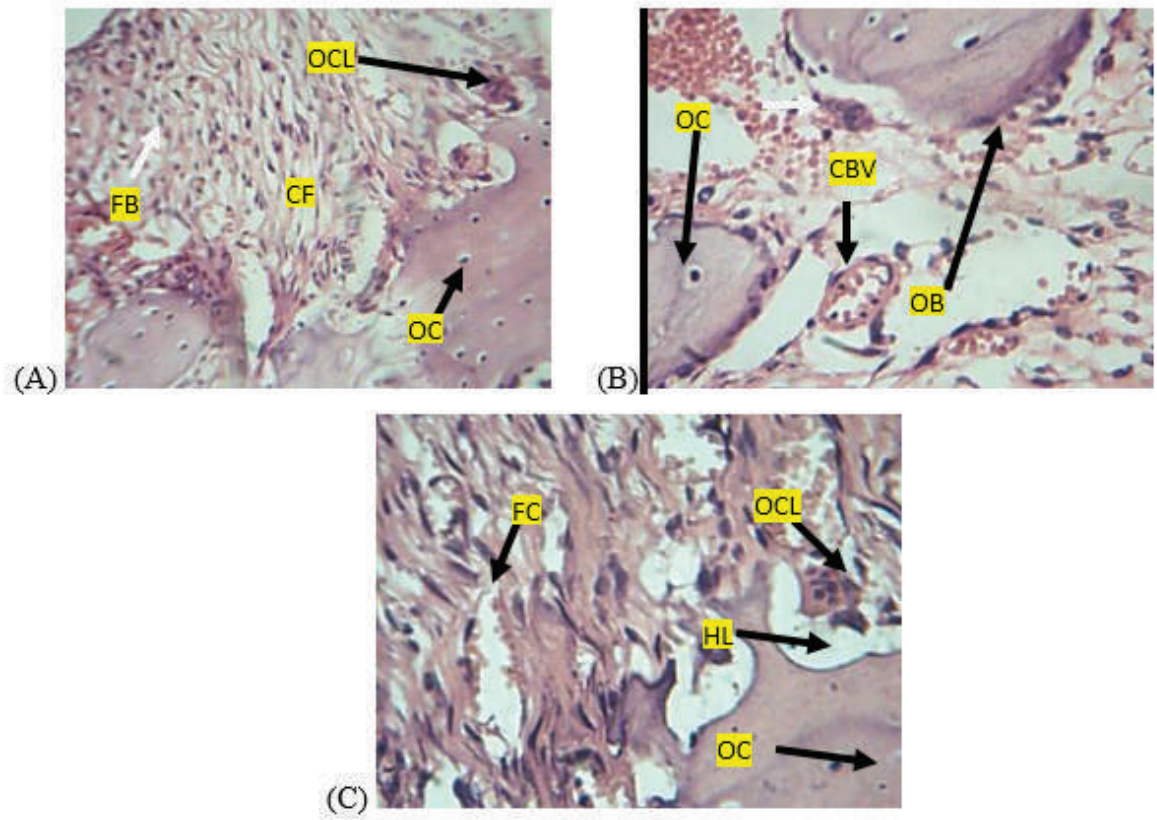
After four weeks, the histological picture of the control group showed congested blood vessels with new coarse of collagen fibers in the periodontal ligament that is attached to the newly formed bundle of bone which is surrounded by osteoblasts and filled by osteocytes as shown in fig (1).




**Fig(1):Control group After 4 weeks shows (A): Congested blood vessel(CBV) and fibroblast cell(FC), H&E (X20). (B): Cementum(C),osteocyte(OC), Haversian canal(HV),Blood vessel(BV), and Osteoblast cell(OB),H&E (X20). (C): Cementoblasts (CB), dentine (D), osteocyte cell (OC), blood vessel(BV) and bundle bone(BB), H&E (X40).**

**Experimental group:**

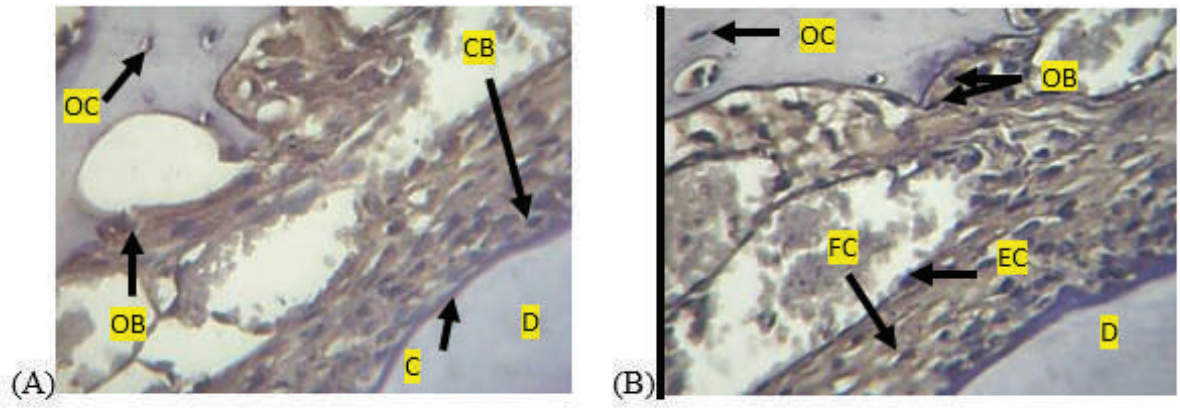
After 4 weeks, the experimental group showed irregular coarse of collagen fibers, numerous fibroblasts, osteocytes, congested blood vessel with numerous osteoclasts and osteoblasts in bundle bone which indicating continues bone remodeling as shown in fig (2) .




**Fig(2): Experimental group after 4 weeks showing (A): Coarse of collagen fiber (CF) in periodontal ligament, osteocyte(OC), osteoclast cell (OCL) and firoblast cell(FC), H&E (X20). (B): congested blood vessel(CBV), osteocyte cell(OC) and osteoblast(OB), H&E (X20). (C): fibroblast cell(FC), osteocyte cell(OC), Howship's lacune (HL), osteoclast cell(OC),H&E (X40).**

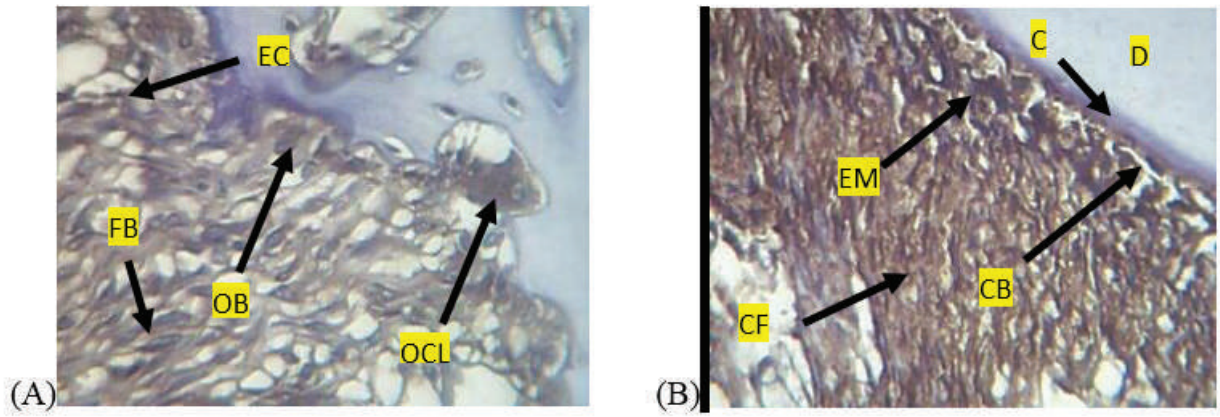
**Immunohistochemistry:**

The control group after 4 weeks, the immunoreactivity to AMELX showed strong positive expression in osteocytes, osteoblasts, cementoblasts, endothelial cells and progenitor cells with collagen fibers fig(3).




**Fig(3): Immunohistochemical view of control group. (A): Showed positive expression seen in Osteocytes(OC), cementoblasts (CB) and osteoblasts (OB). (B): Positive expression in Osteocytes (OC), osteoblasts (OB), endothelial cell(EC) and fibroblast cell(FC). DAB stain with counter stain hematoxylin x40**

The experimental group after 4 weeks, the immunoreactivity to AMELX showed mild to moderate positive expression in epithelial rest cell of malassez , endothelial cells, osteoblasts, o fibroblast cells , osteoclasts, cementoblasts and collagen fibers fig(4).




**Fig(4): Immunohistochemical view of experimental group. (A): Positive expression in endothelial cell(EC), osteoblastcell(OC),fibroblast cell(FC) and osteoclasts (OCL).(B): Positive expression in cementoblast cell(CB) , collagen fibers and epithelial rest cell of malassez(EM). DAB stain with counter stain hematoxylin x40**

## Statistical Analysis

Table-1 illustrates the group comparison differences in immunoreactivity for amelogenin antibodies (AMLX) in bone cells (Osteoblasts, osteocytes and osteoclasts). The results showed a highly significant differences between control and experimental groups in immunoreactivity for AMLX in osteoblast cells and osteoclasts with significant difference in osteocytes. Also the result revealed a higher mean value in expression of AMLX in control group in osteoblast and osteocyte cells than experimental one. Whereas the osteoclasts showed higher mean value in expression of AMLX in experimental group than control.

**Table.1: Group comparison differences in immunoreactivity for AMLX in bone cells by using T-Test .**

| Cell types | Control group mean | Experimental group mean | P value |
|---|---|---|---|
| Osteoblast | 20.56 | 11.78 | 0.00 |
| Osteocyte | 12.52 | 6.34 | 0.05 |
| Osteoclast | 3.7 | 10.72 | 0.02 |

Table-2 revealed the group comparison differences in immunoreactivity for amelogenin antibodies (AMLX) in mesenchymal stromal cells(MSCs) in periodontal ligament. The results showed a highly significant differences between control and experimental groups in immunoreactivity for AMLX in MSCs in periodontal ligament. Also the results illustrated a higher mean value in expression of AMLX in the control group than the experimental one.

**Table.2: Group comparison differences in immunoreactivity for AMLX in MSCs of periodontal ligament by using T-Test.**

| MSCs in periodontal ligament. | Control mean | Experimental mean | P value |
|---|---|---|---|
| | 34.71 | 27.43 | 0.01 |

## Discussion

The periodontal diseases can cause progressive and chronic destruction of the teeth supporting tissues, which is important in the attachment of the teeth to the alveolar bone. The Amelogenin belongs to a large family of extracellular matrix proteins [(9)]. There is a strong evidence for amelogenin role in enamel formation, but there is much less evidences about its expression in the periodontium. A strong evidence suggests that the amelogenin can act as a signaling molecule in these tissues. Also it is proposed that the amelogenin can influence the fate of the mesenchymal progenitor cells [(10)]. A previous animal models studies had shown that the amelogenin is also localized in the root epithelium, so suggesting an additional role for the amelogenin in the root formation [(11)].

The present study illustrated that amelogenin has a crucial role in the processes of bone development and remodeling due to amelogenin expression in osteocytes, osteoblasts and osteoclasts indicating amelogenin induction of osteogenesis and inhibition of osteoclastogenesis and this agrees with [(12)].

The histological observations of the present study revealed that the regeneration process resemble the normal pattern of periodontal development, in which the cementum is first produced, in the regenerated tissue, followed by the PDL and then alveolar bone, and this result confirm previous study[(13)]. The results of the present study showed that the amelogenins active in the regeneration process of periodontitis. Amelogenin expression was detected in normal and regenerating cells of the alveolar bone (osteocytes, osteoblasts and osteoclasts), periodontal ligament, cementum and in bone marrow stromal cells. Amelogenin expression was highest in areas of high bone turnover and activity, and this agree with[(13)].

This study showed that the epithelial rest cells of Malassez in glucocorticoid-induced osteoporosis express the amelogenin, and this confirm previous study[14]. Also the current study had shown a higher mean value of defense cell in the experimental group because of the effect of glucocorticosteriod, also there is a decrease of osteoblast cells in the experimental group because of the apoptosis effect of glucocorticosteroid[15]

The process of bone regeneration requires recruitment of mesenchymal stem cells to the injured area and tight control over osteogenic and osteoclastogenic activity. This study illustrated that amelogenin expression in bone marrow mesenchymal stem cells(MSCs) and its assumed ability to induce regeneration of alveolar bone may suggest a possible role for amelogenin in inducing mesenchymal stem cells recruitment during the processes of periodontal and alveolar bone regeneration and this agree with [16].

## Conclusion

The present study concluded that the glucocorticoid-induced osteoporotic state may cause delay healing of induce periodontitis with mild to moderate immunoreactivity to amelogenin when compared to control group which revealed a strong immunoreactivity.

**Conflict of Interest:** No

**Source of Funding:** Self funded

**Ethical Clearance:** Not Required

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# Trajectories of Salivary Hormones in Pregnant Women with Anxiety and there Effect on Gingival Health Condition

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## Abstract

**Background:** Hormonal fluctuations during pregnancy caused several of the physiological changes designed for promoting growth and delivery of a healthy child. A delicate balance between immune tolerance to fetal antigens and immunity against infectious pathogens needs to be maintained.

**Aim:** Assessing salivary cortisol and progesterone hormones in anxious pregnant women and their impact on gingival health status.

**Subjects and Method:**A sample of 80 womenwith healthy pregnancy divided into two groups including 40 pregnant women with high anxiety levelmatch in age with40 pregnant womenhavea low level of anxietyin Baghdad Teaching Hospital of Baghdad city in Iraq were participated in this study. Plaque index was used for assessing dental plaque accumulations.The gingival conditionwasmeasured by the gingival index.Unstimulated saliva sample was collected for measuringsalivary cortisol and progesterone.

**Result:** The strong validity and reliability of Beck Anxiety Inventory (BAI) Arabic translationscale make ita good indicator for measuring anxiety in Iraqi pregnant women.Data analysis of this study revealed high anxiety group has an elevated level of salivary cortisol, decrease salivary progesterone withsignificant differences. The correlations coefficients between salivary cortisol and progesterone among the high anxiety group was negative strong significant correlation while non-significant correlation in low anxiety group.The plaque indexwas higher among the high anxiety group with significantdifferences. Concerning the gingival index; there isno significant differences among two group. Positive strong significant correlation between plaque and gingival indices in high anxiety group.Regarding the correlation between gingival index and salivary progesterone in low anxiety group, it waspositive strong significant.While a positive significant correlation between plaque index and salivary cortisol in both groups.

**Conclusion:** This study reported increase stress-hormone cortisol among a high anxiety pregnant women which hasan effect ongingival health subsequently. Gingival inflammation in the high anxiety group was a plaque induce gingivitis, while a pregnancy gingivitis was found among the low anxiety group.

***Key words:*** *Cortisol, progesterone, gingivitis.*

## Introduction

Pregnancy is one of the most important events in a life of women, since it involves both biological and psychological changes.While this period is full of pleasant emotions for many women, others feel a considerable lot of unpleasant and negative feelings[1]. Although anxiety is common during pregnancy, it may become severe and have a detrimental influence on behavior, causedsignificant harm to pregnant ant child[2].Gestational period is not a state of disease but it is a healthy sign. Alterations in hormonal levels of pregnant women have a direct impact on oral diseases[3].Cortisol is a steroid hormone with catabolic effectarise from the adrenal cortex of the kidney[4].Several studies have found a link between maternal cortisol levels and anxiety levels during pregnancy[5].Cortisol levels were positively associated to progesterone[6],the latermeasured in saliva of pregnant women at the end of pregnancy.Dental plaque is the main cause of the oraldiseases which are the main problem of public health[7].The prevalence of gingivitis during pregnancy varies among studies from 30% to 100%[8].Pregnancy-related periodontal disease usually appears in the third trimester and resolves three months following birth [9].

## Subject and Method

This study conducted among primigravida pregnant womenage (20-29)in third trimester at 29-40 weeks with a healthy baby, without any illness or threaten abortion, medication intake included psychological treatment. This study was approved by the scientific committee in College of Dentistry/ University of Baghdad (number: 313). Pregnant women who recruited in this study distributed according todemographic factors which included age, educational level from (illiteracy to college) based on WHO, 2013 classification after some modifications to compensate the study population, and the week of gestation from 29-40 divided them into three groups from (29-32), (33-36) and (37-40 week).Beck Anxiety Inventory (BAI) byAaron T. Beck in 1988[10] in Arabic version was employed for assessing anxiety during the gestational period. Validity and reliability was measured for this scale (cronbachs alpha is 0.939). Standardization for scale was performed and two items was dropped when measures validity bypsychologist's comities in Psychological Research Center/ University of Baghdad to accommodate the present study. It was applicable for current study participants.Unstimulated saliva sample was collected in 9-11 A.M (to avoid circadian rhythm of hormones), for assessing salivary cortisol and progesterone by drooling passively method. Plaque index by Silness and Löe(1964)was used to assess plaque thickness[11] .The gingival indexbyLöe and Silness, 1963was used for assessing the gingival condition[12].

Statistical analysis used as statistical package for social science (SPSS version 21, Chicago, Illinois, USA);descriptive statistics as mean and standard deviation,frequency and percentage. Inferential statistics as chi square, fisher exact,independent sample T test and person correlation.

## Result

After measuring the items validity of BAI scale,it showed strong significant correlation between each item and the total score (p-value < 0.05).

The distribution of study participantsbased on the demographic variables isillustrated in table 1.

**Table (1): Distribution of subjects based on demographic variables**

| Variables | | Number | Percentage |
|---|---|---|---|
| The age (years) | 20-24 | 38 | 47.50 |
| | 25-29 | 42 | 52.50 |
| Education level | Illiteracy | 7 | 8.75 |
| | Primary | 17 | 21.25 |
| | Secondary | 21 | 26.25 |
| | High school | 9 | 11.25 |
| | College | 26 | 32.50 |
| Gestational week | 29-32 | 2 | 2.50 |
| | 33-36 | 4 | 5.00 |
| | 73-40 | 74 | 92.50 |

Chi-square was used to verify whether there is a significant association between anxiety groups with age factor, the results was not significant (Chi square=0.201, p value=0.654). While fisher exact was used to show any significant association between anxiety groups and educational level, the finding was not significant(Fisher exact=1.924, p value=0.784). Linear by linear association was 3.174, p-value 0.144 and the result was no significant association between anxiety groups and gestational week.

The table 2demonstratedthe plaque index (mean and standard deviation) among anxiety groups. High anxiety group has a high mean value of plaque index than low anxiety group with significant differences.

**Table (2): Plaque index among anxiety groups**

| | Anxiety | | | | T- test | P-value |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | The mean | Standard deviation | The mean | Standard deviation | | |
| PlI | 1.077 | 0.384 | 1.518 | 0.385 | 5.134 | 0.000Sig. |

Df=78

Concerning the gingival index which is illustrated in table 3; a higher gingival index in low anxiety group than in high anxiety group with no significant differences.

**Table (3): Gingival index among anxiety groups**

| | Anxiety | | | | | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | The mean | Standard deviation | The mean | The mean | T test | P-value |
| GI | 1.296 | 0.277 | 1.241 | 0.246 | 0.929 | 0.356 NS |

Df=78

The mean value of salivary cortisol and progesterone was illustrated in table (4). The salivary cortisol was higher in high than low anxiety group with significant differences, while salivary progesteronewashigher in low than high anxiety group with significant differences.

**Table (4): Salivary hormones among anxiety group**

| Salivary hormones | Anxiety | | | | | |
|---|---|---|---|---|---|---|
| | Low | | High | | | |
| | Mean | SD | Mean | SD | T | P value |
| Cortisol (ng/ml) | 9.985 | 4.642 | 20.438 | 10.474 | 5.771 | 0.000 Sig. |
| Progesterone (pg/ml) | 1243.372 | 667.397 | 870.603 | 235.244 | 3.332 | 0.001 Sig. |

Df=78

Regarded the correlations coefficients between the plaque and gingival indices in anxious subjects,it was a positive strong significant correlation in the high anxiety group (p=0.001), whilenon-significant correlation in the low anxiety group.

Table 5shows the correlations coefficients between salivary hormones among anxiety groups. A negative strong significant correlation was existed in high anxiety group, while non-significant correlation in low the anxiety group.

**Table (5): Correlations coefficients between salivary hormones among anxiety groups**

| Anxiety | | s-Prog (pg/ml) | |
|---|---|---|---|
| | | r | p value |
| Low | Cortisol (ng/ml) | 0.200 | 0.215 |
| High | Cortisol (ng/ml) | -0.869 | 0.000 |

The correlation between salivary hormoneswith plaque and gingival inflammationis illustrate in table6. In the low anxiety group, the only significant correlation was found between salivary cortisol with plaque index; salivary progesterone with gingival index which wasa positive significant correlation and strong positive significant correlation, respectively. While in the high anxiety group, the only significant correlation was found between salivary cortisol with plaque index which was a strong positive significant correlation.

**Table (6): Correlation between salivary hormones with plaque and gingival indices**

| Anxiety | | mean PlI | | mean GI | |
|---|---|---|---|---|---|
| | | r | p value | r | p value |
| Low | Cortisol(ng/ml) | 0.441 | 0.004 | -0.228 | 0.157 |
| | Prog (pg/ml) | -0.038 | 0.815 | 0.51 | 0.0008 |
| High | Cortisol/ (ng/ml | 0.534 | 0.0004 | 0.256 | 0.110 |
| | Prog (pg/ml) | -0.038 | 0.816 | -0.159 | 0.327 |

## Discussion

Among the most effective diagnostic tools for assessing anxiety during pregnancy arethe questionnaires of Beck Anxiety Inventory scale[13]. Age, gestational week, and level of education were not considered as troubling variables. Plaque index was more in high than low anxiety group with significant differences, It's possible related to increasing negligence of oral hygiene and reduced propensity for healthier behaviors due to exhaustion with restless condition resulted from anxiety, this was agree with a study[14]found a negative correlation between total distress scores and brushing frequency, while disagreeing with a studywhich found low values of plaque in women during pregnancy[15].The finding of current study revealed that both groups have gingival inflammation but nota significant difference, it disagree with the other study[16] which was explored that did not confirm each of the depression, stress and anxiety are associated to periodontal disease.Positive significant correlation between plaque and gingival indices in high anxiety group which supported by the study which revealed the gingivitis was respond for elevating dental plaque around the gingival margin[17] and this caused by increased level of salivary cortisol due to anxiety, which was directly association with the plaque accumulation in pregnancy[18], that lined the positive strong significant correlation between salivary cortisol and dental plaque in high anxiety group. Because of positive strong significant correlation between plaque and gingival indices, furthermore, no significant correlation between salivary progesterone and gingival index, so this illustrated that gingivitis in high anxiety group mostly was a plaque induced gingivitis. While no significant correlation was found between plaque and gingivitis in low anxiety subjects because of dental plaque may not be the only causative

factor for gingival inflammationin pregnant women. The hormonal effect on the periodontal cells includes changing the effectiveness of the epithelial barrier to bacterial insult and affecting maintenance and repair of collagen fibers resulting inelevated the risk of gingival inflammation[19]. With increasing ovarian hormones, compromised defense mechanisms and certain bacteria harmful to the oral environment increase during the third trimester[20],that could beexplain the positive strong significant correlation between the salivary progesterone and gingival index in low anxiety group. The immune-inflammatory process alterations by hormonal imbalance suggest that ovarian hormones are potential determinants and responsible for the development of pregnancy gingivitis[21], this couldbe explained the gingivitis in low anxiety group could be a pregnancy related gingivitis.The elevated level of salivary cortisol and decrease progesterone in the high anxiety group with significant differences, agree with a study[22]revealed thecortisol is a hormone of anxiety and stress due to its responsiveness to stress stimulation. An inverse curvilinear relation between anxiety and progesterone[23] that lined with the present study finding, but not support by a studyreveals a positive association between elevated progesterone and anxiety[24].Negative strong significant correlation between salivary cortisol and progesterone.Anxiety reduces progesterone during pregnancy[25].This might be due to ovarian steroidogenesis being impaired; resulting from increased glucocorticoid synthesis in response to elevated stressors this lined the result of high anxiety group. Positive significant correlation between salivary cortisol and plaque in high anxiety group, this explained bystudy showed the anxious situations within pregnancy caused increased levels of cortisol and lead to decreased number of immunoglobulin A and IgG and other antimicrobial proteins, which caused growth of oral bacteria in dental plaque and consequence of local inflammation [26]. Strong significant correlation between salivary progesterone and gingival index in low anxiety group, which could explained as the progesterone increases vascular dilation, permeability which caused accumulation of inflammatory cells; increased bleeding tendency; increased catabolic effect of folate that caused inhibition of tissue repair[19], and cause gingival inflammation.In low anxiety group of the current study showed the periodontium was a focus tissue for ovarian hormones effects, this was disagree with a studysuggested no progesterone receptor (PgR) expression in human periodontal ligament cell (HPDLC)[27], but agree with a China study which expressed the PgR in this cells at the gene and protein levels [28].Non-significant correlation between salivary cortisol with gingival index in high anxiety group, this result may not be correlated directly to the gingival inflammation, but rather it may be contributed to other local factor ascortisol caused increase mean value of dental plaque that could be a major factor for gingival inflammation in this groupand that explained by a studyshowed elevation of cortisol by stressors from psychological domains serve as risk factor for developing periodontal disease[29].

## Conclusion

In general, salivary hormonesaltered during pregnancy, but anxiety has aggravated effects. In turn affected cortisol hormone and caused plaque building resulting in gingival inflammation in high anxiety group.

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**Conflict of Interest:** No

**Source of Funding:** Self funded

**Ethical Clearance:** Not Required

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# Male Children Sexual Abuse in the Transkei Region of South Africa

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## Abstract

**Background:** Reporting of male-to-male sexual abuse is associated with stigma and discrimination. It is not only trauma to a child but also to a family. It is under researched and under estimated in a community. Even when abused children have grown up and become adults the abuse remains a painful secret in their lives. This scar of child sexual abuse stays for a life time. It also fuels the spread of HIV/AIDS in society.

**Objective:** To study the sexual abuse among male children in the Transkei region of South Africa.

**Method:** This is a retrospective study, carried out between 2007 and 2011 at the Sinawe Centre of Mthatha General Hospital, Mthatha, South Africa.

**Results:** There were 38 cases of male child sexual abuse (MCSA) reported between 2007 and 2011. There was only 1 case reported in 2007, 3 in 2008, 6 in 2009, 10 in 2010 and 18 in the year 2011. Of these, 3 (7.9%) were 5 years old, 17 (44.7%) were 10 or less years, and 9 (23.7%) were between the age of 11 and 15 years of age. Of the perpetrators 20 (52.6%) were known to the victims, 16 (42.1%) were unknown 2 (5.2%) were family member of the victims. There was delay in reporting. Genital injuries were observed in 8 (21%) cases, and physical injury in only 2 (5.2%) cases. All the victims were HIV negative and post-exposure prophylaxis compliant.

**Conclusion:** There is an increasing trend of male children sexual abuse in the Transkei region of South Africa. It requires urgent attention by the law enforcement authorities.

***Keywords:*** *Anal penetration, HIV infection, child abuse*

## Introduction

Male child sexual abuse (MCSA) is a worldwide phenomenon, but its existence and legality are different in different countries. The word 'sodomy' carries a lot of baggage and has a long and colourful etymological, social, and political history dating all the way back to the biblical cities of Sodom and Gomorrah, both destroyed by God for their wickedness.[1]Consensual sex between adult men is criminalised in about 80 countries in the world. It is an alien legacy. Sodomy laws thought out Asia and sub-Saharan Africa have consistently been colonial impositions.[2]Sexual intercourse between men was historically prohibited in South Africa as the common law crimes of "sodomy" and "unnatural sexual offences", inherited from the Roman-Dutch law.[3] Myburgh has described in 1974 that in the past those branded as public enemies were those who committed incest, sodomy, bestiality, and rape.[4]Homosexuality

and heterosexuality are two extremes and bisexuality is the central point where sexual expression is equal for both sexes. In between these two nodal points are persons who are either more hetero-than homo- or more homo- than heterosexual.[5]

Homosexuality is the greatest taboo in black communities. The sweeping scope of this taboo runs from 19thcentury slave quarters to post-apartheid South Africa. The mythical perceptions of black sexuality and prowess are centred on heterosexuality, not homosexuality. Therefore, any deviation from this norm is seen as unacceptable in many sections of the black community. Homosexuality is a European cultural imposition on Africans.[6] Men having sex with men is forbidden in almost all countries in Africa except South Africa. The maximum penalty is death or life imprisonment.[7]

Victims of MCSA run a very high risk of HIV infection. In the United States, the estimated lifetime risk for HIV infection among men-sex-men is one in six, compared with heterosexual men at one in 524 and heterosexual women at one in 253.[8]This risk is considerably higher - one in two and one in four respectively - for HIV infection among African American/black men.[9]The purpose of this report is to highlight the problem of men-sex-men in the Transkei region of South Africa. It will also discuss HIV transmission as well as psychosocial, social, and legal aspects.

**Method and subject**

This is a descriptive study. Victims of MSM were examined and tested for HIV at the Sinawe Referral Centre, Mthatha General Hospital. Sinawe Centre is the only unit in this area that deals with cases of anal penetration. It renders services to about 400 000 people. It is staffed by 15 people, including medical consultants, professional nurses, social workers, and police officers on duty. The Centre offers a 24-hour service.

HIV testing with patients' informed consent forms part of the management of sexually abused victims, in view of post-exposure prophylaxis (PEP) and antiretroviral treatment if they are found to be positive. The National Department of Health Guidelines are that a patient who refuses HIV testing or one who presents more than 72 hours after the incident should not be given PEP (DOH, National Management Guidelines for Sexual Assault Care, March 2005). On obtaining consent, a rapid test is performed in accordance with the National Guidelines (2005).A blood sample is also sent to the laboratory for an ELISA test for confirmation. The test results presented here are from the initial screening test and the ELISA test from the laboratory. To maintain patient confidentiality, HIV test requests and results were coded, and data were analysed and displayed in table form.

## Results

There were 38 cases of MCSA reported between 2007 and 2011. There was only 1 case reported in 2007, 3 in 2008, 6 in 2009, 10 in 2010 and 18 in the year 2011 (Table 1). Of these, 3 (7.9%) victims were 5 years old, 17 (44.7%) were 10 or less years, and 9 (23.7%) were between 11 and15 years of age. The perpetrators were known by 20 (52.6%) of the victims and unknown by 16 (42.1%), while 2 (5.2%) were family members. There was delay in reporting the cases of sodomy: more than 72 hours 15 (39.5%), 24 hours 10 (26.3%), and 12 hours 13 (34.2%). Genital injuries were observed in 8 (21%) cases, and physical injuries in only 2 (5.2%) cases. Only in 1 case was a victim sodomized at his home, and in only 3 cases were perpetrators under the influence of alcohol. All the victims were HIV negative and post-exposure prophylaxis compliant.

**Table 1: Incidence of sodomy in Mthatha area of South Africa (2007-2011)**

| Year | 2007 | 2008 | 2009 | 2010 | 2011 | Total |
|---|---|---|---|---|---|---|
| <5 | 0 | 0 | 0 | 0 | 3 | 3 |
| 6-10 | 0 | 1 | 0 | 7 | 9 | 17 |
| 11-15 | 0 | 0 | 0 | 3 | 6 | 9 |
| 16-20 | 1 | 0 | 2 | 0 | 0 | 3 |
| 21-25 | 0 | 0 | 2 | 0 | 0 | 2 |
| 26-30 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31-35 | 0 | 0 | 1 | 0 | 0 | 1 |
| 36-40 | 0 | 1 | 0 | 0 | 0 | 1 |
| 41-45 | 0 | 1 | 1 | 0 | 0 | 2 |
| Total | 1 | 3 | 6 | 10 | 18 | 38 |

## Discussion

This preliminary study on the MCSA is probably the first report in this country even though a lot of research has been carried out on sexual abuse of female children. This indicates a difference in the mind-set of people: either they are not willing to report MCSA or there are very few cases in the community. This is yet to be proved one way or the other. In the literature the words 'sodomy', 'men-sex-men', 'male homosexuality' and 'male child sexual abuse' (MCSA) are used interchangeably with almost same meaning. The author is in concerned with all aspects of male children sexual abuse in this paper. There are several research articles published on rape and sexual assault among females, but hardly anything has been published on the sexual abuse of male children, despite of the fact that they are of equal important. Male child sexual abuse is not only stigmatised among the lay public but also among educated professionals, even those who are dealing these cases in health care settings. Therefore, there is gross under reporting of male child sexual abuse. Very few research papers have published on homosexuality and MCSA in South Africa. MCSA has a complex relationship with mental health as it has with HIV with numerous health consequences in both cases.[10]

An average of 7.6 cases of male child sexual abuse (MCSA) were found in this study (Table 1). The highest number of cases (18) were found in the year 2011, and the lowest (1) was in 2007 (Table 1). There is an increasing rate of sodomy among children from 2007 to 2011 (Table 1). This is, however, the tip of an iceberg as reporting is very poor, especially in adult cases. Therefore, in more than half the cases (52.6) that were reported the victim was under the age of 10 years. Of these only 3 (7.9%) presented under the age of 5 years as children could not express to

their parents what had happened to them. It is difficult to estimate the reason for this number, but it can be presumed that at this age the child can describe the physical trauma cause by their perpetrator. Most of the time the mother is concerned about her child and does report the matter to the police or brings the child to the Sinawe Centre when she feels that something abnormal is happening to her child. A child of above 10 years may, however, start understanding the stigma attached to the deed and may remain silent. It is again difficult to quantify the number of cases of MCSA in a community, but it should be much higher.

Alcohol and cannabis consumption among Xhosa people is very prevalent. A study carried out by the author showed that alcohol related traumatic deaths are high in the Transkei region. There is a need to control alcohol.[11]**Tracing overt expressions of intolerance towards MCSA back to the colonial period, it focuses on ways in which notions of appropriate, respectable, exclusive heterosexuality within the 'cowboy' culture of White Southern Rhodesia trickled into the African nationalist movement.[12]**

More than half (52.6%) of the MCSA were known to perpetrator, and a few (5.2%) of them were family members. Many times, the perpetrator was an uncle, a cousin, or a brother. This is because the people are living in crowded houses, and there are not enough beds for everyone. The rural children of South Africa are at greater risk of sexual abuse as they are poor. The poor are facing all kinds of risks in their life. Transkei is an area where people are poor and living on meagre resources.[13] The Eastern Cape has the highest percentage of poor (24%), and this figure rises to 92% in the Transkei region.[14] Delay in case reporting was found to be more than 72 hours in 15 (39.5%) cases. This is similar in all other rape cases as people could not reach in time.[15] Victims who report an incident to a clinic after 72 hours are not given HIV post exposure prophylaxis according to the South African HIV guidelines. It is also not followed up to find out how many of them become HIV positive. In a low resource health centre setting is difficult to carry out any follow up.

There are multiple reasons for reporting late but the most common one is that people cannot afford the taxi fare.[15]People are living in far-flung areas of the former Transkei where roads and infrastructure are poor and health centres are not close to their homesteads. A funnel depressed, patulous anus and a central hole on inspection.[16] There were only eight cases presented with genital injuries and only two with physical injuries in this study. Few cases of sexual assault in children will have clear evidence of a sexual nature. A lack of physical evidence does not rule out sexual assault therefore, finding physical evidence during an examination is the exception rather than the rule.[17] It is difficult to demonstrate spermatozoa as the patient presents to the clinic late. Sometimes foul-smelling discharge with poor hygiene has been observed in a few cases.

The experience of being an MCSA victim can cause trauma not only to the child but also to the whole family, leading to anxiety disorders, depression, post-traumatic stress disorders, and suicide ideation and action.[18] Many black Zimbabweans believe that homosexuality was brought by white people to their country.[16] Sodomy is probably the most grossly under reported crime in the society.[19] Perpetrators are close by or mostly known persons. They always feel free as nothing one can stop or control them. To charge a person is so difficult in such an impoverished society, as this needs a serious police investigation. This is the reason these kinds of cases remain unreported.

**Limitations**

The cases are few and this report is also old as it is reporting a case that occurred ten years earlier. It is, therefore, not covering the recent situation regarding

MCSA. Despite these shortcomings, this report gives some insight into the work that is being done in this context.

## Conclusion

There is an increasing trend of male children sexual abuse in the Transkei region of South Africa. Five- to ten-year-old children are most vulnerable to sexual abuse according to this study. HIV infection also cannot be controlled without control of child sexual abuse. Poverty is an underlying cause of sexual abuse among children. The law enforcement authorities must be vigilant to prevent and protect this kind of abuse in the community.

**Ethical Issue:** The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# An Experience on Facts about Teaching Forensic Medicine to Undergraduate Medical Students in South Africa

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## Abstract

South Africa is struggling with an enormous amount of forensic pathology services because there is an extreme pressure on forensic pathologists to deal with the high number of medicolegal autopsies in the country. There are only five dozen forensic pathologists in South Africa who have to handle at least 80 000 autopsies per year. Medical officers, who have only received training as undergraduates in medical school, are expected to conduct these autopsies. Therefore, undergraduate teaching and training must be strong enough so that these young graduates can handle cases without any compromise in the quality of the outcome.This report is going to highlight the necessity of teaching forensic medicine at undergraduate level in South African medical schools. It will also discuss the shortcomings in medical school teaching programmes.

***Keywords:*** *forensic medicine, pathology, clinical & community*

## Background

The number of murders in South Africa increased by 303 from 21,022 in 2018/19 to 21,325 in 2019/20.[1]On average, 58 people were murdered every day. South Africa is ranked at number one in Africa in terms of unnatural deaths.[2]This rate is higher than the African continental average of 139.5 per 100 000 of the population and nearly twice the global average of 86.9 per 100 000 of the population.[3] South Africa has also one of the highest rates of rape in the world, but this barely seems to surprise anyone. It is extremely disturbing, especially when children and elderly women are raped.[4]Homicide is an extreme form of violence contributing to loss of years of expected life. More than 20 years after the end of apartheid, South Africa continues to experience excessive levels of violence. [5]The criminal use of firearms is widespread and is an important reason why the country has the third highest homicide rate in the world.[6] South Africa's homicide rate is 31.1 per 100 000 of the population, which is about three times higher than the African region.[7]There only five dozen forensic pathologists in South Africa who are delivering a service to 58 million population. [8]The case load is 80 000 medicolegal autopsies in South Africa.[9]Rape has increased to 53,293 in 2019/20 from 52,420 in 2018/19.[1]The majority of these cases are dealt with by a medical officer (appointed by the health department) who only has undergraduate training in forensic medicine.

There are only approximately fifty to sixty qualified specialist forensic pathologists in South Africa.[8] There are hundreds of medical practitioners who are disposing these high number of autopsies, who are simply a graduate in forensic medicine.The resources in previously disadvantaged area mortuaries are limited with poor hygienic conditions. These are mainly in rural areas of South Africa.The history of

apartheid of South Africa is known to everyone where there was prevalent abuse of services of forensic medicine, described in the book *An Ambulance of the Wrong Color*, published by University of Cape Town press in 1999. This book exposes the widespread human rights abuses by health professionals and the apartheid government.[10] South Africa cannot forget the death of Steve Biko who died in detention in 1977. The doctor told the author in a meeting that he obeyed the instructions of the police. It is an issue of ethics with life and death.[11] It is difficult to estimate how many of deaths and disabilities were caused during the apartheid era. The South African Truth and Reconciliation Commission have revealed some cases only such as missing man of Nelson Mandela University Mission Vale Campus, was reburied in a dignified manner in 2007.[12.]This report will highlight the facts in forensic medicine teaching and services in South Africa. It will also provide some solutions.

## Discussion

The history of South African human rights abuses during apartheid was under-investigated, poorly researched, and therefore grossly under-published. The available literature is very scarce. Forensic Medicine specialty is stigmatized not only by fellow professionals, but also by public.[13] It has observed that there has been minimal change in the level of forensic services especially in black homelands area, and thus they are still left as disadvantaged.[14] It is always under-resourced in service delivery in South Africa.[14] There used to be problem-based teaching of forensic medicine in rural medical school to undergraduate medical students in their 4$^{th}$ year of curriculum by trained teachers.[15]

The purpose of keeping this specialty independent is twofold. Firstly, it will audit the work of hospital and region to which the services are rendered. Secondly, it will also improve the quality of care by giving feedback to the clinical staff who managed the patients. It will also help to policy makers regarding number of unnatural deaths in the region, so the policing can improve accordingly. The author has demonstrated a maternal mortality case in a rural hospital along with medical students. The staff of the hospital presented their findings and students presented an autopsy report. It was concluded that this kind of medical misadventure will never be get repeated. After that, the author has not received a single case of maternal mortality from that hospital. This high number of maternal deaths must be reduced through auditing and publishing these forensic cases.[16] So, there is always scope to improve the hospital services at rural hospitals.

Merging forensic medicine with pathology means one is reducing focus on the subject, and that is a disaster. Medicolegal autopsy is an unbelievably valuable tool which is under-utilized in South Africa both in teaching as well as for research purposes.[17]

It is difficult to compare the importance of specialty with developed countries as medicolegal problems are different. South Africa is ridden with a lot of forensic pathology in community. There are high number of unnatural deaths,sexual assault, drunk and driving cases in hospitals.

The author tries to emphasize is that forensic pathologists must cover all the aspects of medico-legal services so that they can effectively teach this to undergraduate students.

There are examples where medical officers committed life threatening errors in their judgment due to a lack of knowledge of forensic medicine. An example as follows,

**"***There was matric schoolgirl was raped in middle of night. She resisted but the rapist cut her wrist to subdue her. The girl was brough to hospital causality and the doctor on duty refer her to orthopedic center, as he thought it is a tendon injury and repair must*

*do orthopedic department. The orthopedic center was about 10 kms and patient was taken there. She was admitted in orthopedic center. She keeps on bleeding from radial artery whole night, and next morning, orthopedic surgeon came, and he refer the patient to rape crisis center. Patient in collapsing condition to a rape crisis center with lot of difficulty. Sister was clever enough and recognize the problem of hypotension and taken patient to the same causality and immediately drip started, and patient saved."*

The casualty doctor considered tendon repair to be the most important; the orthopedic surgeon underestimated the bleeding from the radial artery and saw it best to refer the patient to the rape crisis center. This is something of a life and death situation and can only be emphasized in teaching of forensic medicine. The specialty of forensic medicine is undermined and stigmatized but it is an excellent learning platform to educate and train undergraduates. This specialty may not be much useful in developed countries where medicolegal cases are too little. However, the number of cases presented in South African hospitals that needs forensic knowledge in their management, is large. The author has examined some autopsy reports written by the medical officers where there was only give a cause of death. In most of cases the cause of death is obvious such as gunshot, stab injury and blunt force trauma which can be even identified by a layman, then where is needed to mutilate bodies by autopsy.

This is another case where lack of knowledge led to death of child,

*"A child who had a coarctation of aorta, felling difficulty in walking. The doctor who attended the patient in a rural hospital ask for X-ray of lower limb. He shows non-united epiphyseal center of head of femur and diagnosed as fracture. Consider it as a suspected case of child abuse and referred to orthopedic center. Patient was remained for two days and then died in hospital. Autopsy was carried out along with anatomical pathologist and found that it is a case of coarctation of aorta which is a correctable condition."*

Unfortunately, forensic medicine teaching and training is shrinking in South Africa as some medical schools are even running their course on an ad-hoc basis without any proper staff – like a factory worker who comes in on a shift, completes his duty in order to get a salary. The Health Professions Council must take cognizance of this kind of medical school in the country. This kind of deteriorating trend in teaching has started in last few years. Medical schools need a duly well-established department of forensic medicine as teaching and learning of undergraduates is a dynamic process and revolves around interactive teaching. Merging forensic medicine with National Health Laboratory services is another mistake that some medical schools have made, as it is just like attempting to fit a square into a round hole. They consider that forensic pathology is like anatomical pathology. Forensic pathology has some larger ramifications, and clinical implications which has proven beyond reasonable doubt in a court of law.

The undergraduate medical students must be trained in writing case reports, because through this they will grasp the fundamentals of research and the value of carrying out research from their early age. Forensic medicine is the only specialty which has account with from patient admission to burial or even sometimes exhumation. Motivate students to get documents, correlate present findings by the students in clinicopathological meetings and then get it published. This exercise is not carried out in most of the rural medical schools because either the department is not academic, or their hierarchy is not having research mind. It has been said that if you want to destroy a country, one does not need a bomb or long-range missile, just lower the level of teaching

at universities.[18] Remember, to break the trend of inactivity or lethargic position is challenge, because people get their bread without doing anything. The author has experience of how most staff in a medical school who were non-academic. Those who do some research work, get trouble as there is culture of non-academic. Therefore, it is important to not appoint a head who is not having interest in research.The quality of forensic services is crucial as wrong decisions are dangerous and can mislead the judgement.[19] This is because the effect of services is not directly visible to the public. In some rural schools, almost everyone is called a professor, without any scrutiny in their appointment. Some of them are even not registered as a specialist, yet they hold the position of professor.

Integrating forensic medicine with other subjects means diluting the scope of forensic medicine both in teaching as well as in training of students. Forensic medicine teaching is necessary for their critical thinking skills, and will help in auditing of cases, so that any mishaps in medical and surgical management could be identified. It forms part of improving the clinical care in hospitals as treating doctor will take this feedback.Most of the forensic pathology is related to trauma, which is referred from surgical departments. It is an import to have clinico-pathological meetings where clinical findings are given by the clinicians and pathology is demonstrated by a forensic pathologist alongside students. It depends on the culture of the hospital management and their academic interest in the discussion, but it is a very fruitful and educational exercise to all staff of the hospital.

## Conclusion

There is a necessity for forensic medicine in South Africa as it is a driver of clinical teaching, ethics, and research in medical schools. It is not only a Health Professions Council requirement but also a need of public especially in rural areas of South Africa. The infrastructure and platform must improve for clinic-pathological meeting so that clinicians will also getbenefit as patient care improves.

### Ethical Issue

The report has been prepared to highlight the problems in the teaching of forensic medicine in the undergraduate medical curriculum. It is not intended to bring down any person or any institution. The author has ethical permission for the case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# Repeated Sexual Assault and HIV Seropositivity: A Case Report

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## Abstract

**Background:** Sexual assaults are on an epidemic level in the Transkei region of South Africa, despite the government efforts to bring it down. Human Immuno-deficiency Virus (HIV) is also proportionally high in this region of South Africa.

**Objective:** To highlight the problem of sexual assault and HIV infection in the Transkei region of South Africa.

**Case history:** This is a case report of 15-year-old teenagers (SD) who was a schoolgirl in standard 6 when she was assaulted. SD was sexually assaulted repeatedly by an older man of 28 years. She was found sero-converted on HIV testing. On physical examination the introitus was red and bruised. A copious amount semen-like infected fluid was observed in her vagina. The case history, findings, beliefs, and compliance with HIV drugs are discussed.

**Conclusion:** There is high risk of repeated sexual assault and HIV infection in the Transkei region of South Africa. Government must look deeper to prevent these rape attacks as well as HIV infection.

***Keywords****: Sexual assault, HIV infection, antiretroviral drugs*

## Introduction

South Africa has one of the highest rates of sexual assault in the world[1] and it also has the highest prevalence of HIV infection.[2] Several models are currently underway to provide Post Exposure Prophylaxis (PEP) following sexual assault at public health services, but it varies from center to center and province to province. There has been an increase in the number of sexual assault incidences reported at the Sinawe Centre in the Mthatha area of South Africa. Rape has emerged as the most serious public health problem in South Africa.[3] This may reflect either an increase in sexual assault reporting awareness or a real increase in actual cases or both.[3] The HIV prevalence rate is 13.7% in Mthatha area, and just over a half (7%) of HIV positive rape victims were aged 16-25 years.[4] There are thus challenges that remain a high priority in the rural regions of South Africa, including HIV education and prevention of HIV infection.[4]

A study conducted on the state of sexual assault services in the country found there are many gaps in the services provided to sexual assault survivors as well as a large amount of variability.[5] There is an inconsistency in the performing of an HIV test before initiating PEP, differences in the types of drug regimens being offered, differences in prescription of the drugs and the type of support services provided.[5] Christofides et al. (2006) found that only 50% of the

patients who were given seven days of PEP drugs returned for their next supply and this led to a change in policy to give patients the full one-month supply.[6] This low level of completion (15%) was confirmed in a separate study that included one of the Western Cape rape centres.[6] Even lower rates of adherence have been reported in the Eastern Cape. At the Sinawe Centre in Mthatha, Meel reported adherence as low as 3%.[7] A recent cost effectiveness study of providing PEP to prevent HIV transmission in South Africa has shown that the cost of averting HIV transmission was dependent on completion of PEP.[6] The purpose of this case report is to highlight the problem of repeated sexual assault, HIV infection and PEP adherence in this region of Transkei. The reasons for non-compliance to PEP are also discussed in this presentation.

## Case history

SD, a 15-year-old schoolgirl in standard 6 was presented at the Sinawe Centre with a history of sexual assault by an older man of 28 years. The perpetrator used to call her his girlfriend. Nursing staff on duty took the case history and carried out voluntary pre-test and post-test counselling. SD was kept for three days at the perpetrator's house and experienced repeated sexual assaults. The accused endured this relationship for a long time and underwent repeated sexual assaults on several occasions. On genital examination, there was an old, ruptured hymen. The introitus was red and bruised. Copious amounts of a semen-like substance were observed in her vagina. SD was found to be HIV seropositive.

## Discussion

It is very unpleasant to hear about rape cases, especially those of child rape like that of SD, and they are difficult to research. Rape has different meanings for different people. It is also attached to a lot of stigma and discrimination especially in socio-economically poor communities. Rape is under-reported and, therefore, under-estimated. The statistics are either not available or if they are available are generally incomplete. Transkei was a former black homeland and mainly Xhosa-speaking people are staying here. It produced a lot of national and international leaders but remained grossly underdeveloped. There were a lot of women who were raped during the apartheid era and promiscuous behaviour still exists. Probably, the culture of rape was a legacy of apartheid, and it will take time to recover from this culture of rape of women.[8]

It is known to everyone that the people are very poor in the Transkei region of South Africa. There is poor infrastructure and a high illiteracy rate. The society is very fragmented, and the use of alcohol is very common.[9] In such a situation, children such as SD and women in general are weak and therefore the most vulnerable individuals in poor communities. This case of SD is thus just the tip of an iceberg. SD was taken into captivity for days and sexually assaulted repeatedly because of masculine power. Rape is associated with a close linkage between the concepts of sex and power.[10]

Many girls are reluctant to report rape. If a girl is sexually abused by her father, the mother is usually reluctant to report this to the police. Poverty takes away this right and it remains a family secret. Poverty even forces girls to have sex in exchange for food. Moreover, the father of a child is often the bread winner. Awareness of child abuse in the community is low, reporting of crime is poor, and prosecution of rape is difficult.[11]Sometimes a child is forced into marriage in exchange for lobola (gift in the form of money or cattle or sheep). Poverty alleviation is not only important to feed human beings, but it is also important to control HIV infection by reducing the incidences of rape.[11] It is not clear in the case of SD whether the mother or father played any role in sending the child to an old perpetrator. Even so, it would still be considered statutory rape as she was only 15 years

old. Responding to the need to avert HIV infection, the South African government adopted a policy in December 2002 to provide anti-retroviral treatment as part of a comprehensive service for people who have been sexually assaulted, but the government is not doing enough to prevent rape.[11]

A 'think tank' meeting on AIDS prevention in the high HIV prevalent countries in Southern Africa concluded that 'high levels of multiple and concurrent sexual partnerships by men and women with insufficient, consistent, correct condom use, combined with low levels of male circumcision are the key drivers of the epidemic in the sub-region.[12] The rate of rape reported to the police in 1996 was 240 cases per 100 000 women. The research suggests that this represents the tip of the iceberg of sexual coercion in the country. A representative community-based survey found that in the 17-48 age group there are 2070 such incidents per 100 000 women reported per year.[1] HIV transmission arising from the widespread rape and forced sexual abuse is not preventable by condom use and male circumcision. The police and criminal justice system should be tough with criminals. Hardly any rapist is using a condom, and probably they are HIV positive themselves and want to spread HIV infection. Hospitals cannot help as they are under-resourced. SD was only offered a course of antibiotics along with psychotherapy. Social workers cannot reach the rural areas where there is not even a proper road.

The 'think tank' should also think about the widespread prevalent myths of virgin rape as a cure for HIV/AIDS.[13] HIV infected subjects are supposed to transmit the infection to only one subject during his lifespan, but this is not true as evidenced in the victims of rape. SD was exposed to repeated cycles of rape. This behaviour is fueling the HIV epidemic in South Africa. The forecasting of the future of HIV/AIDS in South Africa depends upon forecasting the incidences of rape such as that of SD. Effective patient support must be in place to improve compliance to PEP after a sexual assault.

## Conclusion

There are life threatening risks associated with repeated rape and the government must look deeper into the underlying cause of rape and HIV infection.

**Ethical Issue:** The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# Ethical Issue Related to 'Save the Life of the Patient' at Mthatha General Hospital in South Africa

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## Abstract

**Background:** Resuscitation of a patient with life threatening conditions, before referral to another department or hospital, is a legal obligation on the part of the health professionals. Several deaths occur every year in hospitals and health care centres without an emergency management.

**Objective:** To highlight the ethical issue related to 'Save the Life of the Patient' at Mthatha hospital in South Africa.

**Case History:** This is the case (AM) report of a 16-year-old Grade 10 learner who was assaulted physically as well as sexually. She was bleeding from a stab wound to her when she was brought to the Mthatha General Hospital's (MGH) casualty. She was then referred to the Bedford hospital for repair of the tendon of her right wrist as recommended by the doctor on duty. The patient was referred to Sinawe Centre for forensic management, where she collapsed during an interview. The patient was taken on a stretcher to the MGH casualty for an emergency treatment of her blood pressure. The history of the patient and the care duty of the health professionals are discussed. Ethical issues are also highlighted in relation to the 'preserve the life first' principle in this case report.

**Conclusion:** There was a serious breach in duty of care by medical officers in the Mthatha hospital, South Africa. It is an ethical priority to save the life of the patient.

***Keywords:*** *Resuscitation, life-threatening, legal obligation, emergency management, and death.*

## Introduction

There is a lack statistics worldwide regarding the number of deaths related to medical negligence. A study by Healthgrades found that an average of 195 000 hospital deaths in each of the years 2000, 2001 and 2002 in the United States were due to potentially preventable medical errors.[1] Researchers examined 37 million patient records and found that there were 98 000 deaths annually in USA because of medical errors and this situation should thus be considered a national epidemic.[2] Medical errors are a major concern regardless of patients' life expectancies. A study carried out by Hayward et al. (2001) in the USA showed that almost a quarter of active-care patient deaths were rated as possible preventable by optimal care.[3]

There are four main principles regarding patient care: legal duty of care, breach in duty, injury because of lack of care, and ultimate death or disability of a person.[4] The provision of health care service in resource-poor settings is associated with a broad set of ethical issues. These are related to 'act of omission'

and 'act of commission'. Immediate treatment of a victim of injury on arrival of the patient in a hospital casualty is sometimes lifesaving. Avoidance of unnecessary movement of the victim prevents further injury.[5]Conditions that require immediate attention to avert death include cessation of breathing, severe bleeding, poisoning, and heart attacks. The essential first aid is bandaging of a wound in case of injury.[5]

There is a scarcity of literature on medical negligence in South Africa, but it seems to be a major problem. The dysfunctional public health care system in South Africa is taking a heavy toll on the life of patients especially in former disadvantaged community. People in these communities are most vulnerable to being raped and cannot afford to get private health care. To address this situation, social and structural inequities in South Africa must be changed. The purpose of this case report is to highlight the ethical issue related to 'Save the Life of the Patient' at Mthatha hospital in South Africa.

## Case History

AM, a Grade 10 learner, was referred from casualty with a history of being physically and sexually assaulted by two unknown men in 2009 at about 2 am. She was alone at home sleeping as her parents were in the rural areas. Two men entered her house and demanded money. She gave them R130. They demanded more but she did not have more. They beat her up. She asked them to give her a chance to get out and get money from her neighbour, but they did not allow this. She managed to get out, and went to the neighbour's house, but they followed her. They also demanded money from the neighbour (girl G), and they robbed her of her cell phone. After robbing them, they forcefully asked them to undress, and they raped both. They took turns at raping. The girl resisted but they stabbed her with a knife.

The patient was brought to casualty. The doctor on duty bandaged her wrist and referred her to Bedford hospital to get her tendon repaired. She was bleeding continuously. Bedford hospital referred her to Sinawe Centre for examination of her sexual assault injuries. At about 1 pm (after almost half a day), the patient was presented at Sinawe Centre with multiple superficial stab wounds and bleeding profusely wrist. She lost lot of blood. Her bleeding wounds were not managed at the casualty. She was just referred to Sinawe Centre without being assessed. The patient's condition changed during the process of interviewing her. She was sweating heavily and was in shock. She was taken to casualty on a stretcher for management at about 9 pm (almost three quarters of a day later) the patient was examined for a sexual assault. She was pale and hypotensive (90/62 mm hg), and her pulse was rapid and feeble. Intravenous fluid was given, and her wounds were bandaged. Prophylactic treatment was advised, and they asked her to come to the Sinawe Centre the next morning for counselling.

On genital examination, there were signs of bleeding with fresh rupture of the hymen. Margins of hymen are irregular, and the point of bleeding was visible. There was bruised introitus, indicating that there was a recent forceful sexual assault. She was non-reactive on HIV rapid testing. A pregnancy test was also negative.

## Discussion

This report reflects the tip of an iceberg, one situation of many where a patient could have been saved from a near death in a public hospital in Mthatha. There are lot of prehospital preventable deaths in this hospital.[6] Very little research has been carried out in this regard, and therefore very few are published. The Transkei region of South Africa is one of the poorest areas, where a very poor health care system is in place. The history of Transkei sheds light on the factors behind the high incidence of rape in

this region. Apartheid made violence an instrument of control and violence became the norm in these people's day-to-day life. The Transkei region suffered under the rigorous apartheid system and women were asked to produce more children to keep on supply the fighting force needed to defeat the apartheid regime.

Rape is a stigma in the community, but it is also so among health professionals. The moment they get a case of rape, they simply they want to get rid of it. This is what happened in the case of AM. She was presented in causality as she was raped, and the medical officer, without assessment of her injuries, assumed that her tendon was cut and referred her to an orthopaedic hospital. So, the doctor had 'done his job' without knowing the consequences for the patient's life. The patient (AM) was admitted to the orthopaedic hospital about ten kilometres away without a diagnosis and was given a bed. In the morning the doctor came on his rounds, heard her history, and referred her to the rape crisis centre. AM was sent from one centre to another centre for about a day, bleeding from cut artery in her wrist. AM was at the casualty for her injury as well as for the trauma of rape. She should have been treated by the casualty doctor for her injuries first, following the basic undergraduate teaching curriculum dictum of save the life first. Her wrist artery was cut and bleeding, a life-threatening situation, but doctor on duty was more worried about her tendon injury.

It is common sense, and has repeatedly been taught to undergraduate students, one must save the life of patient first, and then look for other things to treat. It is surprising that the doctor in the orthopaedic hospital did not pay adequate attention to the referred patient from casualty. As a result, the patient kept on bleeding the whole night from her cut wrist. The next day the patient was referred to the rape management centre, and there a sister picked up the problem and took her to casualty where she was treated for hypotension. It is sad that the doctors were negligent in their actions. There are two hospitals in Mthatha within about 200 metres of each other. The lower hospital has casualty, and the higher hospital is called the Accident and Emergency hospital, although both are carrying out same type of work. Patients are referred from the lower hospital to the higher hospital, and sometimes they die on the way to superior hospital. The procedure of transfer is so slow that it will take a lot of time, and when the patient is in a critical condition, he, or she in between these two hospitals.

There are a high number of rapes and a high risk of unnatural death among children in Transkei.[7,8] A study carried out by the author showed that about 12% of prehospital deaths are preventable. [6] It is not sure how many of these deaths could have been avoided if the patient was not admitted to hospital. There are several deaths that have occurred by the act of commission as well as by the act of omission. They could have been counted as surgical mishaps, but it is difficult to find out if an overdose or the wrong use of medication in hospital was the cause of death. This is because there is no instrumentation to measure the drugs and patients' blood levels in hospital. About 1% of hospital admissions have an adverse event due to negligence in the United States of America.[9]This is much higher in Transkei hospitals. Medication errors are common throughout the health care system and result in significant human and financial cost.[10]

A nursing sister who was in the rape referral centre saved the life of this child. She immediately picked up that the patient was going hypotensive and immediately organised a trolley and took her to casualty where intravenous fluid was given to the patient and therefore the child survived. This is not recognised by the hospital management. The practice of those who are doing right must be appreciated and those who are doing wrong must be reprimanded but this is not in practice in this region. The author has written to Chief Executive Officer of one hospital to

carry out this practice, but she was in fact favoured the politically sound medical officer who was frequently committed the same negligence.

It is a serious ethical issue and doctors must be penalised if there is some responsible governance of the hospital in place but unfortunately it is lacking. The hospital management is generally ignoring this kind of mishap and unethical practice of medical officers in hospitals. It is ironic that they pay more attention after the death when they organise a memorial service and console the family. There is a mechanism in place for reporting to the Health Professions Council, but generally the people are illiterate, and they do not know the system. Transkei people do not have a litigious mind and they do not report to anyone. They accept the death or injury as given by the God.

Mistakes must not happen, but mistake must at least not be repeated in dealing with the life and death of a patient. An independent review of doctors' treatment plans suggests that 14% of admissions can have improved decision-making; many of the benefits would have delayed manifestations.[11] Even this number may be an underestimate. One study suggests that, in the United States, adults receive only 55% of recommended care.[12] At the same time, a second study found that 30% of care in the United States may be unnecessary.[13] AM is not rich to afford private health care. Increasing levels of absolute poverty have been recorded in the Eastern Cape.[14] Seventy-four percent of people in the Eastern Cape live below the poverty line of R800 (equivalent to 105US dollars) or less per month. The poverty level is below 82.3% in the Transkei region.[15]

The rate of sexual assault runs parallel with HIV prevalence. AM was qualified for post exposure prophylaxis (PEP) as she was reported within 24 hours. She lost the opportunity to receive PEP within six hours because of the doctor's negligence as they could not refer her timeously. AM was tense and stress out and in need of counselling and psychotherapy. Some of the victims of rape lead to suicide. A study carried out in UK showed that 20% of victims face psychiatric problems after rape. [16]

The *res ipsa loquitor* (the case speaks for itself) doctrine is currently not being used in the South African courts. [17] AM's case fits the *res ipsa loquitor* context where she was moved to a different hospital which was not needed and, because of that, she was almost near to death. Her PEP was also delayed whereas it is always good to use it as early as possible to provide more effective protection. Poverty alleviation is not only important to fee a human being, but it is also important to bring morality among people and to control increasing HIV infection in the community.

South Africa is having two faces. On the one hand, it has highly developed areas such as Cape Town and on the other hand grossly underdeveloped regions like Transkei. The health care services are also at two extremes. This has been going on from the time of apartheid without much improvement. The public is also not well informed because of illiteracy and therefore they are powerlessness. Life hardly has any meaning in poverty.

## Conclusion

The victims of rape are also victims of the dysfunctional health care system in Mthatha Hospital in South Africa. There is a shortage of ethics in the care of patients to save lives. It is not only a legal requirement but also a serious ethical issue in the care of patients. The hospital management must take note of it.

**Ethical Issue:** The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa. The author has expressed his personal views at places and have no intention to demean the hospital and its management.

**Conflict of Interest**: None

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# Guilty of Unnatural Death but HIV Positive in Transkei Region of South Africa

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## Abstract

**Background:** HIV infections and crime have a complex relationship. It is difficult to understand its unnatural causation. HIV positivity has never accounted as an underlying cause of death in persons who have died unnaturally.

**Objective:** To correlate HIV infection with non-natural deaths in the Transkei region of South Africa.

**Method:** This is a record of a review study at Mthatha (Umtata) General Hospital. The data was collected from the office of medical superintendent and from the forensic pathology laboratory.

**Results:** There has been an increase in the Mthatha General Hospital mortality rate by almost two- fold in last five years. Suicidal deaths like hanging have increased by one and half times. Fatal poisoning, possibly suicidal, has increased about five to sixtimes. Gunshot injuries, which may or may not be suicidal, have increased by one and half times. The accurate estimate of the prevalence of the HIV/AIDS is a necessity to measure the costs of disease for effective strategic planning.

**Conclusion:** The HIV infection is increasing along with natural and non-natural deaths in the Transkei region of South Africa. It must be considered in the category of diminished responsibility as a mentally sick.

***Keywords:*** *Mortality, HIV infection, unnatural deaths, diminished responsibly*

## Introduction

South Africa is experiencing an HIV/AIDS epidemic of shattering dimensions.[1] About 160 000 to 200 000 people have died of AIDS-related illnesses in South Africa to date, but four million are infected with HIV. The inevitable disruption by HIV/AIDS of all aspects of our society, including the built environment, will be so profound that it is virtually impossible to imagine its dimensions.[2] Transkei is also known for the increasing trend of unnatural deaths in South Africa.[3] These deaths are painful and yet preventable. It is generally assumed that unnatural deaths are prevalent in the urban areas of South Africa,but this is not necessarily so. The rural population is poorer than the urban one, and therefore they take more risks in procurement of food. Poverty along with HIV is the main underlying cause of death.[3]

The Transkei is the poorest and most underdeveloped area in the South Africa, where mortality is very high, and certification of death caused by HIV infection represents a particular difficulty in terms of death certification. The stigmatisation associated with HIV-related disease has made doctors reluctant to specify it as a cause of death.[4]

The majority of deaths (78.9%) were certified as cardio-respiratory failure. Surprisingly, there was no mentioned of HIV/AIDS as the primary or underlying cause of death in the hospital survey of death certificates.[5] This indicates clearly that there is a lack of knowledge and sensitivity regarding HIV/AIDS surveillance among health professionals on the value and practice of filling out the death notification forms. Perhaps the stigmatisation associated with HIV-related disease has made doctors reluctant to specify it as a cause of death. This will provide misleading statistics in the HIV/AIDS related deaths in the area. In such a state, death certification does not reflect the number of HIV/AIDSdeaths.[1] There is need to explore some other predictors of HIV/AIDS deaths. Therefore, the purpose of this study is to explain the high trend of deaths in the Transkei region of South Africa.

## Method

The mortality statistics for 1996 to 2000 were obtained from the office of the Medical Superintendent of the Mthatha (Umtata) General Hospital (MGH). The suicidal statistics were collected from the medico-legal laboratory at MGH. Deaths due to hanging, poisoning and gunshot injuries were compiled from the medico-legal register manually. The analysis was presented in a graphic form.

## Results

The Mthatha General Hospital is a tertiary hospital attached to the University of Transkei's Medical School. This is the only centre in the region for tertiary education serving up to five million people. The trend in hospital mortality has increased in the last five years from 15 percent in 1996 to 25.3 percent in 2000 as shown in Table 1.

**Table 1. Trend of deaths in Umtata General Hospital (UGH) vs HIV infection over a period of five years (1996-2000)**

| Year | Natural deaths | Hanging | Gunshot deaths | HIV Positive (%) |
|---|---|---|---|---|
| 1996 | 15% | 16% | 14% | 14% |
| 1997 | 16.3% | 15% | 21% | 17% |
| 1998 | 18.8% | 21% | 18% | 23% |
| 1999 | 24.7% | 24% | 23% | 22% |
| 2000 | 25.3% | 24% | 24% | 24% |
| Total | 100% | 100% | 100% | 100% |

Poisoning has increased four percent to 28 percent in last eight years. This increase corresponds with the increase in the prevalence of HIV as shown in Table 2. The males are (66%) almost double in number than females (34%).

**Table 2. Incidence of deaths due to poisoning: males vs females (1993-2001) at Umtata General Hospital**

| Years | Male (%) | Female (%) | Total poisoning deaths (%) |
|---|---|---|---|
| 1993 | 3 (3%) | 1 (1%) | 4 (4%) |
| 1994 | 1 (1%) | 3 (3%) | 4 (4%) |
| 1995 | 2 (2%) | 4 (4%) | 6 (6%) |
| 1996 | 2 (2%) | 3 (3%) | 5 (5%) |
| 1997 | 6 (6%) | 1 (1%) | 7 (7%) |
| 1998 | 11(11%) | 3 (3%) | 14 (15%) |
| 1999 | 9 (9%) | 2 (2%) | 11 (12%) |
| 2000 | 10 (10%) | 6 (6%) | 16 (17%) |
| 2001 | 17 (19%) | 9 (9%) | 26 (28%) |
| Total | 61(66%) | 32 (34%) | 93 (100%) |

## Discussion

This is the first report on the relationship between HIV/AIDS and unnatural deaths. There is no literature available even though there are a significant number of people living in society with HIV/AIDS. There is no researched on this correlation of HIV positivity and unnatural deaths. The prevalence of HIV/AIDS varies from country to country, and within countries from region to region. Similarly, the number of unnatural deaths also varies. Crime is increasing in South Africa even though the policing and road traffic control system have increased. Many perpetrators of crime are not guilty but are mentally sick.[6] This sickness could be because they are HIV positive. There is a bulk of literature available on crime where researchers have counted several reasons for its increase. HIV/ADS has not counted as a contributor to unnatural deaths by any of the researchers. Many of the HIV positive people may be perpetrators of crime or vice versa.[7] Very little is known about the underlying reasons for unnatural deaths. Death from HIV/AIDS is accepted as a natural cause of death. It is true that some people do die after a long illness following the development of AIDS, but how many reach that stage, and many of them die while walking on a road as car knock down or are involved in some quarrel in a shebeen (local alcohol selling outlet in a rural area). A few HIV positive individuals are put behind bars because of their involvement in rape. Rape of a virgin is considered as a cure for HIV in this region of South Africa.[7]

Moreover, there is also the situation where death may occur following an accident that arises from drunk driving. There is always a lighter punishment for those who commit a crime under the influence of alcohol but there is no provision for HIV positive people who commit a crime. Being HIV is stigmatized and it is not visible, so it is not considered equal to alcohol, but the effects or consequences are more serious than those for an alcoholic who commits a crime. Alcohol also affects the brain function and HIV also causes brain disorder; therefore, it should be in the same category as alcohol. Alcohol and crime have a closely interconnected relationship, with alcohol abuse being

a contributory factor to many crimes and many crimes being a contributory factor to alcohol abuse.[8] Criminal behavior is so common among alcoholics.[8]Most treatment centres are dealing with the emotional and financial and personal toll of crime,[8] but this is not the case in HIV positive individuals. Therefore, in any accident, there is a mandatory test for alcohol, but there should also be also a test for HIV positivity. Pre- and post-test counselling must be carried out.

The antenatal survey of HIV/AIDS is seen to be a less sensitive method of data collection as the data suffers from under-reporting, especially in the rural areas of the Transkei. Under-registration of deaths has long been known to be a problem in South Africa[9,10] and it is important to estimate the extent of under-reporting. There has been an increase in the morality at Mthatha General Hospital since the HIV/AIDS epidemic started climbing (1996-2000), even though the deaths related to crime have been stabilized but at a higher level.[11]

Unnatural deaths are a serious and preventable public health problem. HIV infection is also a public health issue and preventable. Both have some relationship directly or indirectly, but it needs an in-depth study. Both are having a negative long-lasting effect on families. HIV infections disturb the individual and they have a lot of psychological effects such violence and suicide. HIV infection and psychiatric disorders have a complex relationship. HIV infection can lead to psychiatric disorder, and psychiatric persons are more vulnerable for HIV infection.[12]A high number of HIV positive cases are in a community where there is lack of a support system in place. People are poor and cannot afford to have three meals in a day. Alcohol consumption is very high. It is either the habit of drinking alcohol or drinking because of HIV positivity or both. These people are just like the walking dead without any fear. This could be because of the effects of HIV on brain function, as this leads to behavior changes in the home and outside of the home. This often results in an accident or involvement in some violence; either they kill someone, or someone can kill them. Can one think of an HIV positive person driving a car on the road?

HIV infection has increased from 1996 (14%) to 2001 (25%), and a similar pattern of increase has also been observed in death due to hanging, poisoning and gunshot injuries (Table 1 & 2). Death due to suicide by hangingincreased by one and half times. That is from 16 percent in 1996 to 24 percent in 2000(Table I). Most of the suicide victims were males between 20 to 30 years of age (Table 1). A study carried out by the author showed that there is increasing trend of hangings, especially in young adults between 20 and 29 years of age old.[13] The HIV is highly stigmatized and there are many instances of discrimination against sufferers and their families. This could lead to suicide, both in infected as well as unaffected individuals.[14]There have beenan increasing number of fatal poisonings from 1993 to 2001 in this region (Table 2). It is possibly also suicidal in nature. It has increased about five to sixtimes in this study period (Table 2). A twenty-year studyhas showed that there has been an increasing trend of poisoning in the Transkei region of South Africa.[15] Members of the Xhosa tribe in the Transkei region frequently consult traditional healers and use herbal medicine, and sometimes they are lethal.[15]Firearm-related deaths are high in South Africa, but they are very high in the Transkei region. People have the habit of carrying guns.[16] Gunshot injury related deaths were also increasing in this study (Table 1).The gunshot injuries, which may or may not be suicidal, have increased by one and half times (Table 1).

Statistics South Africa is conducting a mortality study into “secondary” causes of death to assess the true impact of HIV/AIDS. The agency said preliminary indications were that there has been

a marked rise in the number of people between 20 and 40 dying of natural causes, which include disease.[17] This rapid change in the empirical death rates confirms predictions of the profound impact of AIDS on mortality. These shocking results need to galvanize our efforts to minimise the devastation of the epidemic.[1]

There are HIV/AIDS promoting factors in the community like promiscuous behavior, excessive consumption of drugs (like alcohol and cannabis), poverty, disintegrated families, migrant mineworkers, high incidence of child abuse and rape, poor HIV/AIDS awareness programmes, poor service delivery regarding HIV/AIDS, and so on. It is difficult to understand that HIV promoting factors are more prevalent in the Eastern Cape than in the KwaZulu-Natal, but the prevalence of HIV is one and half-times higher in KwaZulu-Natal than the Eastern Cape Province. It means there is under estimation of HIV/AIDS prevalence in the Transkei.

The outcome factors of HIV/AIDS are an increase in the mortality, especially related to suicidal deaths like hanging and poisoning. Previously the suicide in the black population was uncommon; however, there has been an increasing trend found in recent years.[18] Furthermore, the impact of HIV/AIDS could be further confirmed by various other parameters such as sick leave, students' admissions, and work output. The University of Transkei is the only tertiary institution in the area, which is serving the community of about five million. Most of the students are either from Transkei or adjoining areas. However, the student numbers have not increased during study period. It is not sure whether HIV/AIDS has affected their admission to university. Poverty is also associated with HIV/AIDS, and it makes families poorer because of lack of work and more expenditure in families.

This preliminary report which is also a quite an old study may be a surprised to readers as it also surprised the chairperson in the conference at university where it was presented. The chairperson became violent and could not digest its findings, but the fact is that non-natural deaths cannot be controlled without controlling HIV/AIDS in the community. This is author's firm belief.

## Conclusion

There is high impact of HIV/AIDS in the form of a high crime rate in the Transkei region of South Africa. It places enormous challenges on health care as well as on law-and-order governing agencies. Government must take note that, without controlling HIV/AIDS in the regions, it is difficult to control crime as well.

### Limitations

Although there are several biases in this study, especially the limitation of years, it extends its support for the study conducted by the Medical Research Council, South Africa, 2001.

**Ethical Issue:** The author has ethical permission for a case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa. This report was presented at a conference of university.The author has expressed his views in this study and has no intention to harm anyone or any institution.

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# Post-retrenchment and Retirement of Mineworkers: A Poor Quality of Life in Transkei Region of South Africa

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## Abstract

**Background:** Former mineworkers are sandwiched between scare resources and little hope of getting re-employment. They do not have enough savings to maintain their families. The expenditure is at its highest when they return from the mines, as the children are grown up and are in secondary or senior secondary school. Many are not re-employable because of poor health, and some are disabled.

**Objective:** To highlight the problem of retrenched mineworkers in the Transkei region of South Africa.

**Method:** The case histories of these mineworkers were recorded either at Benefit Examination Clinic (BEC) or at the forensic pathology laboratory which is situated in the chest section of the Mthatha Hospital, Mthatha.

**Results:** There were 2027 former mineworkers examined at BEC between 1997 and 2000. Of these, 172 (8.48%) were re-examined and only 51 (2.5%) received compensated over the period of four years. The examination of mineworkers has decreased from 1997 (29.6%) to 2000 (3.94%). There were 55 (2.71%) died before they received their benefit of examination.

**Conclusion:** Retrenched mineworkers are frequently under psychosocial pressure, including their families and the community. Extreme poverty, sickness, and disabilities co-exist among these retrenched, retired mineworkers in the Transkei region of South Africa.

***Keywords:*** *Retrenched mineworker, bread winner, poverty, sickness*

## Introduction

The migrant labour system has produced very extensive socio-economic effects in the Transkei region. It was estimated that two million of the five million black workers in South Africa at the time of apartheidwere migrant labourers.[1]There are about 600,000 mineworkers employed by the gold mining industry alone in South Africa.[2] The majority of them are from the Transkei region.About 14% of the former mineworkers who visited the Umtata 'Benefit Examination Clinic' between April and August 2000 indicated that they were given no reasons for their retrenchment.[3]There is evidence of a huge accumulation of unrecognised, therefore uncompensated, cases of pneumoconiosis and/or tuberculosis among former mineworkers living in the labour-sending areas such as Transkei.[4] The purpose of this article is to highlight the plight of these former mineworkers and their families. It also provides some insight into the compensation claims in this region.

## Method

The BEC was opened by the author in 1996 as a voluntary service to the former mineworkers to meet the demand as they were not compensated for their disease and disabilities. They were invited by the radio news in this region to come every Wednesday at to the chest section of Mthatha Hospital (it was known as Henry Elliot Hospital in apartheid time). Their mining history, chest x-ray and a compensation form were sent to the compensation commissioner through The Employment Bureau of Africa (TEBA) in Johannesburg at the Medical Bureau of Occupational Diseases (MBOD), National Centre of Compensation. The case histories of these mineworkers were taken either at the Benefit Examination Clinic (BEC) or at the forensic pathology laboratory situated in the chest section of Mthatha Hospital, Mthatha.

## Results

There were 2027 former mineworkers examined at BEC between 1997 and 2000 (Table 1). Of this172 (8.48%) were re-examined and only 51 (2.5%) were compensated over the period of four years (Table 1). The examination of mineworkers has decreased from 1997 (29.6%) to 2000 (3.94%) while at the same time, re-examination has increased from 0% (1997) to 8.48% (2000). There were 55 (2.71%) who died before they received their benefit of an examination (Table 1).

**Table 1. Ex-mineworkers examined, re-examined, compensated and died before they received their claim from 1997 to August 2000**

| Year | Examined for compensation claim | Re-examined for compensation claim | Compensated | RIP |
|---|---|---|---|---|
| 1997 | 601(29.6%) | 0 (0%) | 9 (1.5%) | 9 (0.44%) |
| 1998 | 1181(58.26%) | 11(0.54%) | 17(1.4%) | 18 (0.88%) |
| 1999 | 165(8.14%) | 99 (4.88%) | 18(11%) | 20(0.98%) |
| 2000 | 80 (3.94%) | 62 (3.05%) | 7(9%) | 8 (0.39%) |
| Total | 2027 (100%) | 172 (8.48%) | 51(2.5%) | 55 (2.71%) |

*RIP= Rest in peace are the subjects who were awarded compensation but died before receipt.

## Discussion

Transkei is the labour supplying region for theSouth African mines. TEBA recruit these young boys to take them to different South African mines. It is a job of pride in the beginning but not once they realise how small their salaries to the extent that they are not even enough for Lobola to get married during their service. Lobola is mandatory in the Xhosa culture to give to bridegroom before the marriage ceremony in the form of cash or sheep or cattle. These sick former mineworkers lost their places in society as they could not work anymore. They become a burden on their families to provide them both with food and medicine.

There were 2027 ex-mineworkers who presented to the BEC, and of these only a very few (2.5%) compensated. The number (2.71%) of deaths who were more than the compensated mineworkers were more

than compensated. It is difficult to know why there were so few who were compensated, but probably the staff of MBOD are still following the apartheid practice, and they were excluding black mineworkers. This is confirmed by the fact that before 1994 hardly any black mineworker was paid their compensation claim while white mineworkers who hardly even worked under ground were compensated. Less than 10% were re-examined for their compensation claim. It is because MBOD asked them to get re-examined and send a report back to them. Most of the time, the x-ray plates are of very poor quality so they may need another x-ray plate. Former mineworkers who worked in apartheid times are probably hardly surviving. The slow compensation mechanism of the MBOD is to get the process delayed so there will not be any mineworkers left alive and so the problem will be solved by itself. Doctors in the Transkei region are also not interested in helping these mineworkers as it is a tedious job without the help of government. The Health Department is always under stress and has no interest again in helping these poor mineworkers.

*A young adult of around 20 years of age was recruited by TEBA for a mining job. He looked just like a 60-year-old, although he was just 33 years of age. Here is a description of him by his wife at the Benefit Examination Clinic (BEC): This is an example of a family where wife is the breadwinner, and the husband is sick that he cannot work. He is irritable and shouts at the children with the slightest provocation. This situation is compounded by his impairment in hearing. When young he had been handsome, and the wife was attracted to him and married him. Now she sells household items such as paraffin, candles, and soap. She is now the breadwinner.*

The extremely high burden of lung disease in ex-mineworkers is an enormous challenge to the health services and compensation authorities. An X-ray based study conducted by the author (2002) showed that 78.2% of ex-mineworkers had evidence of lung disease. Pulmonary tuberculosis (PTB) with or without silicosis was evident in 64.2% of the x-rays, silicosis with or without PTB in 34%, chronic obstructive pulmonary disease (COPD) in 7%, and asbestosis in 1.5%.[5] There is the case of a mineworker who went to get his examination at BEC. He travelled by taxi and paid a heavy fare.

*KM is an* ex-*mineworker suffering from lung and kidney disease with unusual skin pigmentation. He was admitted to the local hospital. He traveled 80 km hiring a taxi only to attend the Benefit Examination in Umtata.*

Many mineworkers are not re-employable because of their poor state of health, and some are disabled. They get used to taking alcohol to drown their sorrows.Only 2.5% of former mineworkers received their compensation (Table 1). It is too low, and others who received compensation died (2.71%) before they received their funds (Table 1). The following is such an example.

*BD, a-60-years-old ex-mineworker, died on 6th June 2000 about 6.00 in the morning. His body was lying outside the gate of his house. He had come late from a shebeen. He had the habit of jumping over the gate when it was locked. On this day he was found on the other side. A of duty policeman saw him, took him to his house, and informed his colleagues. The deceased had the habit of going to the shebeen in the evening and returning home the following morning and sleeping it off. Although 60 years of age, he was getting an old age grant and used that money for drinking. His wife left him because of the drinking problem, and he was left with three children. His drinking started from the time he was retrenched. Two of his children were schooling in standard 4 and standard 2 and the third was staying at home.*

The discovery of gold provided the base from which South Africa was able to develop a substantial industrial capacity, but the mines demanded a reliable supply of labour, which could only be met by drawing in migrant workers from distant rural areas both within and outside South Africa. The migrant labour system not only creates situations in which diseases such as tuberculosis and Sexual Transmitted Infections (STIs) flourish, but also serves to disseminate these diseases widely throughout the region.[6] Many of these migrants contracted fatal diseases in the mines. Historian Randall Packards[7] paints a grim picture of this trend.

*GK, a 49-year-old, was retired mineworker from the goldmines. He earned a monthly wage of R1105, 66. He worked from 1977 to 1997 when he took a voluntary retirement package. He was awarded R14 031.48. He has five young - children. In 1989 he was treated for pulmonary TB in the mine hospital and in 1998 for a relapse in the local hospital. At the same time cancer of the oesophagus was also diagnosed. He had been a smoker and a consumer of alcohol. He died because of complications from the cancer.*

Many mineworkers ended up disabled because of mining accidents. Many go home without compensation and RM, the insurers, do not deal with old cases.

*LT a 40- year- old started working underground in a mine in Elandsrand in 1984 and worked until September 1996. He died in 1999. When he left for the mines, he was a healthy young man. He sustained a fracture whilst working underground and was discharged. After recovering he worked for a construction company for a meagre monthly salary of R260/-.*

During an informal interview, a spouse described that the ex-mineworker husband is "no longer the person I married". The man was impotent and could not meet her demands. This was revealed in a case where a woman poisoned her husband. Her daughter revealed this before committing suicide.

*ZZ, a 27-years-old female, hanged herself in Feb 2001. A suicide note revealed that she was taking her life because the mother was not close to her. The father had died of poisoning in 1998, and police were not informed. He had worked in a platinum mine in Rustenburg for 20 years. He was retrenched because of poor vision. The wife, the mother of the deceased, had poisoned the father as he used to sleep away from his wife (the son-in-law of the elder brother of the father narrated this story).*

There was not much attention paid to black mineworkers, even though they performed the most difficult jobs like drilling rocks at deep levels. The Occupational Disease in Mines and Works Act (ODMWA) used racial criteria in the past to determine the amount of compensation paid to workers. White workers with pulmonary tuberculosis (PTB) were paid 5.5 times more than black workers. White workers with silicosis were paid up to 13.6 times more than the black workers.[8] The majority of the ex-mineworkers are now in their fifties (mean age 51years), capable of re-employment if they are fit and healthy. However, many of them are sick and even if they are employed will get very low wages.

*The family of an ex-mineworker was admitted to the local hospital with food poisoning. On inquiry it transpired that they had eaten meat of a cow found dead. They decided to consume this meat because of poverty because they cannot afford to buy meat from the butchery. Many ex-mineworkers live below the poverty line. Some get occasional work and earn a few Rand which is hardly enough to run a family.*

There are ex-mineworkers who are suffering from mental illnesses. It is difficult to manage their illnesses, as there is lack of health facilities in the

rural areas of Transkei.

*On 11th May 2000, a 51-year-old ex-mineworker was knocked down by a car. It was a hit and run accident. He had a history of mental illness. He had been a mineworker in President Steyn Mines. Since he left the mine, he was living without any money. He had been acting strangely, sometimes even running naked in the locality and collecting trash. He had once been admitted to hospital in Queenstown in 1997.*

Many ex-mineworkers have been suffering from hearing impairment. Some of them are completely deaf. It is difficult to apply for compensation, although their hearing loss is due to their mining job. If a miner is applying for compensation, it must be done within a year of retrenchment or retirement. This in most cases does not take place. As a result of this most ex-mineworkers who end up with hearing loss get nothing as compensation. A recent study conducted by the author showed that there is a high prevalence (54%) of hearing loss among ex-mineworkers. Of them 33% were between 40 to 59 years of age.

Twenty-two percent who had worked in the mines for 10-20 years had indicated loss of hearing.[9]

The returning miners impact their families and communities in two main ways. One is measurable and can be compensated. Disease and disability belong to this category. The other is issues such as psychosocial impact. Diseases and disabilities could be assessed by age and the level of skills. The compensation commissioner's office makes use of such indices for playouts either as a lump sum or in instalments. Only about 8-10% of the mineworkers have been compensated. The unpaid liability of compensation for occupational lung diseases to the mineworkers and their families should be paid without any delay. There is a need for more clinics in rural Transkei so that mineworkers have easy access to medications for the chronic diseases such as silicosis and chronic obstructive pulmonary disease that they are suffering from. The doctors in public service are not fulfilling their legal obligation to submit the claims for living and deceased ex-mine workers.[8] It is difficult to estimate the financial costs to the families because of sick ex-mineworkers.

The process of getting compensation is tremendously slow and inadequate as shown in Table 1. Only 2.5% of ex-mineworkers were compensated between 1997 and 2000. In the meantime, many ex-mineworkers have died of tuberculosis or silicosis or combined plus HIV infection. There is only one clinic in this area that serves the ex-mineworkers in this region. There is a heavy burden of silicosis among young ex-mineworkers in the Transkei region. The strong association between silicosis and tuberculosis in southern Africa combined with the HIV epidemic make elimination of silicosis an important public health issue.[10] Farming is the only productive work that most returning miners can perform. However, they lack land and the skills in farming. For as long as they worked in the mines the families were supported,but on their return, they become dependent on others, mainly because of poor and failing health. Most end up destitute.

## Conclusion

The pride of being a male breadwinner sometimes is lost, and the wives must take the role upon themselves. Some take to drinking alcohol and run the risk of premature death. Everyone is healthy at the time of recruitment, but many return diseased. Pulmonary tuberculosis overshadows underlying silicosis among mineworkers. Oesophageal carcinoma is associated with silicosis, and this could be the reason for its high prevalence in this region.It is not only the ex-miner who is usually under psychosocial pressure, but the families and the community as well. Extreme poverty exists in many families of ex-mineworkers and this needs to be addressed by the government as a priority.

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**Ethical issue:** The report has been prepared to highlight the problems in the teaching of forensic medicine in the undergraduate medical curriculum. It is not intended to bring down any person or any institution. The author has ethical permission for the case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# An Unusual Case Report on Co-Morbidity with Sexual Assault in the Mthatha Hospital, South Africa

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## Abstract

**Background:** Comorbidity or the co-occurrence of mental disorders and substance abuse disorders is common among victims of sexual assault. Occasionally life-threatening conditions have been observed in these patients which need immediate medical attention.

**Objective:** To highlight the unusual case report of co-morbidity with sexual assault in a rural hospital in South Africa.

**Case History:** A seven-year-old girl was referred from a health center to a rural hospital with a history of sexual assault over four days by an unknown man. She was threatened to be killed in the case of disclosure. Her aunt suspected that she had a problem as she was not walking normally. Then she opened and described the whole incident. She was having a history of vaginal discharge with vomiting and diarrhea along with mild fever. She was also depressed. On physical examination, genital injuries including a ruptured hymen were confirmed. She was having muscle guarding of abdominal muscles. The victim was refused admittance as she was labeled a case of rape, but after persistent persuasion of the staff, she was admitted and later operated on for acute appendicitis. This case history, her physical examination, and the difficulty in getting admission to a surgical ward are discussed in this report.

**Conclusion:** Sexual assault may be associated with co-morbidity like acute appendicitis. Doctors must be vigilant in identifying such life-threatening co-morbidity to save the life of a patient.

***Keywords****: sexual assault, abdominal pain, comorbidity*

## Introduction

Co-morbidity or co-occurrence of disease conditions is not uncommon among victims of sexual assault, especially mental disorder and drug or alcohol abuse.[1] Sometimes there are some serious fatal conditions which need urgent medical or surgical attention which one cannot expect.[2]Getting diagnosis right is a key aspect of health care - it provides an explanation of a patient's health problem and informs subsequent health care decisions. The diagnostic process is a complex, collaborative activity that involves clinical reasoning and information gathering to determine a patient's health problem.[2]There is clearly a need to improve diagnosis in health care as diagnostic errors and inaccurate or delayed diagnoses persist throughout all settings of care and continue to harm an unacceptable number of patients.[2]

The victim of a rape feels pain from physical trauma in the aftermath of sexual assault, but there might be a micro biological complication as well caused by stress from the terrifying crime.[3]It is very important to recognise the early symptoms

of appendicitis, for example, so that one can seek medical treatment. Having ruptured appendicitis is a life-threatening situation. The risk of rupture rises dramatically after 48 hours of the onset of symptoms.[4]

It is likely that most people will experience at least one diagnostic error in their lifetime, sometimes with devastating consequences. Diagnostic errors may cause harm to patients by preventing or delaying appropriate treatment, and providing unnecessary or harmful treatment, or by resulting in psychological or financial repercussions.[2] The diagnosis is a collaborative effort. The stereotype single physician diagnosis is not true. The diagnosis process often involves intra-and inter-professional teamwork. Nor is diagnostic error always due to human error; often it occurs because of an error in system.[2] Child rape is becoming more common in South Africa. In 2000, over 52 550 cases of rape or attempted rape of women were reported to the South African police services. [5]Of these 21 438 victims were children under the age of 18 years.[5] Child rape increased to 24 387 in 2018/19 according to children institute of the University of Cape town.[6]

Tremendous challenges remain in the field of prevention of rape, especially in the former black homelands in South Africa. The history of South Africa sheds light on the factors behind the high incidence of rape in the country. Apartheid made violence an instrument of control and violence became the norm in people's day-to-day lives. There was rigorous apartheid in the black homelands. [7] Poverty is also a legacy of apartheid. This trio of poverty, sexual assault and HIV are complementary to one another.[7]Poverty forced people to use children for sexual exploitation.The purpose of this case report is to highlight the unusual co-morbidity of with sexual assault in a rural hospital in South Africa.

## Case History

SR, a seven-year-old Grade 2 female child from Libode area, was brought to Centre with a history of sexual assault on 17 February 2006. An unknown man approached her while she was coming from school with her classmate. He was wearing dark glasses and driving a white car. The other child ran away, but the victim was caught and was promised to be given sweets and told she must not tell anybody. She was threatened to be stabbed if she disclosed. The man stripped and sexually assaulted her. The child did not tell as she was fearful of this man. Her aunt suspected she had a problem as she was not walking normally. When she asked child, she opened and told the entire story. The aunt found that she was having a discharge from a genital injury. There was also a history of vomiting and diarrhea of four days duration. She was also feverish, coughing, and depressed. The child was referred from the health center to the Sinawe Centre.

At the Sinawe Centre she was examined, and the finding was consistent with the history given by the victim. There were genital injuries with ruptured of hymen. Thorough investigations were ordered, and comprehensive management was carried out as per protocol. She was having muscle guarding of abdominal muscles, fever, and diarrhea and vomiting. The patient was also referred to a pediatrician at the Nelson Mandela Academic Hospital, but she was refused admittance as she was labelled a case of rape, but after persistent persuasion of the Sinawe staff, she was admitted and later operated on for acute appendicitis.

## Discussion

This is an only case reported at one centre which is attached to a rural hospital. This centre caters for about 500 000 people in the region, and it provides services around the clock to all the victims of sexual assaults. It has trained nursing staff who are capable

of handling rape victims. They provide pre- and post-test counselling and give post-exposure prophylaxis to the victims. Delay in reporting is very common. This is because of lack of taxi fare or no transportation and sometimes it is because of threatening by the the perpetrator as in this case of SR. She reported after four days and, therefore, she lost the opportunity to get PEP, and is now vulnerable to get HIV infection.

SR was a victim of rape and at the same time suffered from acute abdomen pains. Rape victims are generally stigmatized not only in the eyes of laymen but also among health professionals. Doctors generally ignore it and shift the responsibility without taking a proper history and doing a physical examination. There is need to build a safer health care system so that victims of rape will not be neglected. It is difficult to establish whether SR got appendicitis because of the rape or if it is just a coincidence. After an extensive search of the literature, there was no evidence found that is suggestive of any association between rape and acute appendicitis. However, rigorous rape of a young child may induce the inflammatory process in the gut. SR received a serious genital injury as she could not even walk.

SR was refused admittance to hospital and returned to the one stop centre twice despite the note from a nursing staff member saying that it was a case of acute abdomen pain that needed surgical attention. Examination of the rape could have been carried out later but saving the life of the patient needed urgent attention. Anyhow, the doctor on duty examined and admitted her and subsequently operated for appendicitis. Unfortunately, doctor often just take the history of the rape and do not consider that there could be something else as well. A patient may have two or sometimes three acute conditions at the same time. Doctors must thus be vigilant regarding these associate conditions. A recently reported case of acute pancreatitis associated with severe acute respiratory distress syndrome in coronavirus-2 infection is an example of it.[8]SR was fortunate that she was diagnosed at the centre by the nursing staff. They felt that there was something else other than the genital injuries causing the lower abdominal pain. The health professionals are just carrying out genital examinations without looking for other morbid condition of victims. The completion of a J88 form or a rape crime kit is the only aim which is important to them. They must, instead, always examine a case of sexual assault holistically. The doctor is not just a genital technician and must not examine just the genitalia without assessing the patient thoroughly.

It is not clear how many patients are dying in rural hospitals because of errors of doctor. There is not much research carried out in this field, and nobody even discusses these issues in hospitals. It remains a secret of doctors and sometimes of nursing staff. Death certification is completely very poorly, and it is difficult assess the real cause of death. The cause of death is always a hidden affair known only to the treating doctor. If the cause is not clear, why do they write a fictitious cause of death on the death notification form? There are only two possibilities, either they were not able to diagnose the condition of the patient, or they hid it to avoid the litigation. In public hospitals there is no fear of individual doctor litigation. Patients may sue the hospital or health department. A study carried out the author in 2003 showed that doctors at hospitals are not experienced in completing a death certification form.[9] Most of doctors are certifying death as a cardiorespiratory failure, which is neither a cause of death nor a mechanism of death. In fact, it has no meaning.[9] Therefore, it is difficult to pick up any death which as caused by the negligence of a doctor. Very few cases were reported where the negligence of doctor was document and published by the author.

Reporting cases is also difficult as one doctor generally should not complain about another. Instead,

it is the management who must be vigilante. A case report by the author on inadvertent intrathecal administration of potassium chloride during routine spinal anesthesia was documented.[10] It had no impact on the services of the hospital. Several maternal deaths were also highlighted in this hospital because of it is mandatory to get an autopsy. A paper was published in the South African Medical Journal (2004) on maternal deaths, where doctors' serious negligence was reported,[11]but it just remained in the journal, and no action was taken by the hospital even though it is in the public domain. This is a dual tragedy in hospitals in this region of South Africa. Firstly, doctors are not skilled and sensitive to human beings and, secondly, there is very poor management of the hospital, lacking in managers who can function in the interests of patient care. Doctors must take responsibility for each death in hospital but unfortunately this does not happen. The management must develop a mechanism to ensure that the patient interest as priority. Weekly mortality meetings would be an important step to look at the cases so that the weaknesses in patient care could be discussed. It is sad that most of the time management is turning a blind eye to the problems of the hospital. Recently, some lawyers have shown an interest to ask for compensation from the health department in a few cases after whistle blowers have pointed out cases of neglect, but still there is not much change in behaviour in care of the patient.

Human errors are associated with the deaths of omission as well as commission. The act of omission deaths is difficult to recognise as there is hardly any proof but some of the deaths caused by act of commission may be picked up in a forensic pathology laboratory. There is not a reporting system in hospital at present for any possible cases of neglect. During the time of the previous Transkei government, there was a form which one had complete, but it is no longer in use. The only way to make a report is through publication in scientific journals but who will read these journals? Secondly, the individuals concerned remain anonymous so hardly anyone can be get punished. Ultimately the poor, illiterate and voice less patients are left to the mercy of God.

This rural hospitals in former Transkei region are situated in a very fascinating area in a mountains range with lush green fields. This region is historically known for its high crime rate, and hardly any white South African doctors are prepared to settle to work there. White South Africans were advantaged by the apartheid regimen and therefore they are skilled. They are not contributing to the development of rural hospitals in this region. There is a serious gap between those who have and those who have not. Similarly, this gap is also visible in the care of patients. There is a medical school but that is also staggering with a shortage of trained teachers. Therefore, adequate training of students and doctors is lacking. Government must organise some method so that those skilled in an urban setting can be available in rural hospitals as well.

## Conclusion

Victims of sexual assault may be associated with co-morbidity. It could lead to or exaggerate or precipitate an acute appendicitis, although its mechanism is not clear. Therefore, rape victims must be examined holistically to avoid life threatening conditions.

**Ethical Issue:** The author has received ethical permission for a case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa. This report is kept anonymous as much as possible. However, if someone comes to be identified directly or indirectly, the author has no responsibility as his intention is to improve the patient care in this region, not to defame any hospital, region, or any individual.

**Conflict of Interest:** None

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# Sexual Assault, Pregnancy and HIV Infection among Young Girls in the Transkei Region of South Africa. Case Reports

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## Abstract

**Background:** Rape continues to be one of the biggest challenges facing South Africa, alongside poverty & joblessness that the government is trying hard to eradicate. Transkei region of the Eastern Cape can best be described as deeply rural with bad roads, unclean water supply, limited electricity, few telephone connections, very limited access to transport and health services. Violence including sexual assaults is a common problem in this region.

**Objective:** To highlight the problem of sexual assault, pregnancy, and HIV infection among young girls in Transkei region of South Africa.

**Case History:** This is a retrospective case report from the register of sexual assault at Sinawe Center of Mthatha General Hospital, Mthatha, South Africa.These are reports of two young girls who were repeatedly raped. Histories and findings of the physical examinations are described. Consequences of teenage pregnancies and HIV transmission are discussed. The issue of consent and teenagers' partners' responsibility is highlighted. Solutions to reduce re-victimisation are suggested.

**Conclusion:** There is problem of sexual assaults, pregnancy, and HIV infection among young girls in the Transkei region of South Africa. There is a need to control this epidemic of sexual assaults to prevent HIV spread, and to prevent unwanted pregnancies.

***Keyword:*** *Teenage pregnancies, sexual assault, Human Immune-deficiency Virus (HIV), rape, child abuse*

## Introduction

South Africa is a country with a rapidly escalating HIV/AIDS epidemic. There are also high levels of rape reported from various sources.[1]Here, as elsewhere, statistics capturing the true magnitude of sexual violence are difficult to obtain. Those released by the South African Police Service note that in 2001, 52, 860 rapes and attempted rapes were reported.[1] Only about 15% of women who were forced to have sex against their will reported the incident to the police.[2]Many women do not go to the police because they do not think that the perpetrator will get punished. The evidence suggests that their concern is justified. Few rape cases go to the courts, and of those that do, only 7-13% results in conviction.[3]

The widespread rape and forced sexual abuse of children is a serious social and health issue. With a teenage pregnancy rate in South Africa of 330 per 1000 women under 19 years, it is of concern that these same women are likely to have illegal and unsafe abortions.[4] Among 13-17-year-old females in the rural Transkei area of South Africa, 28% report

first sex as forced.[5] One of the motives behind this unsocial and unhealthy epidemic is the strong belief in a myth of achieving a cure for a person's HIV/AIDS status through sexual intercourse with a virgin. This is also a contributory factor in the transmission of HIV/AIDS in the Transkei region.[6]The author reports a case of a 13-year-old girl who was raped repeatedly, and as a result of it, she was HIV seroconvert.[7] More than 90% of the victims are HIV negative at the time of the incident in a very high prevalent community-it is clear that the HIV post-exposure prophylaxis coverage is indicated.[8]

The purpose of these case presentations is to highlight the problem of teenage pregnancies, gang rape, and repeated rape of young children. In terms of the Child Care Act 1983 of South Africa (Act No. 74 of 1983), minors of 14 years and older may consent to their own medical examination and treatment without the assistance of parents/guardians.

## Case History 1

On 31st May, 2005, a 13-year-old pregnant girl who is already the mother of a 2-year old child presented at the Sinawe Centre following sexual assault. She was raped twice by a young man in her location, and then he forced her to go to another location and raped her again. He also attempted to strangle her and had assaulted her with a knife. He did not wear a condom whilst having forced sex. She was allowed to go home the following morning when the matter was reported to the police.

On examination, there were nail marks on the front of the neck, and a cut on her left middle finger. On genital examination, there was a whitish discharge and marked tenderness. Genitalia were congested as in a pregnant woman. Vaginal speculum examination was avoided, and limited medication was given to avoid the adverse effects on the foetus.

She was sodomised in December 2004 and a course of anti-retroviral drugs was given as she had tested negative for HIV, RPR negative, but positive for Hepatitis B and pregnancy.

## Case History 2

On 8thFeb 2005, 14-year-old MT was returning home from church with her friends. A car drove up to them and three men who were in the car abducted her. She was shouting and screaming but they drove her to another location. They put a black cloth over her face and all four-gang raped her without using condoms. She was dropped late in the evening, and she returned home around 8 pm. She confided to her mother of the incident only after two days.

On clinical examination, she had an erythematous vulva, with outpouring of a thick vaginal discharge. She had completed a course of antiretroviral drugs about two years prior to that, following another incident of rape. She was gang raped again on 10th May 2005 by four men, three of whom were also involved in the previous incident. She was HIV negative and hence antiretroviral drugs were prescribed to her.

## Discussion

Rape continues to be one of the biggest challenges faced by South Africa today. These young rape victims are just the "tip of an iceberg" of a huge problem of rapes in the community. In a previous record review, 68% victims of sexual assaults were found to be less than 20 years of age.[8] Sexual assaults are generally underreported and such assaults on children still less.

A recent study by the author showed that there was an increase in adult rapes from 55.3% in 2000 to 59% in 2004. Although sexual assault of children is down from 44.7% (2000) to 41% (2004), the overall number has increased from 34 (2000) to 97 (2004). The total number of rapes has increased by more than three times among children and adults from 2000 to

2004.[9] The conviction rate of perpetrators is very poor in South Africa. Police data indicates that in 2000, only 45% were referred to courts.[10] The first case was raped for the second time and the other for the third time, in the latter by the same perpetrators. In either case police failed to bring them to courts. It takes long time to get a conviction and often cases are withdrawn. In 2000, 47% of cases referred to courts were withdrawn in courts and only 16.5% resulted in a guilty verdict.[10]Police must do more work to protect the victims from revictimization. The first victim was just a 14-year-old schoolgirl, and already a mother of a child of 2-years. In legal terms, she is still a minor, and not eligible to give consent. She left the school when in grade 5 because of the pregnancy. It was a bitter experience for her to leave the school. She had not planned the pregnancy. Most teenage pregnancies are stressful as they are cases of children having children.

Early sexual activity, early pregnancy, induced abortions, and the increase in HIV infections have become major concerns in Sub-Saharan Africa. Efforts, though, to understand their sexual behaviour and to prevent reproductive health problems are almost non-existent. Adolescent girls are normally seen as victims and easy prey of (often older and married) men's sexual exploitation.[11] Teenage pregnancies are currently one of the major problems facing communities.[12] According to the law, sex with a minor is a criminal offence even with consent. The perpetrator is considered a rapist and must be tried in a court of law for this offence. Unfortunately, the law in South Africa is good enough, but not well implemented.

The risk of HIV transmission is high among victims of sexual assault than to consensual sex. Fortunately, both reported victims are still HIV negative. The first girl had a vulval wart, but this might be a signal of worse things to come. In Black Africans the consequence of child sexual abuse is a high prevalence of HIV infection.[13] The first victim completed a course of antiretroviral drugs in January 2005, and now needs another course for the second rape. The pregnancy is precluding her from getting PEP. There are no clear guidelines regarding pregnancy and PEP. The second victim had just finished an anti- retroviral course in March 2005 and needs another having being gang raped. There is a need to control this epidemic of sexual assaults to prevent HIV spread, and to prevent unwanted pregnancies. The police should carry out extra-ordinary investigations to apprehend the culprits and offer protection to the victims.

## Conclusion

There is a serious problem of sexual assault of young girls in the Transkei region of South Africa. There is a need to control this epidemic of sexual assaults to prevent HIV spread, and to prevent unwanted pregnancies.

### Ethical Issue

The author has ethical permission for the case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# External Examiner's Report for the 4th Year Medical Examination in Forensic Medicine: Is It A Magician With a Wand?

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## Abstract

**Background**: Externalexaminers are generally considered people of integrity, and honesty. The right to pass or fail students is within their discretion. The examination report that they produce must be congruent with their actions. The post examination comment by the external examiner for the 4th year medical examination in Forensic Medicine stating that students were weak in Forensic Medicine was not consistent, however, with the marks he awarded to the students.

**Objective**: To validate the report of external examiner with his action.

**Method**: Every year an external examiner is invited to conduct an examination of 4th year MB. ChB students in Forensic Medicine. There were three specialist examiners who conducted an examination of medical students. The external examiner (Mr. X) has been invited from a pioneer institute for the last three years. Mr. X also made changes, and approved course contents at the beginning of the year.

**Results**: There were 97 students who sat for the examination. Of these, 93 (96%) passed and none of them failed. Only 4% were required to write a supplementary examination. All scripts were marked by all three examiners. One third of the students (31/32%) who received the lowest marks were exposed to the external examiner for an oral examination, together with their scripts. The external examiner made no change of marks in six cases. Of the other 24 students, six students marked were decreased up to 4%, while the remaining 18 students' marks were increased by up to 20%20%. One student has awarded a distinction (75%) by the external examiner (increased from 55% to 75%).

**Conclusion**: The comment of an external examiner was, however, not congruent with his actions. Therefore, the external examiner is a magician, but without a magic wand.

***Keywords:*** *Medical students, external examination, results*

## Introduction

Very little published literature is available on the role of external examiners in the assessment of medical students. The opinion of external examiners is crucial and is the gold standard. This is because they are an independent assessor of standard. Judging educational standards is an important part of the role of external examiners. Standards are judged by the quality and quantity of students' work. They are helped by making comparisons of standards within subjects and across institutions.[1] It has been observed that there are variable standards of medical education

among institutions within countries. This may be due to the uneven availability of resources including quality of teachers. There is also some global variation in accreditation standards, but certain standards are considered essential.[2]

Generally, the educational standard is judged by the marks awarded to the students which reflect the performance of the students. The marks given by examiners range widely.[3] Therefore, the reliability of examination marks has yet to be confirmed, though much work has been done in this area.[4] Although examiners have proved to be far from infallible in their judgments.[5] In the British educational system, external examiners are a part of the examining process. 'External' in this context is taken to mean that the examiner is from another institution and is involved in teaching courses like those which he/she examines.[6]

Success in achieving the desired learning outcomes for a course or failing to do so can have far reaching consequences for the students as well as for teacher. The teachers involved have an interest in whether learning has been achieved since success or failure is going to reflect on their selection and teaching processes.[6] The external examiner report will reflect the performance of students and the teacher. It gives a very bad impression of the teaching staff if the external examiner makes some negative comments on the performance of students. The purpose of this study is to highlight the importance of the external examiner. It will also provide some critical discussion regarding the need for an external examiner.

## Method

Every year an external examiner has been invited to conduct an examination in 4$^{th}$ year MB. ChB students in Forensic Medicine. They used to come a day before of the date of a written examination. There were three specialist examiners who conducted examination of medical students. Only one was an external examiner but he has been examining students for the last three years. He also made changes and approved course content at the beginning of the year.

## Results

There were 97 students who sat for the examination. Of these, 93 (96%) passed and none of them failed. Only 4% were required to write a supplementary examination. All scripts were marked by all three examiners. One third of the students (31/32%) who received the lowest marks were exposed to the external examiner for an oral examination, together with their scripts (Figure 1). The external examiner made no change of marks in six cases. Of the other 24 students, six students' marked were decreased up to 4%, while the remaining 18 students' marks were increased by up to 20%. One student has awarded a distinction (75%) by the external examiner (increased from 55% to 75%) (Figure 1).

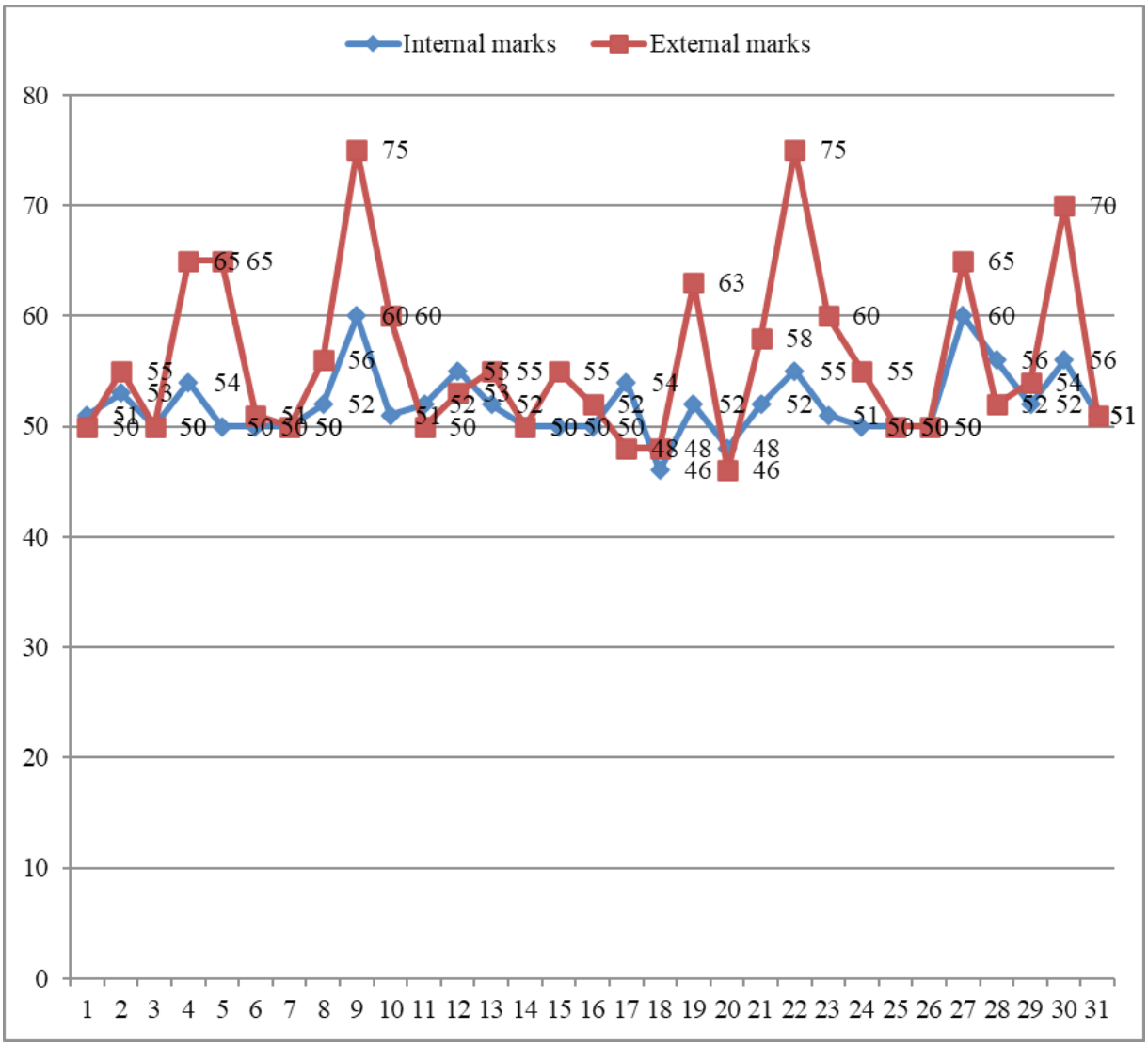


**Figure 1: Relationship in marks awarded to fourth year medical students in Forensic Medicine by an external and internal examiner.**

## Discussion

This case study report is of an external examiner who is named as Mr. X who was approved by the university council of a rural university for the fourth year MBChB students. He came from a so called top urban university where Mr. X is a quality examiner. His feedback, however, contradicted his own marks. He described that students' knowledge of Forensic Medicine is poor and it is not in keeping with other institutions in South Africa. The author received a message through his faculty head and was surprised to read the negative remark made by Mr. X. There are clear contradictions between his words and his actions. He examined all the scripts, and 31 students in oral examination. The marks of the students generally increased, and one student was even given a distinction by him.

Medical education in South Africa is grossly divided in medical schools even though country has achieved independence. The mind-set of apartheid is still in existence although it was removed in the Constitution. There are poor communication skills

among the students at the disadvantaged universities and therefore performance is underestimated. The students have gone from problem-based learning to community-oriented problems and have good practical knowledge of the subject. Despite the relevance of communication skills in medical teaching, gender differences in the performance of the students in the OSCE are still under reported.[7]Individualized Personal Assessment (IPA) has been widely adopted as a method to assess students in various disciplines. The validity and reliability of IPA, however, are often questioned. The lack of correlation between marks scored in IPA and other modalities of assessment seems to support this. The inter-examiner variability and a lack of objectivity on the part of an examiner creates the potential for bias. The examiners are not only influenced by the content of answers, but also by students' verbal style, ability to communicate, and the level of confidence. A study conducted by the author showed that Problem Based Learning (PBL) encourages a strong sense of autonomy, flexibility, and openness, and the system seems to inspire the students with confidence.[8] With students evaluating the system, there is also a danger that the evaluation may priorities students' needs, and by giving them a free hand they may be too subjective in their appraisal.[8]

Several methods of assessing the clinical competence of medical students exist. Traditional methods include short cases and long cases and the viva voce examination, all of which have been criticised for lacking structure and standardisation, having poor inter-rater reliability, and not minimising examiner bias.[1] A proposed the OSCE in medical school as a means of overcoming these issues and improving the quality of the clinical performance of the students.[9] In this study, students and examiners reported favourable opinions of the process and organisation of the OSCE conducted during the Medicine and Therapeutics exit exam. However, students felt that the OSCE was stressful and intimidating, and the time allocation was inadequate for the assigned tasks. More practice sessions/mock exams with adequate feedback may better prepare students and create a better environment to assess skills expected of a doctor in clinical practice. Further, multi-centred studies are required to be carried out to assess whether there is any difference in actual clinical performance between students assessed by traditional formats compared to those assessed by an OSCE, and to ascertain the long-term impacts of OSCE on clinical management of patients later in their professional life. [9]

The examiner's questionnaire evaluated the perception of the overall fairness of the OSCE, the range of clinical skills and knowledge tested, the validity of the measure of clinical competence, exam administration, the level of information required and clarity of instructions at each station, the adequacy of the time allocation for each station, and the level of stress experienced by the students to minimise their chances of failing.

The jealousy is not limited to within the department, but it is also interdepartmental and relates to other institutions as well. The head of the faculty abuses his power by suppressing others so that he can get an authorship in gift. This is the reason you will probably see that there is a professor who has published without doing anything. This author has undergone multiple disciplinary inquiries, which has affected not only my health and work but also my teaching. Mr. X commented that MBChB 4 students' knowledge of Forensic Medicine and Forensic Pathology is not in keeping with other institutions in South Africa. He further wrote that the Forensic Medicine MBChB course at the rural university may require significant revision. This was the language of the external examiner – the so-called Mr. Magician – who has external examiner experience in only two universities, but Mr. X claims all South Africa. Furthermore, Mr. X suggested that a more integrated

forensic pathology course is needed with standardized international textbooks. Mr. Magician (Mr. X) does not even know that we are carrying out a teaching programme based on problem-based learning (PBL). It is dangerous to restrict resources in learning. Mr. X's intention is to look for financial gain by offering his services in the rural university as Mr. X is more than willing to assist with the revision of the rural Forensic Medicine course curriculum.

The Faculty of Health Sciences at the rural university has completed the first five years of its problem-based community-oriented teaching curriculum. The use of SWOT analysis to evaluate current and future directions can lead to the successful evolution of any organisation. Most of the student groups indicated that, in terms of quality, the strengths of the system were stronger than its weaknesses. They also pointed out that there were more opportunities than threats. It was concluded that the PBL curriculum empowers the students by increasing skills that are relevant to existing health problems in the community.

## Conclusion

The comment was submitted to the faculty head by the external examiner without any consultation with internal teachers and thus was malicious and damaging. It was unprofessional as prime facie evidence showed that he has promoted students by himself. Mr. X is a magician of coin but not having any wand. This kind of external examiner must be banned from the role of examiner in other universities.

### Ethical Issue

The university's name and the name of the external examiner were kept confidential. If somehow, he or she comes to know, then it is not the intention to defame any individual. It is the interest of the author to improve the quality of the examining process. This article was supposed to have been published long ago but because of time constraints it has been submitted for publication only now. The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

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# Poverty and Non-Natural Deaths among Former Mineworkers and in their Families in Transkei Region of South Africa

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## Abstract

**Background:** Thousands of former mineworkers across former Transkei have already passed on, are disabled, or died due to either mining related diseases or non-natural ways of dying such as accidents, suicide, or homicide. Many ex-mineworkers have died prematurely, placing a strain on their families. This has led to dysfunctional families and has created the conditions for children from these families to commit crime. Compensation could be claimed for mining related maladies, but the non-natural deaths are unbearable for a resource-stricken family.

**Objective:** To highlight the problem of poverty and non-natural deaths among former mineworkers, and to relate the impact on their families.

**Method and Material:** This research, a retrospective qualitative study on former mineworkers and their children, was carried out in 2000-01 at the forensic pathology laboratory of Umtata General Hospital complex, Mthatha, Eastern Cape, South Africa. The records of interviews performed during medico-legal autopsies in 2000 and 2001were reviewed at Umtata (Mthatha) General Hospital mortuary. The interviews were routinely performed in relation to victims who were admitted at the Umtata (Mthatha) General Hospital mortuary.

**Results:** Eighty-four family records were analysed. Of these, 21 (25%) were found to be former mineworkers and their immediate family members. There were five mineworkers and 15 children of mineworkers. Only one was the spouse of a mineworker who had died unnaturally, and one person was unaccounted for in these numbers. Three mineworkers died because of firearm injuries, one was assaulted by someone with a knobkerrie, and another one died because of alcoholic intoxication. Two of them had heavy drinking habits. Three mineworkers were unemployed. The causes of unnatural deaths were as follows: five stabbed, two from firearm injuries, one from a motor vehicle accident, one assaulted with blunt object, and three committed suicides by hanging and poisoning. Most of the victims consumed alcohol.

**Conclusion:** A high number of former mineworkers died an unnatural death. Poverty could be an associated as an underlying cause of death.

***Keywords****: Poverty, unnatural death, socio-economic effect*

## Introduction

Approximately 57% of individuals in South Africa were living below the poverty income line in 2001, and the proportion of people living in poverty in South Africa has not changed between 1996 and 2001.[1] However, those households living in poverty

have sunk deeper into poverty and the gap between rich and poor has widened.[1] Limpopo and the Eastern Cape had the highest proportion of poor with 77% and 72% of their populations living below poverty income line, respectively.[1] At the same time, South Africa had 59 935 deaths due to injury in 2000, which is an overall death rate of 157.8 per 100 000 population. [2] This rate is higher than the African continental average of 139.5 per 100 000 population, and nearly twice the global average of 86.9 per 100 000 population.[3] Nearly half of South Africa's deaths were due to injury caused by interpersonal violence,[2] four and half times the proportion worldwide.[4]

Violent and/or traumatic deaths in the Transkei region accounted for an average annual rate of 162 per 100 000 members of the population per year. The common causes of these deaths were as follows: motor vehicle collisions, 63; firearm injuries, 43; stab wounds, 32; and 18 blunt trauma.[5]Non-natural deaths are high among young males between 21 and 30 years of age. Poverty has been a major risk factor in the causation of these deaths.[6]The rural people of South Africa are poor. Transkei is an area where a significant number of people are poor and live on meagre resources. The Eastern Cape has the highest percentage of poverty (24%), and this figure rises to 92% in the Transkei region[7] where most of the people are migrant workers in far-flung areas such as Johannesburg. Poverty plays a significant role in committing crime. The poor are both the perpetrators and the victims of crime. They are vulnerable to dying once they are injured, as they cannot get care in a hospital. In rural areas people live with poverty, violence, abuse, poor health, and unemployment.[8] HIV is also the cause and effect of poverty, crime, and unnatural deaths.

Poverty needs to be alleviated to curb the spread of HIV in rural communities. There is a high risk of HIV infection among migrant workers of all ages.[9] A study conducted on former mineworkers by the author showed that financial difficulties are a common underlying cause of suicide.[11] The burden of having a family and lack of self-esteem probably contributed to their suicidal state. Alcoholism is associated with some mineworkers. Most victims are either unemployed or inadequately employed.[10] Transkei is one of two regions from which most South African mine workers used to be recruited, and the destination to which many return. When they returned home, they face a lot of challenges such as unemployment, alcoholism, and financial difficulties. This is a vicious triad that leads to more poverty. The purpose of this report is to describe the manner of non-natural deaths and their relationship with poverty amongst mineworkers and their families in the Transkei region of South Africa.

## Material and Method

This retrospective descriptive study (see my comments in the abstract) reviewed 84 non-natural deaths at Umtata General Hospital (UGH) mortuary during the period April 2000- May 2001. The interviews were routinely performed in relation to victims who were admitted to the Mthatha General Hospital forensic pathology laboratory. These interviews always included a discussion about the deceased's socioeconomic conditions, alcohol intake and about their children. The Mthatha Hospital complex is the teaching unit of Walter Sisulu University (previously known as the University of Transkei) in the Eastern Cape Province of South Africa. The hospital mortuary provides services to Mthatha (Umtata) and Ngqeleni magisterial districts, which together have a population of approximately 400 000. Nearly 1000 medico-legal autopsies are carried out in this mortuary per year. All deaths from unnatural causes in this region are notifiable to the police, who then request medico-legal autopsies. Twice a week the medico-legal autopsies are conducted at the hospital mortuary. In each case a close family member who was present

was interviewed before carrying out the autopsy. This practice is carried out routinely. The histories of the deaths were recorded including the names, addresses, and ages of the deceased, together with the causes of death, and circumstances of the death. All the autopsy histories for the specified period were reviewed, compiled, and collated manually.

## Results

There were 84 families that were interviewed. Of these 21(25%) members were found to be a former mineworker or their children who were involved in non-natural deaths. There were five mineworkers and 15 were children. Only one was the wife of a mineworker. The cause of death was unnatural in all the cases. A majority victims were very poor and drank alcohol. Three of the mineworkers died because of firearm injuries, one was assaulted by someone with a knobkerrie, and another died because of alcoholic intoxication. Two were alcoholics. One was employed as a security guard, another was running a business, and three were unemployed. Five children were stabbed to death, two were gunned down, one was involved in an accident, one was assaulted, two were hanged, and two poisoned themselves. One died of electrocution, and another drowned. Most of the victims consumed alcohol.

## Discussion

There is plenty of literature on mineworkers' health conditions, but hardly any on non-natural deaths. It is under researched and therefore under published. Transkei has a very high population density. Most of the former mineworkers reside in the far-flung and remote rural areas of the region. Their homes are scattered in the wide barren tracts of the Transkei. The poverty and unemployment levels are very high.[1] Transkei is known for its supply of labour to the mines. Miners are respected because they earn more money than other people in this area.Mineworkers are known for their promiscuous behaviour at the mines. Here is a case of one who had four wives and was killed. *ZY, 53 years ex-mineworker was shot in his place and died in hospital. He was the owner of a shop in a location. Police failed to capture the culprits. Nothing was stolen from the shop. He had four wives and they all lived at home. He was an ex-miner who returned from the mines in 1974 and had many children.*

Ex-mineworkers' families are dysfunctional. This is because they are poor. Here is case of suicide as the victim could not get a pair of shoes. The mother is poor, and the ex-mineworker father was non-supportive. *LL, a 15-year-old male scholar, hanged himself in the room he was studying. He was a grade 8 pupil. He used to go to school every day and there were no complaints from the teachers. He was a quiet boy who lived with the father and stepmother. The biological mother married someone else and lived separately with her children. The mother had visited him. The boy was found hanged. He had requested a pair of shoes from the father several times and he had not responded to his request and so he decided to take his life.*

The migrant labours from the Transkei region created a situation where women described that their ex-mineworker husbands were not the person they married. The men were not strong anymore and could not meet their demands. This was revealed in a case where a woman poisoned her husband. Her daughter revealed this before committing suicide. *ZZN, a 27-year-old female, committed suicide by hanging. The reason was written in a suicide note left by her which was that the mother was not in close contact with her. The reason has been that the mother had poisoned the father when he returned from the mines. The daughter had known the secret. The father's death was not reported to the police as an unnatural one. Father was working in platinum mine in Rustenburg for 20 years but had not saved any money for a rainy day. He had been retrenched because of poor vision.*

Trauma is a leading cause of death in the Transkei region of South Africa.[2,3,4] Road traffic accidents contribute substantially to the number of such deaths, and more than one-third have been pedestrians. *NN, a 7-year-old boy, was knocked down by a car while he was crossing the road. He died instantly. The driver paid money to the family for his burial. The father of the deceased was an ex-mineworker. He was sick and coughing (and) had two wives and seven children. All were staying at home and unemployed.*

The migrant labour system in the Transkei region has contributed very extensively to socioeconomic effects. Former mineworkers are now sandwiched between scarce resources, and there is little hope of getting re-employment. They do not have enough savings to run their families. *LD, a 48-year-old ex-mineworker, was shot dead by an African man. He died instantaneously. He had been working as a guard and it appears that the motive for his killing was to steal his firearm. A bystander called the police, and the perpetrator was arrested. He used to work in gold mines for about 20 years and left the mines to get married. He was jobless for a year as his health was poor but was forced to join a security company to earn some money. The money he saved was utilised to pay lobola (marriage gift). He left behind a wife and a son.*

Ex-mineworkers were strong and powerful when they were taken to the mines but lost their physical strength when they retired from service. Most of them were retrenched as they were weak and non-productive. *WS, a 31-year-old ex-mineworker was shot dead. He was working as a security guard. The criminal grabbed him and tried to take his firearm. He struggled with him until he shot him in the head. The purpose of the crime was to take the firearm. One of the culprits was arrested. He was not married and did not have a girlfriend. He had worked in a gold mine for seven years in Gauteng. He was retrenched about four years ago and on returning began to work a security guard.*

Mineworkers produce children from their traditional wives back home but fail to maintain them. Although they go to work in the mines with the hope of earning a substantial amount of money, they return with meagre savings. Lack of support of the children make them grow with no education and, as they cannot get decent work, they start taking to alcohol or drugs and end up as a victim or a perpetrator of crime.[6] *SIB, a 26-year-old male, was stabbed in a shebeen (local pub). He had a quarrel with a friend who stabbed him. They were both drunk one evening. The deceased was not educated and was a builder in the locality. The culprit was also uneducated and unemployed. They were quarrelling about liquor. The police arrested the culprit. The father of the deceased had worked in mines for about ten years. When he was discharged, he did not come home. So, the relatives went and fetched him from where he was staying. All the money he received he had spent there. He remained sick after he returned and finally died of a stroke.*

Former mineworkers are now sandwiched between scarce resources, and there is little hope of getting re-employment. They do not have enough savings to run their families. Their expenditure is at its highest when they return, as the children are grown up and are in secondary or senior secondary school. These children need to go for tertiary education, and that is not possible because of poverty. Therefore, they end up as bitter, angry individuals who indulge in crime. The discovery of gold provided the base from which South Africa was able to develop a substantial industrial capacity, but the mineworkers in return got poor health and disability. *KG, a 50-year-old, was a retired mineworker from the goldmines. He earned a monthly wage of R1100. He worked from 1978 to 1998 when he took a voluntary retirement package. He was awarded R14000. He has five young-children.*

*In 1989 he was treated for pulmonary tuberculosis in the mine hospital and in 1998 for a relapse in the local hospital. At the same time cancer of oesophagus was also diagnosed. He died because of complications from the cancer. He left his all his five children on the street.*

Financial difficulties were the main cause of suicide in 87% of victims.[11] The poor state of health, unemployment, and alcohol abuse were contributory factors and inter-linked in the causation of financial difficulties.[10]

### Study limitations

There are several limitations to this study. The small sample size and history lacking in detail are two important ones. This study has, however, provided some insight into some issues leading to non-natural deaths and poverty among mineworkers of Transkei in South Africa.

## Conclusion

The migrant ex-mineworkers have undergone serious psychosocial trauma, which is the legacy of mining work. The journey to the mines systematically stripped them of their expectations, and made them helpless, disabled, and dependent on their family members. This probably led to the high number of unnatural deaths among mineworkers and in their family members. The pride of mineworkers is stripped off. Extreme poverty in their families is exposed, leading to both natural calamities as well unnatural risks of life, and many of them died because of it. It is difficult to number how many non-natural deaths have occurred in this community, but definitely a very high number. Most of them could be linked to poverty; however, there is no proof and there will never be a way of getting proof, but this is the truth.

### Ethical issue

The author has ethical permission for the case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

**Conflict of Interest**: None

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# A Case Report on the Obstacles in Research Publications in a Rural University, South Africa

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## Abstract

**Background:** Universities are the powerhouse; they generate knowledge through research all over the world. A university without research is a black hole that swallows the taxpayers' money without contributing much to them.

**Objective:** To highlight the obstacles to carry out research in a rural university of South Africa.

**Case History:** Ms. X was enthusiastic to carry out the research in the university She had so far produced about 100 research articles in peer reviewed journals, despite all odds. She was appreciated nationally and internationally for her work, but the university under study has humiliated and victimized her. This report on Ms. X highlights how this rural university is being disadvantaged by not allowing people to go further. The author will try to present his personal views after informal discussions with various academics and researchers. The highlights of the de-motivating and inhibitory factors are discussed in this manuscript.

**Conclusion:** The main obstacle is lack of transparency and abuse of power at this rural university. The university functions like a secretive society.

***Keywords:*** *Research, motivating and inhibitory factors.*

## Introduction

Thirteen universities from South Africa feature in the 2021-2022 list of top 2000 universities compiled by the Center for World University Rankings (CWUR). The University of Cape Town ranked 269 globally, followed by the University of the Witwatersrand at 292. Stellenbosch, KwaZulu-Natal, and Pretoria University are within the top five universities in South Africa. The University of Johannesburg ranked sixth. There are, however, no rural universities in the rankings in South Africa. In addition, South African universities are dropping in their rankings.[1]

**Table 1. Ranking of the 13 best universities in South Africa**

| 2021 Rank | 2020 Rank | University | 2021 score |
|---|---|---|---|
| 269 | 268 | Cape Town | 77.3 |
| 292 | 275 | Witwatersrand | 76.9 |
| 435 | 429 | Stellenbosch | 74.9 |
| 483 | 477 | KwaZulu Natal | 74.3 |
| 580 | 578 | Pretoria | 73.3 |
| 674 | 706 | Johannesburg | 72.5 |
| 924 | 922 | North-West | 70.7 |
| 1163 | 1200 | Free state | 69.3 |
| 1239 | 1158 | Western Cape | 68.9 |
| 1313 | 1295 | Rhodes | 68.5 |
| 1416 | 1409 | Unisa | 68.1 |
| 1631 | 1655 | Nelson Mandela | 67.1 |
| 1950 | 1936 | Tshwane | 65.9 |

Research is vital to know the facts of life and it promotes the quality of life as well as the longevity of human beings. It is carried out mainly in universities. There has always been a great emphasis on research in the rural university, and a lot of money has been pumped into it, but it contributed only 0.2% to the research output in 2014 in South Africa.[2] According to the Department of Higher Education (DHET) Report of 2014, the rural university under study is almost at the bottom of universities in terms of research output.[2] In a recent list of rankings this university is not even visible (Table 1).

## Case History

Ms. X was appointed to this rural university. She was excited to do research, thinking that it would help to get promotion. She started publishing significantly and was awarded a research award but soon these research publications and these awards become a problem to her. Subsequently, Ms. X was humiliated time after time, and year after year by managers. They wanted to demotivate her so that she stopped her research activities, although in the university forum these managers encouraged her to carry out the research. Since 1996 Ms. X had also been doing community service, and that also benefitted her in the form of international and national awards. From 2008 to 2018, Ms. X underwent three disciplinary enquiries with two suspensions, one forensic audit, and stoppage of her salary and birthday bonus. Despite all this, Ms. X has continued working but her research output has come down drastically.

## Discussion

Ms. X was a victim of research publications. She was not promoted despite all her achievements, but

that is not enough. The managers tried hard to trouble Ms. X as much as possible. Discrimination is not uncommon in this rural university, but this is limited only to the managers. The people at ground level are very nice and humble people. There are always protests to increase the salaries at the university but there has been hardly any benefit to Ms. X as her salary has hardly increased. The Human Resource (HR) Department is working on the instructions of management, without following any policy and protocol. This practice is to discriminate against people like Ms. X. In fact, there is shortage of policy documents and managers do not try to develop them. This helps them to manipulate people. There are no norms so usually managers turn a blind eye to a problem if someone takes one to them. There is discrimination by powerful people, as they abuse their power. This discrimination was not uniform, as union member did not practice it, but the Human Resource Department practiced it, especially in relation to salary, leave encashment and so on.

Ms. X has undergone challenges during her service to the university which is difficult to describe because of limited space in this article. The university's management tried to get rid of Ms. X from the university as she had become a threat to fellow professionals. In university meetings, it is difficult for these managers to explain why they do not publish. It is something like a concrete house built by someone in the middle of mud houses. These mud houses are the many nationals who are appointed professors. They have lot of influence on local staff. The residents of the mud houses combine to demolish this concrete house. This was because the number of Ms. X's research publications constituted about 50% of that of her faculty, and about 25% of the whole university.[3] This was the report submitted by an independent assessor in 2011.[3] The recommendations of an independent assessor just remain on the books, and nothing has been implemented. If this was the position, then there was no reason to send an assessor and spend funds on futile work. A lot of forensic audits have been carried out in this university, but nothing has been made public. Probably, some of them are buried in the secrecy of university.

Purposely Ms. X was kept all alone in her department, so she might not have time for publication and research. Ms. X took her own time on weekends and holidays to work on research articles. The good part of research publications is that they are measurable and available on the internet. One can count them, and this is a difficulty managers have, how to bring Ms. X down. Generally, universities appreciate their researchers and honour them but in this is not a culture in this rural university. Higher management is generally weak, and therefore the middle management started to abuse power. It may not be known in South Africa how this rural university is functioning as reporting is generally carried out by the same mangers, highlighting all the good things going on. Thus the purpose of this article is to enlighten the honorable Ministers of Education and Health so that they may make a note of it.

There is hardly any introspection as to why some universities are publishing research articles and others are not. The simplest answer is that those university will not promote staff without them fulfilling the research requirements and publications, while this rural university hardly cares and, instead, promotes staff members without them meeting any publication requirements. Research office at this rural university introduced another method to promote their favoured ones, i.e., through inaugural professorial lectures. Of course, it was started with the approval of higher management. It takes hardly one hour to get promotion through this system. Generally, it is said that either you publish or otherwise you perish, but this is the opposite at this university. The development of an unbiased promotion system is important to the legitimacy and mission of a university [4] but at this rural university

research is not a criterion for promotion, and, if it is there, they do not follow it up. There are many professors working in university without a single publication. Generally, there is always an inequality and bias in promotion but not at this high level which is visible in this university. Those who publish are singled out as there is no culture of publications. This is very well evident in the case of Ms. X.

In Ms. X's case, the problem started from the day she joined the department, where se was presented with a case which was a preventable death. Ms. X was new and did not understand their culture. Ms. X has received research awards. She was not allowed to apply again as she received awards for two consecutive years. Anyhow, it was a decision of the higher management of the university, and one cannot challenge them. Again, the university research award application was announced in 2005 and Ms. X applied. She received a platinum award, but this was stopped a day before handing it over her, and a disciplinary enquiry (DC) was started. This was the day when Ms. X became a target to remove her from the university. Ms. X was cleared of that allegation of publishing plagiarized work with the help of the University Union. Fortunately, the chairperson of this enquiry was a top labour law practitioner of South Africa. It was after only a few weeks that another similar enquiry started, which is called as double jeopardy in law. It was a duplication of her own article, but the punishment was more serious, DC with suspension than first enquiry although the charges were much lighter than first one. This suspension was very heavy, as it did not allow the person to come to the university and it did not allow her to leave the city, but her work was displayed as a poster in the corridors of the faculty to get appreciation from visitors. Similar duplications were found among four other professors in the same faculty, but no action was taken against them. This time the enquiry commissioner was an African expatriate who was appointed telephonically. They certainly wanted to finish off Ms. X.

Fortunately, the university union was very supportive and protected Ms. X. The university head is just like a father of the institution, and he must protect a researcher from outside and inside threats, but this was not the case in this rural university. All her research funds were frozen, and several claims were lost by the Finance Department. The international prize money of USA $5000 awarded to Ms. X was not handed over to her. It was swallowed in the research office. Again, the research award application was announced but could not conclude because Ms. X was an applicant. Meanwhile, Ms. X received an international and a national award which were not in their control, but this had no impact on their behaviour.

Ms. X faced all the challenges of no promotion, financial loss, and humiliation but she did not stop working, even though time was wasted in DCs so the research work could not be carried out as much. They also stopped Ms. X from going to the forensic pathology laboratory so there was no way to collect data, and therefore her research output came down. The faculty head also did not stop troubling Ms. X. They started a forensic audit and that took many years but remained unfinished until the present date. The report of the audit was requested by the research office as well as by Ms. X, but it remained secret. The Occupational Specific Dispensation (OSD) started in July 2009 and was paid to Ms. X in 2009 but then after that it was stopped, and she was not paid despite repeated requests. The OSD scale is supposed to pay all the professionals in South Africa who are serving in public hospitals, but Ms. X was omitted.[5] Again, those favoured professionals who were in the faculty were silently shifted to the health department, but Ms. X was not among them.

The DC with suspension was continued by the head of the faculty. This time a convicted prisoner who was in jail for years was appointed as the commissioner

of enquiry. This time the management of the faculty were certain that they would finish off Ms. X. This commissioner disclosed his intention before the start of the enquiry by stating to his friend in a city that he would punish Ms. X. The charges against Ms. X were very superficial. He promptly declared that Ms. X was guilty without any supportive evidence. It is surprising that such an ex-prisoner was appointed to this position. It is not clear in university policy documents if an individual who has a criminal record can be appointed to this position. This is probably because they themselves were promoted in the same manner. Therefore, a cat with a cut tail would like to see another cat without a tail.

The research office is remotely controlled by the head of the institution, who should get out of this office as there are no policy documents in place. Sometimes they show a policy document which does not have any signature of the council chairperson. The university's research output has slowly declined from 1996 to 2018 while at the same time corruption has kept on growing with the same speed.

Ms. X was a victim of this system, but she never thought this was possible. She was thinking that the only way to get to stay in the university through research publications. There are staff in the university who admire and appreciate the work done by Ms. X. She published a sizeable number of articles but the immediate head in the faculty was not happy. This was because they expected to see their name in these publications. At that time, higher management was not so bad, and the research director was excellent, as she promoted publications. She was pushed to the head of the institution and Ms. X was promoted one step higher despite all the faculty managers being against this decision. There are two types of suspension according to the Rhodes University protocol governing suspension. Ms. X did not fit into those criteria. Rather, Ms. X was not suspended when there was a serious charge of plagiarism, but she was suspended on a lesser created charge.[6]Ms. X cleared all the disciplinary enquiries and retired with full respect in 2018, which was published in the news media.[7]

There is rampant corruption going on in the university and it is known to everyone. A good example is that Ms. X's personal research funds were taken away without any explanations by the research office. It was also not explained by the finance department of the university. After reporting the matter to the university head, no action was taken. It indicates some suspicion regarding his position. When Ms. X raised this question with the head of the institution, he simply said nothing had happened. Ms. X was surprised by his statement. Thousands of rands were taken away from a researcher who was retiring and for her nothing happened. An HR clerk promoted himself, and increased his salary by himself, and all relevant papers were put on the desk of head, but there was no reaction by the same head.

Ms. X wrote dozens of letters to the head of the university and HR, but very few were answered. There is no culture of replying to a letter. Then Mr. X wrote a letter to the institute auditor on her request as follows:

*Dear Mrs. D, I request you to audit all the research funds if you can go back. These funds are generated by me through research publications. These are two accounts. 1. Personal research publications account. 2. Department research publications account. Mr. X is not getting any information, and nobody prepare to help me. It looks like that someone is dosing with bad intention. Attached herewith some examples or copy was issued by research office, but these are not only one. Every year (2001 to till date), I received money from DoE through research publications. Please take audit it from 2001 if possible. Thanks, Mr. X.*

The head of the rural university's intention is not to bring the university in line, and he is ignoring all kinds of proof of corruption submitted to him. This is only a small example of it: Ms. X's reply from head of the rural university was as follows:

*Dear Ms. X, I am concerned about the email below. Ms M reports to me, and no staff member has the power to request her to institute audits. And in any event, this request is not reasonable. Please refrain from this kind of thing in future. Rgds r*

Ms. X has written several letters to all the levels but there has been no progress with her problem. It is difficult to estimate the total financial damage to Ms. X, but it is very heavy.[8] Her pension has reduced because of the low salary paid for a long time.[9] The publish or perish dictums is commonly used in universities but in this rural university a new dictum has developed, "Publish and perish."[10]

## Conclusion

The main obstacle is the lack of transparency and abuse of power. The university functions like a secretive society without any accountability. The control system is just like in Afghanistan as there is multiple organ failure.[3] There is a need to change this culture if you wish to develop a viable university which can serve the community.

### Ethical issue

The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa. The author tried to keep complete anonymity, but if someone anyhow find it out, then it is not an intention to defame any institution or any individual person. It is a system failure, and that tried the author to improve it.

**Conflict of Interest**: None

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# Prevalence of Tobacco Smoking Among Ex-Mineworkers of Transkei, South Africa

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## Abstract

**Background**: The smoking of tobacco is one of the most important confounding factors contributing to lung pathology. It is therefore essential to know the degree of prevalence of smoking in the community of former mineworkers of the Transkei, who constitute one of the groups in South Africa most affected by smoking.

**Objective**: Prevalence study of smoking among former mineworkers of Transkei, South Africa

**Method**: This is a record review study from the Benefit Examination Clinic, which was carried out once a week at the chest section of Mthatha Hospital in Transkei.

**Results:** 466 ex-mineworkers were studied for their smoking habits. Non-smokers were lower in number at 97 (21%), the number of ex-smokers was 226 (48%) and smokers numbered 143 (31%). Little more than three-fifth were smokers - 89 (20%) - and ex-smokers - 142 (30%) - who were in their middle age (40-59yrs). Between 1% and 5% were observed to be in the extreme ends of their age groups (<40 &>60).

It was observed that among the ex-miners who had worked in the mines for a period of 10 to 19 years, the ex-smokers constituted 26%, the smokers 17% and non-smokers 9%. The ex-smokers, smokers and non-smokers were in a proportion of about 5:3:2. The population of smokers peaked from 15% for those with a mining history of nine or fewer year's underground to 42% for those who had been mining for 10 to 19 years and fell almost at the same rate from 42% to 22% in the group with 20 or more years of mining. Non-smokers among the sample increased from 5% (nine or fewer years) to 9% in the 10 to 19 years group, and ultimately settled at 7% when they had worked 20 years or more.

**Conclusion**: The prevalence of smoking among ex-mineworkers is high (79%) in the Transkei region of South Africa.

***Keywords:*** *Prevalence, tobacco, smoking, ex-mineworker, bad effects, and education.*

## Introduction

South Africans have puffed their way through 25 billion cigarettes, according to a "social report" released by British American Tobacco (BAT) SA in June 2002. The report says the tobacco industry and smokers in South Africa contribute nearly R5,5 billion in excise duty and VAT alone to the government exchequer. The total number of cigarettes sold in South Africa was 25 billion (1,2 billion packets).[1]Smoking-related deaths are projected to rise to 10 million a year

by the 2020s, and 70% of these deaths are expected to occur in the poorer countries.[2] Lung cancer, one of the few malignancies for which the main cause is known and can thus be prevented,is on the increase, especially in developing countries that have been targeted by tobacco companies.[3]

The estimate for South Africa is not available, but it seems to be a more serious problem in this country than elsewhere, especially in the ex-mineworkers of Transkei. Patients who visit the Umtata General Hospital's Benefit Examination Clinic (BEC) state lung complaints as their major reasons for consulting a doctor, and most of the patients seen at the hospital with chest complaints are former miners who also have a habit of smoking. Therefore, the purpose of this study was to determine the prevalence of tobacco smoking among ex-mineworkers of the Transkei.

## Patients and Methods

Transkei has very high population density. Most of the former mineworkers reside in the far-flung and remote rural areas of the region. Their homes are scattered in the wide barren tracts of the Transkei. The poverty and unemployment levels are very high. The eighteen districts in the Transkei form the catchments area for the Benefit Examination Clinic (BEC). However, the main bulk of patients emanate from the adjoining areas like Tsolo, Qumbu, Mqanduli, Libode, and Ngqeleni and other localities. The examinations for the purpose of this research were carried out in the morning hours of every Wednesday for a five-hour period at a time. This day was chosen for the convenience of the doctor, as the hospital area remains unutilized by the other hospital doctors.

During a two-year period from May 1997 to May 1999, 2080, former mineworkers were examined at the Benefit Examination Clinic (BEC) at Umtata General Hospital (UGH), a tertiary hospital attached to the University of Transkei in Eastern Cape Province. The UGH is the main hospital serving a population of about seven million in this area. It is under-resourced and the only hospital which serves the mainly black community in this region of the Eastern Cape. The photographs of the X-ray plates were marked as follows for each participant: ex-smoker (ES), smoker (S), or non-smokers (NS). All the data collected from the X-ray photographs was analyzed by Epi6 Info computer programme. The result is displayed in tables.

## Results

The records of 466 ex-mineworkers record were reviewed. The data revealed the following numbers: ex-smokers 226 (48%), smokers 143 (31%) and non-smokers 97 (21%) (Table 1). The highest number of ex-smokers - 142 (30%) - was recorded in the 40 to 59 age group. Similarly, smokers - 47 (10%) - and non-smoker - 89 (20%) - were highest in number in the same age group in this study (Table 1).

The duration of mining between 10 and 19 years was associated as follows with ex-smokers (26%), smokers (17%), and non-smokers (9%). (Table 2). The smoking increased from 15% (9 or less years) to 42% (10-19 years) between the first two age groups of mineworkers (Table 2) and decreased from 42% (10-19 years) to 22% (20 years or more) in the latter two groups (Table 2).

**Table 1. Relationship between different age groups with ex-smokers, smokers, and non-smokers in former mineworkers of the Transkei**

| Age Groups (Yrs.) | Ex-smokers | Smokers | Non-smokers | Total |
|---|---|---|---|---|
| 30 to 39 | 22 (5%) | 19 (4%) | 15 (3%) | 56 (12%) |
| 40 to 49 | 69 (14%) | 48 (11%) | 24 (5%) | 141 (30%) |

***Cont...*** **Table 1. Relationship between different age groups with ex-smokers, smokers, and non-smokers in former mineworkers of the Transkei**

| | | | | |
|---|---|---|---|---|
| 50 to 59 | 73 (16%) | 41 (9%) | 23 (5%) | 137 (29%) |
| 60 to 69 | 46 (10%) | 29 (6%) | 22 (5%) | 97 (21%) |
| 70 + | 16 (3%) | 6 (1%) | 13 (3%) | 35 (8%) |
| Total | 226 (48%) | 143 (31%) | 97 (21%) | 466 (100%) |

**Table 2. Years of mining in relation to ex-smokers, smokers, and non-smokers of ex-mineworkers of the Transkei**

| Mining Groups (Yrs.) | Ex-smokers | Smokers | Non-smokers | Total |
|---|---|---|---|---|
| 1 to 4 | 4 (1%) | 1 (0%) | 1 (0%) | 6 (1%) |
| 5 to 9 | 43 (9%) | 23 (5%) | 23 (5%) | 89 (19%) |
| 10 to 14 | 63 (14%) | 49 (11%) | 24 (5%) | 136 (30%) |
| 15 to 19 | 58 (12%) | 28 (6%) | 20 (4%) | 106 (21%) |
| 20 to 24 | 25 (5%) | 26 (5%) | 10 (2%) | 61 (13%) |
| 25 to 29 | 17 (4%) | 4 (1%) | 7 (2%) | 28 (7%) |
| 30 + | 16 (3%) | 12 (3%) | 12 (3%) | 40 (9%) |
| Total | 226 (48%) | 143 (31%) | 97 (21%) | 466 (100%) |

## Discussion

There are various estimates of life expectancy in South Africa. Statistics South Africa estimates that the life expectancy in 1996 was 52.1 years for men and 61.6 years for women.[4] The Transkei region, being mainly rural and mostly poor, also carries a high burden of sick and unemployed ex-mineworkers; the life expectancy is probably less than the national average. Smoking among ex-mineworkers is quite common as a socially accepted part of Xhosa culture. The prevalence of smoking (inclusive of ex-smokers) among ex-mineworkers is almost double (79%) than that of the general population (46%) in the Transkei (Table 1).[5] The mining job is difficult and stressful. These mineworkers live in a group so there is always group peer pressure. Smoking and alcohol are used as a recreation method when they come out from the underground mines, and to celebrate that they have come out alive from the narrow rocks where they were digging, given the dangers inherent in the work that they do.

About half (48%) of mineworkers have given up their smoking habit because of the condition of their health, probably as advised by health professionals, or their health has deteriorated to such an extent that there is no choice except to stop smoking (Table 1). The fact that a large number of ex-mineworkers are smokers could be understandable as most of the mineworkers are illiterate and not aware of the consequences of smoking; or even when they are aware of the dangers of smoking, they often minimize the effects of it. The task of working underground is very stressful; therefore, smoking and drinking are used to relieve the stresses of hard work and to relax.

Socially too, smoking and drinking are often the only activities available on the mines.

Many former mineworkers (48%) have stopped smoking, however (Table 1), while it is a known fact that smoking is also associated with genetic predisposition. Some individuals will never smoke, and some will never stop, depending upon their spectrum of vulnerability. About one-fifth (21%) of the mineworkers have no smoking history (Table 1).

A lesson could be learnt from the fact that it is not necessarily difficult to stop smoking. By interviewing several mineworkers, it was found that they stopped smoking on their own as they recognized and realized the adverse effects of smoking. Coughing and exacerbated sputum production in many of the mineworkers made them to decide to stop smoking. Twenty-one percent (21%) of non-smoking ex-miners were comparatively healthy in comparison to smokers in the same age groups, although they too had been exposed to dust in the mines just like their other colleagues (Table 1).

Passive smoking is another problem. It was difficult at the time when the sample subjects were working to avoid passive smoke inhalation, as there was no provision for separate hostels for non-smoking mineworkers. The life in hostels was very crowded and no law was in existence to safeguard the rights of non-smokers. Many toxins are present in higher concentrations in side stream smoke than in mainstream smoke and, typically, nearly 85% of the smoke in a room results from side stream smoke.[6]

Only 23 (5%) mineworkers were found to be non-smokers in the middle-aged group. The youngest and oldest mineworkers - of <39 to >70 years - who had no history of smoking were few, averaging only 13 men or 3% of the total sample (Table 1). The number of smokers was more than double at 48 (11%) among the middle-aged group, and almost equals 1%-3% more than non-smokers in the extreme age group (Table 1). It is difficult to estimate the number of demised mineworkers, but certainly in the last six years the corpses that were brought for autopsies were also found mostly to have been smokers. The mortality related to smoking is high, although it remains underestimated. According to the author's experience, the Umtata General Hospital's records indicate that smokers are more prone not only to death from lung disease but also of dying younger than non-smokers. This may not be an irrefutable observation, but there is need for a study on the relation between youth mortality and smoking.

The duration of time-spent mining could be correlated among ex-smokers, smokers, and non-smokers (Table 2). The quitters registered the highest number in all the categories of ex-smokers (26%), smokers (17%), and non-smokers (9%) among mineworkers who had worked in the mines for periods ranging between 10 and 19 years. After this the number of quitters decreased in frequency in all the categories of smokers. The ex-smokers, smokers and non-smokers were in proportion of about 5:3:2. It means that when five mineworkers decided to quit smoking, for instance, three were still smoking, and only two could be non-smokers. These non-smokers were in the minority of course but could be smoking passively in the overcrowded hostel environment. The relationship between smokers (including ex-smokers) and non-smokers was not consistent and was statistically insignificant (Table I). The longer the period the former miners had spent mining, so too did the mineworkers tend to acquire the habit of smoking, but after developing lung problems they dropped their levels of consumption of tobacco. The pattern of smoking was found to increase markedly with the increase in the duration of mining and tapered off later in the same period. The population of non-smokers was at an all-time low and remained more or less constant in all the years of mining.

A maximum number (52%) of ex-mineworkers fell in between 10 to 20 years of mining history, while 20% had worked nine years or less than that. The mineworkers who worked more than 20 years constituted 28% of the total number of mineworker (Table 2). The number of smokers rose slowly from 15% among miners who had spent nine years or less in that occupation and peaked to 42% among those who had been mining from to 10 to 19 years and again fell almost in the same proportion from 42% to 22% in their next 20 or more years of mining experience. The number of miners who had never smoked increased from 5% to 9% over a mining period of ten years, and ultimately settled down at 7% when they had worked 20 years or more (Table 2).

Increased risk of developing lung cancer depends upon the age when a person starts. The younger a person is when he starts smoking, the greater the risk of developing lung cancer. Death rates increase approximately in proportion to the duration of smoking: doubling the duration of smoking from 10 to 20 years increases the incidence of lung cancer 16 times if daily cigarette consumption remains constant. [12] The amount of tar in cigarettes is also important but less so than the number smoked or the duration of smoking. Chronic bronchitis and industrial bronchitis are similar, but the latter is not associated with cigarette smoking. Both are associated with mucus gland hypertrophy and goblet cell hyperplasia in large airways.[7]

The cheap rate for hiring labour in South Africa's mining industry also meant that mining employers hardly cared for the dangers associated with smoking among its employees in a hazardous environment. The bad effects of tobacco smoking are underestimated. The two commonly used legal drugs, alcohol, and tobacco, are more frequently consumed than all other illegal drugs combined among miners, with disastrous consequences for their health. [15] Smoking is even more acceptable than alcohol, and often people have an indifferent attitude about its harmful effects in the community.

The burden of proving that disease is occupational in origin lies with workers. They must find physicians who are convinced that their illnesses are occupational in origin or that their illnesses were aggravated or hastened by occupational exposures. Physicians must then be able to convince referees who hear the cases that the diseases are indeed work-related. Since occupational lung cancer does not have distinctive clinical features, if an ex-mineworker with lung cancer has smoked cigarettes, has had diagnostic X-rays, and has also been occupationally exposed to silica dust in gold mines, an expert medical witness, using clinical judgment, still cannot say that the disease is without question occupational in origin.[8]

Among the ex-mineworkers who have been examined at the Umtata General Hospital, there is evidence to show that many of them have lung disease in one form or the other, mostly due to dust inhalation,[9] arising from when they were still working, or from tobacco smoking or from a combination of both. However, there is also clear evidence to show that most of the miners who are sick are also smokers or have been smokers, but for the latter the decision to quit smoking has been made late because their former habit of smoking has already done some damage to their lungs. But apart from the clinical evidence of the consequences of smoking, many ex-mineworkers have smoked while they were employed and continue to do so. For reasons that we have not necessarily pursued in this study, there is clear evidence nonetheless that tobacco smoking is a prevalent habit among the ex-mineworkers of Transkei.

## Conclusion

The prevalence of smoking is very high (79%) among ex-mineworkers in the Transkei region which

is twice higher than that among the general population. Health education with emphasis on the bad effects of tobacco smoking is an important step towards curbing the widespread habit of smoking among ex-mineworkers.

**Ethical Issue:** A formal ethical permission was taken by the author from the University of Transkei, South Africa. I would like to disclose that this article has previously been published in a book of conference proceeding.

**Funding:** Self-funded

**Conflict of interest**: None

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# A case report on alcohol and crime in the Transkei region of South Africa

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## Abstract

**Background:** Alcohol and crime are two-in-one, inseparable from each other as most of the crime in South Africa is carried out under the influence of alcohol. Many deaths as well in South Africa are attributed to alcohol consumption but, despite that, alcohol is available everywhere in country.

**Objective:** To highlight the problem of alcohol and crime in the Transkei region of South Africa.

**Method:** This case study is based on the case report histories which were obtained from the Mthatha Forensic Pathology Laboratory.

**Results:** These are ten cases that provide examples of alcohol related deaths. All these deaths were caused by stabbing (except one) where the perpetrator or victim or both were intoxicated. The primary underlying cause of these fight was either money or girlfriends. The history, culture and circumstances of these deaths are discussed in this manuscript.

**Conclusion:** Alcohol is a major cause of crime in the Transkei region of South Africa.

***Keywords****: crime, alcohol, stabbing, and death.*

## Introduction

The global burden of alcohol related deaths and disability was between 1.6% and 9.2% of the population in the year 2000.[1] This was high in developing countries and low in the developed world. The total disability adjusted life years accounted for 4.5 billion years lost.[1]South Africa is a high mortality country. A preliminary estimate of the total burden of alcohol related crime in South Africa is 6-7%, ranking it third after unsafe sex and interpersonal violence.[2] The amount of alcohol consumption per capita in South Africa is the highest in the world. [3] Roughly, one in four males and one in ten adult females experience symptoms of alcohol problems in this country. The burden of alcohol mortality and trauma is extremely high, with just under half of all non-natural deaths in 2002 related to alcohol consumption. The blood alcohol concentrations are greater or equal to 0.05g/100 ml and were found in up to two-thirds of all cases tested annually at trauma units in three cities between 1999 and 2001. [4]

Both quantitative and qualitative studies conducted among adolescents and young adults in Gauteng Province between 2002 and 2003 point to strong links between drinking and engagement in risky sexual behaviours. Specifically, frequency of alcohol use, the quantities consumed, and problem drinking are associated significantly with the number of sexual partners a person has had and engagement in sex that was later regretted.[5] Almost one in five HIV

patients studied at a large infectious disease clinic in Cape Town in 2003 met criteria for an alcohol use disorder. These patients were more likely to have symptomatic HIV infection.[6]

In one of the small towns with a population of less than 7 000 people, there were 64 known illegal outlets for the sale of alcohol. It is confirmed by the police that over 90% of crimes in this place were related to alcohol abuse. The alcohol is often purchased with social grant money and, as the illegal shebeen owners were also micro-lenders, many were, in fact, paying huge interest on their alcohol consumption. In line with data elsewhere in the country, the connection between alcohol abuse and victimization was clear.[7] Alcohol makes people very vulnerable; young girls, for example, become vulnerable to rape as a result of getting drunk, very often in illegal shebeens where underage drinking is allowed. [7]The purpose of this article is to highlight the problem of alcohol and crime in the Transkei region of South Africa.

## Case Histories

**Case No. 1**

LM, a 32-year-old male, was stabbed to death on 20/07/2000 in the evening. He was stabbed by three people at Polla Park where they lived together. They had a quarrel and stabbed him. The killers had alcohol and were drunk, and they met on the way to the deceased who was doing business selling fruit in a tuckshop. All the perpetrators were unemployed. The victim was an ex-mineworker, and his mother is a housewife. He had four siblings, three of whom were dead.

**Case No. 2.**

ZM, a male of 19 years, was attending a party at a festival on the campus of the University of Transkei. It was during night on 29/04/2001. He was intervening on behalf of someone when another person turned to him and stabbed him. He was brought to casualty and died there. A stab wound on the right ventricle was seen through the 3rd intercostal space on the left side. His father had previously died from a stroke, and his mother is a clerk in the Department of Education.

**Case No. 3**

BN, a 20-year-old male, was in the tavern (place of selling liquor). He was hired by a certain father to look after his cattle/sheep. He met his friend in a liquor shop as he went to buy liquor. His friend became hostile, started fighting stabbed his friend. The deceased died in Qunu at 12H00. His father had previously died in a mining accident and his mother is employed.

**Case No. 4**

SB, a 26-year-old male, died from a stab wound in a shebeen. He had a quarrel with his friend and was stabbed by him. They were both drunk at 16H00 on Friday in Ngcengane location in Umtata Magisterial area. The deceased was not educated and was a builder in the locality where he schooled until Grade 4. The culprit was also uneducated and unemployed. They were quarrelling about liquor. Police arrested the culprit and bail was paid for him. The father of the deceased died due to a stroke. He used to be a dipping man and worked in a gold mine for ten years. He was discharged and didn't come home so they went to fetch him as they don't know why he wanted to remain there. He didn't have any money as whatever money he earned he spent there. After about five years he died at home. He was sick and was coughing and used to drink alcohol. The mother of deceased is alive and is a housewife. The deceased was helping the family with work as he was the eldest son.

**Case No. 5**

TM, a 20-year-old male, was stabbed to death on 16/9/00 and he died on the spot. Since he was stabbed

by a known person, the culprit was arrested. He was stabbed in Palo location in Ngqeleni in a witch doctor's ceremony where they slaughter goats and cattle and brew liquor to drink. The deceased went to his mother's home where there was a party. He was stabbed by his cousin under the influence of alcohol. They were quarrelling about a girl who belonged to the accused. The father of the deceased was not working. Previously he was working in Cape Town as a labourer for a carpenter. He also worked in a gold mine for 16 years and came back from the mines in 1966. He was working underground as a foreman. He used a spade to lift stones. He became sick after leaving mines and was diagnosed with TB and he was treated in Tygerburg Hospital. He used to live in hostel and there were 20 people in one room. He is now a pensioner. The decease's brother was shot dead in Umtata in 1995 when he intervened during a robbery, and one of his sisters died early. He leaves behind six sisters, two of whom are working in Cape Town, one is married, two are in school and one is a mentally retarded sister.

**Case No. 6**

LR, a 33-year-old male was drinking in a sheeben along with a friend. Both were injured but he died. It happened on Wednesday 31$^{st}$January 2001 in Bongeeni location in Umtata. It was about 24H00 (midnight) to the early hours of Thursday on 01-02-2001. After stabbing him his attackers threw him in somebody's garden. The body was found on Thursday at 2h00 (01-02-2001). The Headman informed the family and the cousin of the deceased identified him. The deceased was working as a part-timer in Telkom. He was a labourer. He was unmarried and had three children with his girlfriend. He left his mother when he was two years old and was brought up by some relative's mother somewhere in Johannesburg. His mother has never seen him since then. He was brought to casualty as dead.

**Case No. 7.**

AM, a 23-year-old male, died on Saturday 27 August 2000 in the morning. He was with a friend who friend left him in his place to go with a girlfriend. When friend returned in the morning at about 5H00 he found him in the bed dead with blood. There was a gun near him. The house was closed but not locked. They had been drinking in the night. They are suspicion as they had had some quarrel with a Zulu person two weeks ago in a shebeen. They called him "Ngingin." After checking on the computer, the police said that the gun was a stolen gun from Durban. Andile did not have a gun. There was also a problem in the area (Zimane) among men in the location. There were two groups who were quarrelling. Andile helped his father in his job like plastering, painting, etc. Andile wrote Grade 12 twice about four years back and then stopped studying. He had quarreled with the Zimbane men.

**Case No. 8**

SQM, a 61-year-old of male, was involved in a fight between a father and son. All of them were drunk. There was no ceremony. The fight took place at the home of the father and the son. It is a locality in the Umata area called Kaplani. The deceased was on a pension doing nothing. Previously he was working in a firm making cardboard. He left five children and wife, all of whom do nothing. After being stabbed three times, he died on the spot and the culprit ran away. Now he has been arrested and the police took him away to the police station.

**Case No. 9**

A 21-year male was stabbed on Christmas day. He was on a street in a location Umtata at 15H00. He was drunk (25/12/2000). He was taken to hospital and died on the way to hospital at about 16H00. He was a student in Grade 11 at school.

**Case No. 10**

SG, a 22-year-old male, was stabbed by three men at the municipality tip in Simpane on 8/05/2001 at 17H00. There was a quarrel between the deceased and the three people. All three were drunk, except the deceased. He died on the spot and was taken to funeral parlour. The deceased did not have a house and lived in a shack. His father was died in motor vehicle accident, and his mother was drowned in a river. He had three siblings; two were alive but unemployed.

## Discussion

The association between alcohol and crime is widely prevalent, under researched and therefore is not documented in the literature. It is very closely link with culture as anyone is allowed to drink and anywhere. It is written in bottle stores: Alcohol is not for sale to persons under 18 years, but this regulation is not implemented. A lot of school children are in the habit of drinking alcohol, according to a study carried out with rural school children in South Africa.[8] Alcohol is freely available even on the side of roads. Ladies selling fruit are also have alcohol for sale, especially at taxi stands. The nights are more difficult in this region as many people are found drunk. The cases described in this report are based on ten case studies, but this is justthe tip of an iceberg. Alcohol is the mother or father of crime. Criminals, before committing robberies and murders, generally consume alcohol. Most of the quarrels and killings are associated with some kind of alcohol use by one or other or both people involved at times. Transkei was a former black homeland which was merge with South Africa, when the country got independence. People are poor and living on very limited resources.

The families are fragmented as a large number of children do not know the name of their father. Fathers also do not know where their children are living. The burden of bringing up children is on the mother or on grandmothers. Mother are either single mothers or divorced. Mothers are feeding their children from social grants. Alcohol consumption is very high, and it is the cause of family violence.[9] People are roaming the streets at night drunk. The lives of the people have no meaning as they are taking risks every day. This is the reason a lot of people die unnaturally. An autopsy study carried out by the author between 1993 and 2015 showed there were 26 855 unnatural deaths, and of these 5205 (20%) were caused by stabbing.[10] It is very common to see drunk people on the roads and sometimes killed by motor vehicles. One will find people quarrelling over small things. There is a culture among Xhosa people to carry a knife with them, and therefore it is a readily available weapon to use. It is dangerous when a person is drunk. They kill or get killed by someone. This is also a reason why the majority criminals will not be traced. It often becomes a case of an unknown man killed by another unknown man. Alcohol is contributor to a high number of homicides. A record of 12618 (5%) autopsies on victims of homicide between 1993 and 2015 were studied and showed the average murder rate was 85 per 100 000 of the South African population, which is the highest in the world.[11]

There is a trend of using alcohol for celebrations such as when matric results are published or at circumcision ceremonies or even sometimes when a child passes Garde 10 in school. People always try to find an occasion to drink alcohol. It is a very common practice, especially at Christmas, although even long before Christmas they start celebrating Christmas with a braai party with alcohol. Xhosa people also have the custom of attending funerals which is a very good custom to pay last respects to a dead person. They fly from far away to attend the funeral services. Xhosa people take life very philosophically and therefore they enjoy it by taking alcohol. They do not believe much in tomorrow. This is a reason they spent their money very fast. This also comes from the apartheid

era when they could have got killed at any time by the ruthless regimen of apartheid practitioners.

## Conclusion

Alcohol is a major cause of crime in the Transkei region of South Africa. It leads to a high number of premature deaths. Alcohol is imbibed in their culture, but this must change. The availability of alcohol must be reduced, so that many lives could be saved. It would also prevent economic losses in the country. If one calculates the total cost of the consumption of alcohol, is astronomically higher than the benefits.

### Ethical Issue

The author has ethical permission for collecting data and publication (approved project No. 4114/1999) from the Ethics Committee of the university, South Africa. The contents in this article, especially that related to the culture of the people, is based on the author's own experience. It may not be correct. However, it is highlighted for good intentions in this article.

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# Why Do Women Not to Go for Abortion in a Designated Legal Abortion Facility in Transkei Region of South Africa?

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## Abstract

**Background:** The implementation of choice arising from the Termination of Pregnancy Act of 1996 (Act 92 of 1996) is a challenge in South Africa as there are many abortions that are still carried out by illegal abortionists in the Transkei region. This raises the serious question as to why the women do not prefer to go instead to a designated legal abortion clinic which carries out safe abortions in South Africa.

**Objective:** To study the underlying factors which prevent women from going for an abortion to designated legal abortion facilities.

**Method:** This qualitative research was carried out by medical students who visited both the legal and illegal facilities in Mthatha in 2015 for their assignment during the rotation of their subject of speciality.

**Result:** The legal clinics are not user-friendly, the staff misbehave with the girls, they cast doubt on their confidentiality, and they take a long time to do the abortions, while the illegal abortionists charge money for their services but they are quick and keep confidentiality.

**Conclusion:** Legal abortion clinics are not user friendly.

***Keywords:*** *Abortion, confidentiality, illegal abortion, abortion clinic*

## Introduction

Each year about 44 million induced abortions occur worldwide, according to the World Health Organization.[1]Around fifty percent of these abortions are carried out in unsterile conditions and, therefore, they are unsafe, contributing substantially to maternal morbidity and leading to approximately 13% of maternal mortality.[1] Every year, about 19-20 million abortions are done by individuals without the requisite skills, or in environments below minimum medical standards, or both.[2]Most of the unsafe abortions (97%) occur in developing countries,[2] mostly in Africa, Latin America, and South East Asia where abortion laws are more restrictive, the unmet need for contraception is high and the status of women in society is low.[3]

The Choice on Termination of Pregnancy Act, promulgated in 1996 in South Africa, provides for abortion on demand up to 12 weeks of gestational age, and under certain circumstances between 13 and 20 weeks of gestation age, and under limited circumstances after 20 weeks.[4]The Medical Research Council (MRC) found that 425 women died as a result of unsafe abortions prior to the implementation of the Choice on Termination of Pregnancy Act.[5] The sole

aim of this Act was to improve women's health and to prevent unnecessary deaths. A study conducted by the MRC showed that there had been a dramatic decrease in maternal mortality (91%) and maternal morbidity (50%) after this Act was implemented.[6]

The circumstances under which women obtain unsafe abortions vary and depend on known traditional methods and the types of providers present. Health professionals are prone to use instrumental procedures to induce the abortion, whereas traditional providers often make a brew of herbs to be drunk in one or more doses.[7]The purpose of this study is to understand the factors which are stopping women to go to legal clinics despite the fact that they are free, hygienic and the risk of complications is minimal.

The research was conducted in the Transkei in South Africa, a region where there is widespread poverty and lack of education. The prevalence of many local beliefs regarding treatment also sometimes creates health problems in this region.[8]

## Method and Material

During the time when medical students at the University of Transkei were doing two weeks of rotation, they were given an assignment to compete their course of study. Two of these students voluntarily chose to find out about legal and illegal abortions. The legal abortion site was a local hospital which was not difficult to access given they were medical students, but it was more difficult to access illegal abortion places to find out how they conduct abortions. They planned a strategy and in two days they obtained first-hand information about illegal abortions. One student become a client (using an informer) of an abortionist and the other became a friend to accompany her. The conversation with the illegal abortionist was recorded when the site was visited, including how they carry out an abortion. An interview was also conducted with a 15-year-old client of the abortionist. She had undergone an illegal abortion. This research was carried out voluntarily by the two medical students, keeping full confidentiality and ethical values in mind.

## Results

The only government clinic that does abortions in rural hospital is the X women's clinic. When informers first went to the clinic to get some information about the kinds of patients that they see they had an unwelcoming response from the nurses. They did not pay attention and they were unwilling to attend to them as they asked them questions about their service. All that they were told was to go to the procedure room where the evacuation was done by a doctor. They were wearing lab coats, so the staff just thought they were part of the group of students who had a task to observe the procedure. They then went to the procedure room to observe. It is a big room with about four patients inside who were separated from each other by curtains, but they could see each other because the curtains only covered their lower bodies. So, there was no privacy at all and that could be a reason patients do not go to the public clinic (legal clinics) to have an abortion.

When they went to the clinic on another afternoon, they were not wearing lab coats. At the clinic door there was a notice saying that people who wanted to be served at the clinic should bring their identity documents. They went inside the clinic and the nurses were busy with their own conversations and they attended to the students only after some minutes. The first thing that they said was, "If you are here for the abortion, your peers have left already." It was around 14:00 and they explained to the nurses that they were medical students, and they wanted to know about the kinds of patients that they see there every day. They were given a book where they recorded the details of patients coming in for the purpose of statistics and they then asked about the notice that was on the door. The staff said that people used to come to the clinic

and give them false names and then the real person whose name was used initially would find it difficult to get the required services. In addition, they also ended up with false statistics.

The clinic usually sees about 70 patients a day. Patients from as young as 13 years to patients as old as 49 years of age come to the clinic. The gestational age of the patients was from 6 weeks to 18 weeks. Some patients however had an empty uterus. They also do an HIV test for every patient they see but the kit was out of stock the first time the students went there. They do two procedures: medical abortion and manual vacuum aspiration. Most of the patients had manual vacuum aspiration according to the statistics that they gave them. The women's clinic is in an isolated place behind other departments so that patients feel comfortable because they are not seen by other people when going for an abortion. The waiting room is big enough to accommodate many patients so that they do wait outside where they can be seen by people passing the clinic.

## Discussion

During the apartheid regimen white women had several options when an unwanted pregnancy occurred. Many procured abortions from their private practitioners, who would perform a dilation and curettage in the office. In contrast, the relatively low paying, and insecure jobs available to black and coloured women limited their ability to seek termination of unwanted pregnancies. Besides the difficulty of financing a safe abortion, finding a trained doctor was difficult.[9]

Poor women who could not afford a doctor's fee often sought the aid of less skilled midwives, lay practitioners or non-registered doctors who had not completed medical training. Many of these practitioners offered their services without adequate technical knowledge or access to proper facilities and clean instruments.[9] Women who did not want a backstreet abortion, or could not afford one, often tried to terminate their own pregnancies, endangering their lives by attempting an abortion using dangerous methods.[9]In late 1997 the first official report on maternal death in South Africa cited only nine deaths compared with 400 deaths in 1994.[10]

Approximately 30 000 abortions have been performed per the year since the implementation of the Act, while the number of women presenting for treatment of severe complications resulting from an incomplete abortion has decreased significantly.[11] While this decrease is evident in metropolitan cities where abortion clinics are maintaining some standards in terms of confidentiality and privacy of women, this may not be true in rural areas like in the Transkei. Illegal abortion is a serious and preventable public health problem in the Transkei region of South Africa.[12]

This is the first qualitative research carried out in the Transkei region of South Africa on the issue of legal abortion. There are few papers published but they are mortuary based and address only dead foetus recovers in metropolitan cities. This does not provide the reasons so many illegal abortions are carried out. This qualitative study has provided us with first-hand information on the functioning of a legal abortion clinic in a rural hospital. A dysfunctional abortion clinic forces women to go to an illegal abortionist to terminate pregnancy. The two medical students who visited the hospital abortion clinic and narrated the story identified three main concerning issues which are as follows:

1. Privacy and confidentiality of patient

Privacy and confidentiality are essential components of any medical practice, but it is very crucial in abortion clinic. There is supposed to be a code number for each patient and this coding is known

only to the senior nurse in charge and she keeps this register under lock and key so that the confidentiality of the patient is always kept secret. Unfortunately, breaches in this confidentiality are the main reason women do not wish to come to this legitimate clinic for an abortion even though it is free. The nursing staff take the ID document of the client, and that is very bad practice in this rural clinic.

The students assessed the clinic and found that it has its shortfalls, but it is not a very bad place to be. The privacy may not be fully there but the fact that there's a big waiting room at least protects patients from outside contact. The community also has a culture of distant families who meet and support each other. The privacy of the place is a very important issue in an abortion clinic. The issue of privacy is a big problem as well and the students suggested that the department should attend to that problem to prevent patients from going for illegal abortions. This will be possible only when the legal clinic fully guarantees the privacy of the women.

**2. Behaviour of the staff**

The nursing staff are jittery and misbehave with the women as they see them as people with low morality. This is another negative aspect of the government-designated abortion clinic, and it discourages people from going to it. The nursing staff are also forced to work in this clinic although they do not wish to do so. This is because they believe they are taking the life of someone. Some nursing staff are against the abortion law, and yet they are appointed to this clinic. They take out their frustration on the clients.

The student informers reported that the nurses were not friendly to the patients though they never shouted at them in front of them. They observed, however, that the nurses did shout and were rude to the patients who came into their clinic. Patients may not want to go to public women's clinic because of the horrible attitude that the nurses have. The stigma that the public health sector has for not delivering good services to the people may be another reason patients prefer the illegal abortion services. There are many illegal clinics in a town, indicate that the legal clinic is not working properly. Illegal places are in demand in town. People are not well informed about the legal clinic and the women thus end up at one of the illegal places that are advertised widely. There are hardly any pamphlets distributed on the street about the legal clinic, while a lot of illegal clinics are advertised widely.

**3. Time taken in the abortion clinic**

Legal clinics are taking a lot of time to conduct an abortion. This is because the nursing staff were appointed by the government, and they are taking their time as most of staff in other services do the same. The women who come from home and travel by taxi wish to go home before sunset. This is not only so that they can look after their children but also because they want to keep their abortion confidential. As the Transkei is scattered over a wide area from the Kei Bridge in the west to uMzimkhulu in the east, it is often difficult to reach clinic in one day and go back home if the nursing staff have not terminated the pregnancy timeously. This is a reason the women go to illegal facilities for an abortion, although they charge money for this service, but they provide the services immediately. They usually finish the procedure fast and confidentiality remains intact.

There are always dangers associated with illegal abortions so women must advice to go to legal clinics in hospitals. This is the reason of bringing abortion Act of 1996, as it is saving the life of women. Legal clinics have provision for immediate intervention if severe bleeding or another emergency develops during or after the procedure. Legal clinics also provide post abortion check-ups and care of their patients. They use sterile instruments, so the chances of infection are very

limited. Most people performing these abortions are trained so they can manage serious complications such as shock, air embolism, amniotic fluid embolism, deep venous thromboembolism, disseminated intravascular coagulation and infection.[13] An illegal abortionist does not make any such provisions as they generally carry out abortions in a dark corner of town without any facilities. They do not have enough knowledge and do not have hygienic places to conduct such an abortion. Therefore, several women get infections and may die because of their complications. A study carried out by the author on maternal deaths showed that there are several preventable deaths in this region which require an improved service.[13] There is a poor accountability of maternal deaths despite the fact that there is a confidential enquiry going on in south Africa. This is because of poor governance of the hospital and poor death certification. There are several deaths because of post abortion complications, either not reported or wrongly certified.

## Conclusion

The designated abortion clinics are lagging in their duties. As a result of this, the women are forced to go to an illegal abortionist to save their pride and honour. Confidentiality is main concerned in these clinics. This is failing the mission of the Abortion Act of 1996. Therefore, the legally designated clinics must improve their services so that women do not decide to get an illegal abortion and thereby run the risk of suffering from post-abortion complications and even death. No woman enjoys a termination of pregnancy, but the current circumstances are compelling many of them to terminate with the wrong people and in the wrong places.

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# A Study on Chronic Obstructive Lung Disease (COPD) in Ex-mineworkers of the Transkei. A Misunderstood Clinical Condition

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## Abstract

**Background:** There is no diagnostic indicative mark for chronic obstructive pulmonary disease (COPD). It is a general assessment of the patient leading to a diagnose as COPD. In most cases, there is a mixed picture of severe lung diseases including fibrosis, tuberculosis, silicosis, etc. In fact, COPD is the commonest and terminal entity in the majority of ex-mineworkers who were suffering primarily from the dust-lung-disease which is complex in origin.

**Objectives:** To establish the prevalence of chronic obstructive pulmonary diseases in ex-mineworkers of the Transkei, South Africa.

**Methodology:** During the period of 28 May 1997 to 27 May 1999, 2080, ex-mineworkers were examined at the Benefit Examination Clinic (BEC) in the chest section of Umtata (Mthatha) General Hospital (UGH). Physical examination along with standard chest x-rays were carried out; the mining history of each worker was taken, and identification forms were completed.

**Results:** There were 13% of ex-mineworkers suffering from chronic obstructive pulmonary disease. Out of them, 56% of the subjects (COPD) were associated with conditions like chronic bronchitis, emphysema, and bronchial asthma. The rest (44%) were associated with other lung diseases like tuberculosis and silicosis. Out of this number, 33% were associated with tuberculosis and 11% with silicosis. Most of the ex-mineworkers were suffering from an association of diseases ranging from simple tuberculosis to silicosis and their secondary effects.

**Conclusion:** Every seventh ex-mineworkers were suffering from chronic obstructive pulmonary disease. About half of them were having associated parenchymal diseases like tuberculosis and silicosis.

***Keywords:*** *Ex-mineworkers, chronic bronchitis, emphysema, pulmonary tuberculosis, silicosis*

## Introduction

Chronic obstructive pulmonary disease (COPD) is a complex under-diagnosed and under-reported cocktail of lung conditions in the ex-mineworkers of South Africa. A study conducted by the author showed that 78.3% of former mineworkers are suffering from lung diseases.[1] A majority of them suffer from either silicosis or tuberculosis or both. COPD is a component associated with most of the ex-mineworkers' lung condition, but it is often not diagnosed. In fact, it is submerged by the presence of other complications like tuberculosis, silicosis, fibrosis, etc. and as a result remains under-diagnosed even though it exists practically in most of the lung

condition of the ex-mineworkers. With the increase in cigarette smoking, environmental pollutants, and other noxious exposures, the incidence of COPD has increased dramatically in the past few decades and now ranks as a major cause of activity-restricting or bed-confining disability in the United States.[2]

COPD is combination of overlapping diseases: chronic bronchitis, emphysema, and asthma. Chronic bronchitis is defined by the Medical Research Council and occurs commonly, affecting around 10% of the population.[1] In an average group practice of 5,000 patients with a smoking rate of 30%, there will be 75-100 patients with COPD. In an inner-city practice with a predominantly working-class population and a high smoking rate the number will be higher. Many of these patients will be undiagnosed. [3] With the frequent component of reversible airway hyper-reactivity (asthma) in these patients, one can understand the utility and popularity of the word COPD.[2] Smoking is the leading cause of COPD. Although it is still a mystery why more than 80% of smokers will develop it, those who do get COPD must stop smoking. The COPD patient almost invariably has a significant smoking history. COPD is rare in someone with a genuinely light smoking history (less than 20 packs per year).[2] Trends in asthma mortality over the past three decades have attracted considerable interest to understand the epidemiology of this condition and to identify preventable causes. Concern was aroused by a sharp rise in asthma deaths rates in people 5-35 years in some countries during the 1960s.[4]

Little is known about asthma prevalence or mortality in South Africa. A 1983 study reported high asthma mortality rates among African and colored people. These rates were considerably higher than comparable rates in the United Kingdom and Wales.[5] COPD and asthma both cause airflow obstruction. The major difference between the two are that asthma is variable and reversible. Asthma varies in severity from day-to-day, season-to-season, or over long periods. The COPD patient shows little or no day-to-day variability in symptoms and little reversibility. Asking the simple question, "Do you have good days or bad days?" can be illuminating. Pneumonia, bronchiolitis, and other serious lung diseases in early childhood are risk factors for COPD independent of cigarette smoking.[6] During the first two years of life, while alveoli and airways are maturing, insults can have permanent effects. Passive exposure to cigarette smoke, particularly from maternal smoking, puts children at higher risk of serious lung disease.[7] Low birth weight is another independent risk factor for COPD.[8]

There is heavy burden of silicosis among young ex-mineworkers in the Transkei region. The strong association between silicosis and tuberculosis in southern Africa combined with the HIV epidemic makes elimination of silicosis an important public health issue.[9] A study published in Unisa Journal of Psychologica in 2002 showed that many mineworkers were diseased and disabled due to the harsh environments caused by mining operations. When mineworkers become diseased and disabled, they are retrenched and sent home where they must face the psychological and social consequences of unemployment and the stigmatization due to illness.[10]

The purpose of this article is to establish the prevalence of chronic obstructive pulmonary diseases in ex-mineworkers of the Transkei, South Africa.

## Methodology

During the period of 28 May 1997 to 27 May 1999, 2080, ex-mineworkers were examined at the "Benefit Examination Clinic" at the chest section of Umtata General Hospital (UGH). Physical examination along with standard chest x-rays were carried out, the mining history of each worker was taken, and identification forms were completed. The data was collected from

the record registers and analyses.

## Results

There were 270 (13%) ex-mineworkers suffering from chronic obstructive pulmonary disease. Of the 2080 participants 1164 (56%) of the subjects (real COPD) were associated with conditions like chronic bronchitis, emphysema, and bronchial asthma. The remaining 915 (44%) were associated with other lung diseases like tuberculosis and silicosis. Out of this number, 687 (33%) were associated with tuberculosis and 228 (11%) with silicosis. Most of the ex-mineworkers were suffering from an association of diseases ranging from simple tuberculosis and silicosis, and their secondary effects.

## Discussion

This preliminary report on prevalence of COPD in the ex-mineworkers is probably the first report in the Transkei region of South Africa. There is deficiency in diagnosing and establishing COPD as there is no litmus test of diagnosis. It complicated more when there are many other elements such tuberculosis and silicosis which are also found in the same mineworkers.[9] Smoking is also common among mineworkers, and it is considered as one the greatest risk factor in developing COPD. A study carried out by the author (2021) showed that there is a strong correlation between lung abnormalities and smoking among ex-mineworkers of Transkei.[11] Work in the South African gold mines attracts black men from all over southern Africa. Their labours in the mines are poorly remunerated. The men risk developing occupational lung disease to a degree that should encourage the mining companies to improve the underground environment.[1] The gold mining industry employs approximately half a million men and is estimated to indirectly support more than five million people. It is thus to be hoped that the necessary improvements can be made to the mines without altering their labour-intensive structure, which is so vital to the Southern African region.[12]

There were 13% of former mineworkers who were suffering from COPD in this study. An x-ray-based study carried out in 2002 showed that 7% of them were suffering from COPD.[1] It is difficult to diagnose COPD based on an x-ray and it is always under reported. COPD is both a structural and a functional abnormality. The radiologist can pick the structural problem, but it is difficult to assess the physiological function. Therefore, it has always remained under diagnosed without examining the patient. Dust-related disorders are very common among mineworkers as they are exposed to dust underground when there is a dry drilling of rocks in an enclosed space. Although a face mask is given to the miners by the mining company it plays a very limited role in preventing dust inhalation. The larger dust particles reach the larger bronchi and produce a chronic bronchitis symptom complex.[13] These larger and some intermediate size particles that also reach the smaller airways produce chronic airflow limitation.[13] Both the bronchitis symptoms and chronic airflow limitation may also be aggravated by underground environmental pollutants other than silica- containing dust. The smallest, respirable particles, which have until recently attracted the major attention, reach the airspaces and have the potential to cause silicosis if their free silica content is sufficiently high.[10] Chronic, uncomplicated (simple) silicosis in black South African gold miners is associated with significant pulmonary dysfunction. A study conducted by Cowie et al. (1987) showed that 1.4% of mineworkers develop silicosis, [14] which is in contrast with the study carried out by the author, where silicosis with or without pulmonary TB was found in 78.3% of the cases. This study was carried out on the former mineworkers who were retired or retrenched and sent back to the Transkei to die.[1] Furthermore, Cowie et al. (1991) claimed that tobacco smoking produces chronic airflow limitations. It cannot be denied that

smoking is certainly a risk factor in COPD,[11] but it is not the only factor in mineworkers. There is also an association with silica which an underground rock breaker constantly inhales.[12]

A heavy burden of silicosis, tuberculosis and COPD was found in former gold miners.[15] The onset of disability is insidious, and sufferers almost unconsciously adjust their lives to fit their disability. They avoid visiting their GP to be lectured on their smoking habits! There are claims made by all kinds of pneumoconiosis but hardly any claim made because of COPD. COPD can be caused by various environmental factors other than smoking. It is difficult to decide how much smoking contributes to the causation of COPD. There are considerable challenges remaining to provide compensation and prevention services to all mine workers and their families to receive benefits in South Africa.[15]

**Limitation of this study**

There is a limitation of this study as it was undertaken in bits and pieces with very little data, but it should give some insight into the common problem of former mineworkers. It may help them to carry out further study.

## Conclusion

Chronic obstructive pulmonary disease is a hidden disabling component of a mining job which is difficult to isolate from other dust-borne diseases. Silicosis and smoking are the precipitating factors for COPD, and it is again difficult to differentiate between silicosis or smoking induced or both. There is a need for an overall assessment of the lung diseases in the ex-mineworkers, including respirometric studies and gas analysis to understand the reliability of diagnosis. Compensation authorities must provide facilities like respirometers to the individual physicians who are carrying out the examination of ex-mineworkers for their compensation claims. The assessment calls for a more careful evaluation of the physical examination of an individual.

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**Ethical issue:** The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa. This is an old study and could not publish because of the lack of time. Still, it has some relevance to the former mineworkers.

**Conflict of Interest**: None

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# A case Report on the Estimation of Contractual Damage Caused by a Health and Rural University, South Africa

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## Abstract

**Background:** Contractual claims are mandatory for an employee who has either resigned/died or finished his or her contractual obligations. It is legally binding to an institution according to labor law.

**Case History:** BM was such a victim who has been admired nationally and internationally but dehumanized in his own university through the process of three malicious disciplinary enquiries, two suspensions, forensic auditing, and stoppage of salary and birthday bonus. He was not allowed to go to the forensic pathology laboratory despite of the fact that he was all along a forensic pathologist.

The harassment of BM became more rigorous in 2008, although he was victim long before that. The head of faculty was promoted later to a position of a university head. BM had an interest in research so he was publishing prolifically, but that disturbed the balance of the faculty staff as the rest of them contributed very little as per the report of the external assessor.[1] The harassment was not limited only to mental harassment but also at the same time financial losses to Mr. BM occurred. The Occupational Specific Dispensation (OSD) scale is supposed to be given to every health professional in South Africa who is working in the public sector and registered in Health Professions Council. The OSD scale started in 2009 but was not implemented for Mr. BM. Instead, Mr. BM, underwent a series of disciplinary enquiries. He was cleared all the charges and retired in 2018. The history of his research, contractual damages and other collateral losses are discussed in this report.

**Conclusion:** There is heavy contractual damage which the Department of health and rural university has to pay from 1996 to 2018. This damage refers mainly to the short fall of salary and bonuses along with financial losses related to research.

***Keywords:*** *contractual damage, disciplinary enquiries, financial losses*

## Introduction

Contractual damages can be calculated, and an estimation can be assessed as it is written in the contract of an individual as an employee. It is a black and white policy document of the University of Transkei.[2] Direct financial loss could be calculated by an accountant, but the loss in the form of pain and suffering caused by persistent malicious disciplinary enquiries could only be assessed and estimated with the help of an actuarial consultant.[3] Why does this rural university ranks so low on the list of institutions in South Africa ? The University of Cape Town is ranked 269 globally, followed by the University of the Witwatersrand at 292. Stellenbosch, KwaZulu-Natal, and Pretoria University are within the top five universities in South Africa. The University of

Johannesburg is ranked sixth. There are, however, no rural universities in the rankings in South Africa.[4] The higher managers themselves are never serious enough about their work to fix the inherit problems of mal-administration at this rural university. There is a question mark against the functioning of this university administration.[3] The usual answer they give is lack of funds.[3]

The heads of this university have displayed complete amnesia and turned a blind eye to ongoing corruption. An example of this corruption is where a Human Resources (HR) department clerk increased his own salary. The matter was put on the desk of the legally sound head of this rural university, but it was completely ignored. The issue was pointed out several times by a very professional senior HR manager to higher management, but nothing improved. The salaries of staff on the same rank varies from a one individual to another. The university is clearly working like a secretive society. In one case to HR about the salary of a professor who was less than a senior lecturer, but the letter remained unanswered. The breach in contractual obligation by the rural university led to serious damage for which compensated should have been paid. This case report is highlighting the contractual and non-contractual damages caused by the university and estimates the cost of these financial losses.

## Case Report

This case report refers to BM who was employed jointly by both the health department as well as by the university with the former contributing more than 60% of his salary. He was under paid from 1996 as he served as a medical officer in the health department and an acting head of department in university.[2] He was appointed as a senior lecturer in the university in 2001, and then an associate professor leading up to the post of professor. He was running a department single-handedly as there was a scarcity of staff in this scare skill specialty. He has undergone three disciplinary enquiries, two suspension, a forensic audit, stoppage of his salary and birthday bonus from 2008 to his retirement in 2018. He was a prolific publisher in his faculty and contributed almost 50% of the research articles all alone and 25% of the total university output.[1] This has generated professional jealousy and led him to face three malicious disciplinary enquiries.

The contractual losses were calculated with available and undisputable proofs. It is not easy to collect all the information as BM was working almost all alone in the Department without any secretarial help, but BM tried to collect as much as possible. Some claims were not a direct encashment in his contract such as the retracted or unsubmitted articles, but it could have generated more funds if this money was available to the researcher. The experience and productivity in research publications multiplied with age. The research office became a stumbling block to BM from 2010 onwards. It may be a verbal instruction from above and research office just followed it. A layer of permission started with the head of department, then went to the dean, then to the two clerks in the research office and then to the research director and to finance. Sometimes the physical distance between two clerks in the research office is hardly two meters but it takes almost two months to reach a claim. They also had some connection with a travel agent, so it became another way for blocking BM from attending a conference. A dozen letters were written to the research office and copies to higher management but there was no change.

## Results

There are two research accounts in the rural university finance department. One is a personal research account (cost code 5565) which is in the name of the researcher and 60% of the amounts deposited come from DoE recognized publications. No funds can be released from this account without

his permission. This has been a practice going on since he joined the university since 2001. Second account is a departmental research account (cost code 5230: Publication FM), where 15% of the funds are deposits from publications as per the research policy documents.

The basic tool is banks statements, a copy of income and expenditure statements and research policy documents. Both bank account statements from 2011 to 2018 are available, and one can see all the deposits from university reflected in these accounts. All the expenditure supposed to be sign for by BM for his personal use and by the HOD before April 2015. In 2015 two new Heads of Department (HODs) were appointed after a merger with three other departments to become the Department of Laboratory Medicine and Pathology. Both the HOD were clearly told in their letter that the Department funds belonged to BM, as funds were generated by him. Therefore, BM has entitlement on all his Departmental funds.

When BM retired in 2018, he has entitlement to get cash funds as per the minutes of the senate. BM received a statement from finance which showed that they had misused his personal research funds without his knowledge. BM has only attended conferences and meetings, and purchased computers, books, and other electronic devices for his research activity. One hand the mangers were frozen research fund of BM, and other side utilizing his money without his permission from his personal research account. He has never paid for printing, and research material. If it is so, it requires his signature before it gets processed, and then this fund must be deposited into his account, which is not visible in his bank statements. BM informed the auditor of the university and a copy was sent to the head of the university. It was surprising that he did not care enough to investigate such a serious financial misappropriation.

## Results

**Table 1. Contractual damage**

| No. | Type of damage | Amount | Proof |
|---|---|---|---|
| 1. | VC award 2006 | R 10 000.00 | 1,2,3,4,8 also para 29 |
| 2. | Research cash payment | R467991.00 | 5,6,7 also paragraph 29 |
| 3 | Unauthorized funds taken out from personal research account before BM retired | R628363.17 | 9 |
| 4. | 60% of retraction of 12 articles (R 858 960) | R 515376 | 10, Bundle 10 |
| 5. | 5 articles unsubmitted of R 357 900.00 | R214740 | 11 |
| 6. | International Humanitarian award | USA $ 5000 (R75000) | 12 (buddle 6 research awards) |
| 7. | CSIR | R80 000 | 13, bundle 10 |
| 8. | Unpaid claims by the research office (Table 2) | R738574.08 | 14,3 and 4 (Bundle) |
| 9 | Unpaid Departmental research claims 6 processed (Table 3). | R61018 | 15 (Bundle grievances) |
| 10. | Departmental research | R113776.7 | 9,6 |
| 11. | Research awards pending 2007, 2008, 2009 | R75000 | 1,9, bundle 12 |
| | Total | R2979838.95 | |

**Table 2. Unpaid claims submitted to research office between 2013-2018 (14 Bundles)**

| Date of submission | Amount | Proof |
|---|---|---|
| 14.04.2013 | R50 735 | Lost, Bundle 10, 14 |
| 18.09.2013 | R63464 | Lost, Bundle 10,14 |
| 05.12.2011 | R8622 | Bundle 14 |
| 27.08.2014 | 9099.87 | Bundle 14 |
| 11.10.2015 | R13377* | Bundle 14 |
| 19.01.2016 | R7315 | Bundle 4 |
| 29.09.2015 | 42977.36 | Bundle14 |
| 29.01.2013 | 4736.00* | Bundle 14 |
| 01.02.2017 | 24051.71 | Bundle 14 |
| 13.02.2018 | 16000 | Bundle14 |
| 03.02.2011 | 27183 | Bundle 3 |
| 29.08.2011 | 55918 | Bundle 3 |
| 26.01.2011 | 30249 | Bundle 3 |
| 30.03.2012 | 34994.61 | Bundle 3 |
| 26.05.2011 | 64922 | Bundle 3 |
| 21.07.2007 | 11920 | Bundle 4 |
| 29.01.2013 | 38401 | Bundle 4 |
| 14.10.2013 | 46456.11 | Bundle 4 |
| 17.10.2013 | 47737.67 | Bundle 4 |
| 25.11.2013 | 46799 | Bundle 4 |
| 04.08.2014 | 6859.04 | Bundle 4 |
| 28.07.2015 | 1500 | Bundle 4 |
| 27.07.2015 | 21833 | Bundle 4 |
| 27.07.2015 | 2892.40 | Bundle 4 |
| 27.7.2015 | 2892.40 | Bundle 4 |
| 27.07.2015 | 20529 | Bundle 4 |
| 16.03.2016 | 7315 | Bundle 4 |
| | R738574.08 | |

**Table 3. Departmental research six claims processed but not paid 2014-2015**

| Date | Refunded department account | Comment |
|---|---|---|
| 07.08.2014 | R6859.04 | Not visible in bank acc |
| 28.07.2015 | R1500 | Not visible in bank acc |
| 25.08.2015 | R21833 | Not visible in bank acc |
| 29.07.2015 | R2982.40 | Not visible in bank acc |
| 25.08.2015 | R20529 | Not visible in bank acc |
| 16.03.2015 | R7315 | Not visible in bank acc |

## Discussion

The rural university inherited staff from the University of Transkei in a dual employment system which was complicated and seriously affected the lives of staff in both institutions in terms of pension and the morale of retired individuals. Pension is the pride of a retired person. It is linked to the salary, and if half the salary gets paid then the pension is reduced to half.[5] Financial losses as a result of professional jealousy are under researched and therefore under-estimated. It happens in all professions and institutions. It manifests in different forms and patterns, ranging from simple hate to severe punishment such as disciplinary enquiries, suspension and ultimately termination. Universities have policies and protocols in place to deal with researchers and they are protected by the higher management but there are rural universities in South Africa that either do not have policy and protocols or do not adhere to them. In fact, they are getting benefits because of the absence of policy as there is no barrier to the university swallowing the funds.

BM was working hard thinking that research would lead to his promotion, but this was not the case in WSU. Most of the time people hardly get promoted. It was very well conceded by the head of institution that the research award of 2006 was not handed over to Mr. BM, but no action was taken (Table 1). He was surprised by the fact that Mr. BM was also not paid cash payment of his personal research funds, and later these funds were utilized without his permission. It was unbelievable the head of the institution was claiming that nothing had happened. There were all kinds of misuse of Mr. BM's funds including research awards and other research money either not paid or it was used without his permission (Table 1). The assessor's report, issued in 2011, which made some recommendations, were put on the back burner.[1] Most of the staff who were appoint from the outside did not have the courage to speak out and this created a difficulty for those who raised their voices against the justice.

BM was appointed under the University of Transkei policy document as an acting head of department which state under section 9 (subsection 2a) the amount of additional remuneration that may be granted under this sub-rule shall in no case exceed the amount representing the difference between the salary actually drawn by the acting officer and the minimum salary of the post in which he has been appointed

to act.[2] The shortfall of salary must be paid by the health department as well as by the WSU in dual employment.[6] Mr BM was running the department all alone except few years when some working assistance was given to him.

There is the payment of leave salary which was also very discriminatory.[7] HR provided full leave salary to a few people but not to others. This fact only can be verified from the HR; the author does not have any proof of this. This kind of proof is not easy to get but it was told to the author by a responsible person in HR. It is complex and another report is needed to write about it. When someone is asked in HR about this discrepancy, they reply as per the instruction from above. HR is weak as was also highlighted by the external assessor during his inspection report submitted to the Department of Higher Education in 2011.[1] The HR has lost the personal file of BM. It is difficult to understand whether it was done purposefully, or it was simply a mistake, but the matter needs to get investigated by the higher management of the university. This is in fact an issue of serious negligence but still the higher management remains silent, and no action has yet been carried out.

All the claim and payments have gone into the bank account of Mr. BM. It is easy to find this out as Mr. BM can readily show all the bank accounts (Tables 2 and 3). There was a long witch-hunt against Mr BM regarding his accounts and payments, including establishing a forensic audit against him. The university appointed an agent from an audit company, and he looked everywhere to get some clue against Mr. BM but failed to confirm any misdoing by Mr. BM. Surprisingly, that agent has not even submitted any report as per now despite being asking by the research office.

This rural university is covering a wide area and is the only university for these students, who come from a poor economic background. These students are very talented, and need a good medical school to complete their studies. It is unfortunate that those who need education the most get very little. The university managers must intervene and improve the system so that the university does not remain like Afghanistan with multiple organ failure as was stated by the external assessor in 2011.[1]

## Conclusion

Mr. BM was appointed under the university of Transkei (Unitra); therefore, he must abide by the rules of service. He is eligible to get his shortfall of salary from 1996, the day of his appointment as an acting head, until the day he retired from his job in the year 2018. This is a contractual legal requirement to pay his salary and bonuses as per the law. The health department is number one in this dual employment for the occupational specific dispensation (OSD) and overtime payment.[7] The university is mainly liable for the damages cause by malicious disciplinary enquires, financial damaged caused by stoppage of salary, stoppage of birthday bonus, non-payment of leave salary and payment related to research funds which were generated by or awarded to Mr. BM. Because of the underpaid salary, his pension has also been calculated at a lower rate, and therefore in this regard he has been underpaid since his retirement, and thus this needs to be revised.

**Ethical issue:** The author has ethical permission for this research publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

**Funding:** Self-funded

**Conflict of Interest**: None

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# A Study on the Characteristic Features of Covid-19 Deaths in a Regional Hospital in Mthatha in the Eastern Cape, South Africa

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## Abstract

**Background:** Covid-19 is an acute health emergency to human beings all over the world. This pandemic has destabilised the day-to-day life of almost everyone. It is a challenge to scientists and politicians to initiate a return to normal human life from the grip of Covid-19 so that the economies of countries do not stop growing.

**Objective:** To study the characteristics of Covid-19 deaths in a regional hospital in Mthatha in the Eastern Cape, South Africa.

**Method:** It is a retrospective record review study of the cases of patients in Mthatha Regional Hospital (MRH) in South Africa who were not able to be saved.

**Results:** There were 100 deaths in MRH. Of these 57 (57%) were females and 43 (43%) males. The average age was 63.3 years. Shortness of breath was the most common presenting symptom followed by weakness, a cough and fever. More than half of the Covid-19 cases had associated co-morbidity such as hypertension (50%), diabetes (37%) and HIV (15%). The mean oxygen saturation ($SpO_2$) at the time of admission was 75.5±17 on pulse oximetry.

**Conclusion:** The covid-19 mortality was 1.3 times higher among females in the regional hospital under study. The majority of the victims were suffering hypertension and diabetes.

***Keywords:*** *Covid-19, diabetes, hypertension, HIV, oxygen*

## Introduction

Covid-19 is a severe respiratory syndrome. It has been increasing worldwide, and more than 300 000 people died because of this pandemic worldwide.[1] The rapid progression of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic is a global challenge.[2] Corona viruses such as middle east respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) were also associated with severe respiratory consequences with higher death rates among patients with diabetes.[3] There are limited studies, but the majority of them claim that diabetic patients are at higher risk of viral infections with severe catastrophic consequences.[4]

Age and sex and health status of the patient has a prognostic value in Covid-9 patients worldwide. Therefore, these values of age and sex must be utilised in decision-making to make predictions

about Covid-19.[5] There is also a racial difference among patients affected by Covid-19 and this must be utilised in preventive strategies.[6] Mechanical invasive ventilation is common among Covid-19 patients, but the majority will die because of multiple organ failure in hospital**.** [7]There is a published study in South Africa to identify high-risk individuals. The patterns of Covid-19 deaths identified by sex, age, co-morbidities, and provinces point to the need for targeted and localised interventions. The individuals who suffered from high blood pressure and diabetes should be given careful attention during the Covid-19 pandemic across SA.[8] The black race is more vulnerable in comparison to white individuals in United States. The risk of high mortality in Covid-19 was seen in people with low socio-economic status.[9]The purpose of this study was to highlight the characteristics of Covid-19 deaths at a regional hospital in Mthatha, Eastern Cape, South Africa.

## Method and Material

This was a retrospective record review of the first 100 patients who died due to Covid-19 between 10 July 2020, and 31 January 2021 at the Mthatha Regional Hospital (MRH) in the Eastern Cape Province of South Africa. Patients admitted to the isolation ward after confirming a positive result of reverse-transcriptase polymerase chain reaction (RT-PCR) and/or rapid antigen test for SARS-CoV2 virus from a nasopharyngeal swab formed the study participants. MRH is a 302-bed referral hospital designated for Covid-19 management. The hospital has a 36-bed isolation unit to accommodate, and additional beds were repurposed during the peak of the pandemic to accommodate the additional Covid-19 patients. The hospital provides level one and two care to approximately half a million people.

Data were manually collected from clinical health records and included demographic characteristics and comorbid conditions. Comorbid conditions derived from the patients were abstracted from documentation on the clinical health records. An Excel spreadsheet was used to extract the data and every 10th entry was rechecked for accuracy and quality assurance. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 18.0 and displayed in tables.

## Results

The majority 57(57%) of the patients were female and 43 were male with the mean age of 63.3±16 years. Shortness of breath was the most common presenting symptom followed by weakness, a cough, and fever (Table 1). Half of the hospitalised patient who died from Covid-19 had hypertension (50%) followed by diabetes mellitus (37%), HIV (15%) and TB (11%) comorbidity (Table 1). The mean oxygen saturation ($SpO_2$) at the time of admission was 75.5±17 on pulse oximetry. The majority of patients received supplementary oxygen by facemask (66%), followed by nasal high flow (28%), CPAP (3%) and 3% were on room air (Table 1). About two-thirds (60%) of the hospitalised patients who died due to Covid-19 illness were above 60 years and of this 13% were above 80 years of age (Table 2).

**Table 1. Characteristics of Covid-19 deaths at the regional hospital in Mthatha (N=100)**

| | |
|---|---|
| Mean age ± SD | 63.3±16 |
| Gender (n=100) | |
| Male | 43 |
| Female | 57 |
| Presenting symptoms (n=100) | |
| Shortness of breath | 91 |

***Cont...*** **Table 1. Characteristics of Covid-19 deaths at the regional hospital in Mthatha (N=100)**

| | |
|---|---|
| Weakness | 87 |
| Cough | 68 |
| Fever | 33 |
| Loss of taste | 7 |
| Loss of smell | 4 |
| Comorbidity (n=100) | |
| Hypertension | 50 |
| Diabetes | 37 |
| HIV | 15 |
| TB | 11 |
| CVA | 5 |
| CKD | 4 |
| COPD | 3 |
| Epilepsy | 3 |
| Supplementary oxygen methods (n=100) | |
| Face mask | 66 |
| Nasal high flow | 28 |
| CPAP | 3 |
| Room air | 3 |

**Table 2. Age and gender distribution among Covid-19 deaths at the regional hospital in Mthatha (n=100). Age**

| (yrs.) | Male (%) | Female (%) | Total (%) |
|---|---|---|---|
| ≤20 | 1 (2.3) | 0 (0) | 1(1) |
| 21-40 | 2 (4.7) | 9 (15.8) | 11(11) |
| 41-60 | 11 (25.6) | 17 (29.8) | 28 (28) |
| 61-80 | 27 (62.8) | 20 (35.1) | 47 (47) |
| >80 | 2 (4.7) | 11 (19.3) | 13 (13) |

## Discussion

This is probably a first report on the characteristics of death arising from Covid-19 in the regional hospital in Mthatha. The Mthatha (Umtata), the capital of the Republic of Transkei in the apartheid time, has given rise to national and international leaders from this region, but it is still poverty stricken. People are very poor and extreme poverty is chronic in nature as there is no scope for employment. There is already a triple burden of unnatural death, HIV, and tuberculosis in this region. Covid-19 has added one more.

The mean of Covid-19 patients in this study is 63.3 which is almost comparable with other studies (Table 1). A systematic review and meta-analysis exploring different age, sex, and health differences by Mesas et al. in 2020 showed the average age was 60

years which is a little lower than in our report.[5]An earlier study carried out by the author showed that the mean age of hospitalised patients was 55.5 years.[10]About one-fifth (19.3%) died at 80 or above years. About half (47%) of the patients died between 61 and 80 years of age in this study (Table 2). This is the most vulnerable age group reported.[5] People less than 65 years have very little risk of Covid-19 death if they were not suffering from any morbid conditions.[11] Strategies to focus on protecting high-risk individuals in managing the pandemic. [11]

It is surprising that about two-thirds (57%) were women who died in this hospital of Covid-19. This is higher than in other published studies.[5] The men are more vulnerable to Covid-19 infection than females.[5, 12] Why, then, are the woman having almost one and half times (57/43) higher death rate due to Covid-19 infection? It is because the women are probably higher in number in this region than men with a ratio of 55:45.[13] This could not be explained alone, as there is 1.32 times higher mortality among females. Men smoke tobacco and cannabis and use alcohol in abundance a lot in this region, and therefore men should be more at risk of Covid-19 infection.[5]The Covid-19 produces more severe symptoms and higher mortality among men than women.[14] It is not clear how the immune response differs with sex difference during Covid-19 infection against severe acute respiratory syndrome. [14] Male patients have a higher level of innate immune cytokines such as IL-8 and IL-18 along with more robust non-classical monocytes.[14] Female patients have more robust T cell activation than male patients. By contrast, higher levels of innate immune cytokines were associated with worse disease progression in female patients, but not in male patients. [14] These findings provide a possible explanation for the observed sex biases in Covid-19 and provide an important basis for the development of a sex-based approach to the treatment and care of male and female patients with Covid-19.

The majority victims who died were suffering from hypertension (50%) and diabetes (37%) in this study (Table 2). Thirty-one percent (31%) of victims were suffering from both hypertension and diabetes, but the outcome is same. Diabetes and high blood pressure increase the risk of death in Covid-19 patients.[3] The average admission period was 6.7 days among all patients who were victims of Covid-19 deaths. There was not much difference in survival time in patients who were suffering from hypertensive (6.5 days) and diabetes (6.6 days) in this study. The Covid-19 infection outcome is unpredictable as it drops the oxygenation level rapidly.

HIV infection is prevalent in this community and recorded 15% in this study, which is almost equal to the national average. The estimated overall HIV prevalence rate is approximately 13,7% among the South African population.[15]Only one patient was recorded who died was less than 20 years old in this study (Table 2). She was HIV positive stage 4. The Covid-19 infection has probably precipitated her death much faster, although there is no study found to substantiate this claim in literature. The majority (25/36) of the patients were females who were admitted to the thirty-six bedded Covid-19 ward in this hospital. All were African black subjects so one can does not compare the racial predisposition for Covid-19. They were also poor as those who cannot afford to have medical insurance generally come to public hospitals in South Africa.[13] The black race is more vulnerable in comparison to white individuals in United States,[6] but this may not be true of South African blacks. The risk of high mortality from Covid-19 was linked with low socio-economic status.[9,12]This discrimination has been more prevalent in American society.[9] Therefore, those who are poor become of victims of all kinds of vulnerable situations and Covid-19 is one of them.

The most common usual clinical findings in this study are dyspnoea, cough, and fatigue.[3] Shortness

of breath was found on almost all the patient (91%) records. Respiratory tract involvement is the most common finding where there is involvement of the lungs.[2] This is the reason the first thing in hospital is to do an X-ray chest or MRI to know the level of lung involvement. The lungs get opaque because of the Covid-19 infiltrate and this leads to pneumonic consolidation. There are various mechanisms but the one most accepted is cytokine storms, where the body's own defence is used to heal the damaged organ and system.[16]The cytokine storm is fatal in Covid-19, and leads to multiple pathological characteristics such as ARDS, coagulation, and multi-organ dysfunctions.[16] Blood IL-6 levels are highly correlated with the lethal complications of Covid-19 and is a marker of disease severity. It is presumed that the body's immunity must not be too strong as that leads to cytokine storms, or too weak low that one can does not fight a virus.[16] There are lot of things that are still not clear which are displayed on television channels nearly every day. It is a droplet viral infection so it is different from some other viral infections because an individual may look healthy but at the same time be infectious. Therefore, it is an infection that spreads without any notice. Those people who are suffering from hypertension and diabetes are more at risk of hospitalisation as well as a poor outcome.[3,4,11]

Obesity is very common among African women in this region. Most of the patients admitted to the Covid-9 ward were obese. However, the body mass index was not estimated. A growing number of evidence connects obesity with Covid-19 as a major cause of death. There are several mechanisms from immune system activity which lead to inflammation.[17] It is not clear what causes this but certainly about three-fourths of the women in this study who died because of Covid-19 were obese.

This hospital is a poorly resourced hospital where there is lack of either ventilators or skilled staff to deal with ventilators. Therefore, not a single patient was found in this study who was intubated. A majority of patients wore a facial mask or were on high oxygen flow.[7] The situation is not different in a private hospital in this region. A colleague of mine has died without putting him on a ventilator in a private hospital. It is considered that the chances of death are very high for those who are kept on ventilators but not zero. There is a sizeable number of patients who have survived in different hospitals; therefore, one must try hard to win against this Covid-19 epidemic.

## Conclusion

The Covid-19 mortality among females was 1.3 times higher in the regional hospital in Mthatha. Three-fourths of the victims were associated with hypertension and diabetes as a comorbidity. The survival period was around five or six days in this study.

**Ethical Considerations:** The Ethics Committee of the Walter Sisulu University has approved this study (Reference number: 098/2020) and approval from the Eastern Cape Department of Health (Reference number: EC_202010_027) along with hospital management have also been received.

**Conflict of Interest**: None

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# Correlation of Oral Health Status with Chronic Obstructive Pulmonary Disease in a Tertiary Care Hospital

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**Abstract**

**Background:** Oral health is considered to be an important factor in respiratory diseases like Pneumonia and chronic obstructive pulmonary disease (COPD). Poor oral health and abusive habits like smoking have been implicated as an independent risk factor for the development of COPD, but few studies have evaluated the association between oral health and COPD.

**Aim:** To assess the oral health and habits of COPD individuals visiting our OPD for their regular checkups.

**Subjects and Methods:** We performed a case-control study of oral health among patients with COPD exacerbators and healthy non COPD controls. Cases had experienced ≥1 exacerbation in the previous 12 months, while controls were healthy patients reporting to the dental OPD for a regular dental checkup. We evaluated oral health status, recorded dental symptoms/habits, and Pulmonary Function Test (PFT). In a subset, we performed blinded dental exams to measure bleeding on probing, probing depth, clinical attachment loss, periodontitis severity, plaque index, gingival index, and carries risk. We evaluated associations between oral health and COPD using logistic regression.

**Result:** Self-reported oral health status and objective dental findings had variations between cases and controls. Participants with COPD had multiple missing teeth, a higher amount of plaque, and calculus indicating poor dental health. Oral candidiasis, keratotic white lesions, and oral melanosis were also present.

**Conclusion**: In the present observational study, we found that participants with COPD have poor oral health that compromised their quality of life probably precipitating an acute exacerbation. The incidence of COPD can be reduced by good oral hygiene measures and preventive oral care.

***Keywords:*** *COPD, Oral Health, Oral Hygiene, Chronic Periodontitis, Candidiasis*

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## Introduction

The mortality status of Chronic Obstructive Pulmonary Disease (COPD) in India is showing an alarming growth as mentioned in the WHO Global Infobase and is estimated to be approximately 556,000

out of a world total of 2,748,000 cases annually[1,2]. The Global Initiative for Obstructive Lung Diseases (GOLD)[3] defined COPD as a "preventable and treatable disease with some significant extra-pulmonary effects that may contribute to the severity in individual patients. The pulmonary component is characterized by airflow limitation, which is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, such as cigarette smoke[4].

The new concept on the pathogenesis of COPD believes that interactions between multiple environmental exposures and genetic factors determine lung function and COPD risk. It also states that impaired lung function growth in early life associated with a decline in lung function in adulthood induces COPD [5]. COPD has been strongly associated with smoking and is clinically characterized by symptoms like cough, sputum production, and/or dyspnea.[6,7]

The therapeutic line focuses on reducing the symptoms and preventing exacerbations of the disease. Bronchodilators are the mainstay which includes $\beta_2$-agonists and anticholinergics, either used alone or in combination with corticosteroids.[8]

Smoking tobacco is an important risk factor for the development of COPD, also oral changes associated with tobacco consumption are well known. Research associates tobacco as a major etiologic factor for potentially malignant disorders and periodontal diseases [9].

COPD and its associated chronic morbidity and mortality in our country have led to a big burden and a huge impact on health care. More than half of COPD exacerbations are attributed to bacterial infections and recent studies have demonstrated increased microbial load and microbial diversity in COPD patients compared to healthy adults. The oral and nasal bacteria have been identified in the lung tissue of COPD patients suggesting aspiration of oral secretions as the major cause for this disease. These findings highlight the potential impact of oral health in patients with COPD and the role of oral lesions in acute exacerbations.[10]

The present study was carried out to investigate the oral health condition in terms of gingival inflammation, periodontal disease, and tooth loss in subjects with a confirmed diagnosis of COPD and for comparison, a control group of healthy individuals with a history of smoking was included.

## Subjects and Methods

The present study was a cross-sectional observational study carried out in the departments of Oral Medicine and Radiology at Manipal College of Dental Sciences, Mangalore in association with Kasturba Medical College Hospital, Mangalore, Karnataka. The study protocol was approved by the Institutional Ethics Committee of the institute. Before enrollment, informed written consent was obtained from each subject.

Subjects in the age group of 40 to 70 years, were selected from the outpatient and inpatient departments of the institution.30 subjects in each group are described as follows. There were more males than females in the study group and hence we had to select a similar control group.

**Group: 1**

Healthy smokers who served as control. They were smokers with no history of respiratory illnesses.

**Group: 2**

Subjects with COPD who have been smoking for the last 10 years with a minimum of 1 pack per day [10 cigarettes} and suffering from COPD.

The diagnosis and staging of COPD were done by Pulmonary Function Tests (PFTs) using GOLD criteria [3]

**Assessment of Lung Function:**

Lung functions - vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in one second ($FEV_1$), residual volume, and total lung capacity (TLC), was measured and chest images were obtained by Computed Tomography technique and verified by the senior pulmonologists in consensus for the diagnosis of COPD.

**Oral Examination:**

The COPD subjects and control groups were examined in the Department of Oral Medicine using a mouth mirror, gauze, and universal periodontal probe. Lesions were recorded in the case record form, custom-made for the study. The dentists were blinded to the case-control status of the participants. Assessments included periodontitis severity, bleeding on probing (BOP), gingival index (GI). Periodontitis severity (mild, moderate, and severe) was determined by probing depth (PD, scored as ≤3, >3 to <5, ≥5 to <7, and 7+ mm) and clinical attachment loss (CAL, scored as <1, 1 to 2, or 5+ mm) based on the involvement of at least 30% of the entire dentition. Pocket depth was measured with a millimeter graduated probe at four sites per tooth and a mean value was calculated based on all remaining teeth, third molars excluded. A mean pocket depth of 4 mm was taken as a cutoff point to define periodontitis. Missing teeth and other soft tissue lesions were recorded as significant findings in these cases.

## Statistical Analysis

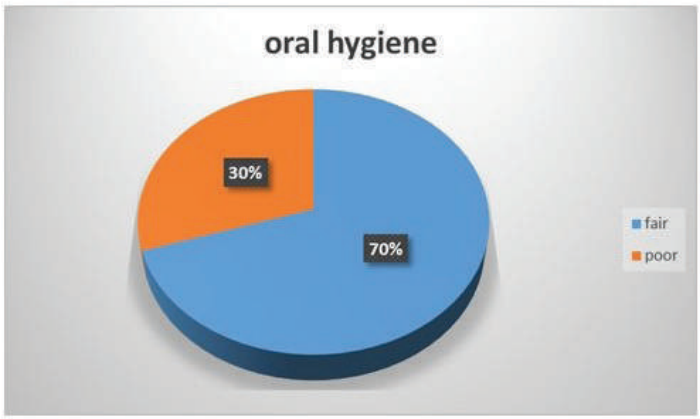
We used Chi-square tests for categorical variables and two-sample t-tests for continuous variables to test the differences in demographic and clinical characteristics between cases and controls.

## Results

***Oral Health status***

Both COPD participants and controls had similar teeth brushing habits, with both groups participants brushing once daily. Oral health status, in general, appeared neglected and there were ample dental deposits that induced early gingival bleeding, and pocket depth was found to be increased in the study group when compared with the control group (Figure 1).

Figure 1: Oral Hygiene status of patients with COPD



The number of missing teeth was high in the COPD group as compared to the control group. Dental deposits were significantly more prevalent in the cases group when controlled for age and gender. The number of sites exhibiting gingival bleeding and pocket depth was elevated in the COPD group when compared to subjects without COPD. The mean number of remaining teeth was significantly reduced in COPD patients when compared with non-smokers and subjects without COPD controlling for age and gender.

The duration of illness varied in our study group members, ranging from a month to 15 years as and the frequency of acute exacerbations of the disease was recorded as seen in Table 1. The oral mucosal and hard tissue changes were relatively more in patients with COPD than in normal healthy subjects Table 2. In terms of periodontal health, these patients were compromised and a few of our subjects also reported burning sensation and intolerance to spicy food due to oral candidiasis.

**Table 1- Baseline characteristics by COPD cases**

| **Age Range Category** | **No of subjects** | **Valid Percent** |
|---|---|---|
| 40-50 years | 4 | 13.3 |
| 51-60 years | 6 | 20 |
| 61-70 years | 11 | 36.7 |
| 71-80 years | 6 | 20 |
| >=81 years | 3 | 10 |
| Total | 30 | 100 |
| | **Marital status** | **Valid Percent** |
| Married | 29 | 96.7 |
| Single | 1 | 3.3 |
| Total | 30 | 100 |
| | **Occupation** | **Valid Percent** |
| Agriculture | 3 | 10 |
| Clothings | 1 | 3.3 |
| Coolie | 1 | 3.3 |
| Driver | 1 | 3.3 |
| Iron industry | 1 | 3.3 |
| Service | 2 | 6.7 |
| Unemployed | 21 | 70 |
| Total | 30 | 100 |
| | **Educational status** | **Valid Percent** |
| Degree | 2 | 6.7 |
| Primary | 15 | 50 |
| Secondary | 7 | 23.3 |
| Uneducated | 6 | 20 |
| Total | 30 | 100 |

**Table 2 – Oral health status of patients with COPD**

| | Oral hygiene | Valid Percent |
|---|---|---|
| Fair | 21 | 70 |
| Poor | 9 | 30 |
| Total | 30 | 100 |
| | Dentition status | Valid Percent |
| Dentate | 2 | 6.7 |
| Partially dentate | 28 | 93.3 |
| Total | 30 | 100 |
| | Mobility | Valid Percent |
| Absent | 21 | 70 |
| Present | 9 | 30 |
| Total | 30 | 100 |
| | Gingival bleeding | Valid Percent |
| Absent | 1 | 3.3 |
| Present | 29 | 96.7 |
| Total | 30 | 100 |
| | Presence of Calculus | Valid Percent |
| Absent | 8 | 26.7 |
| 1+ | 16 | 53.3 |
| 2+ | 4 | 13.3 |
| 3+ | 2 | 6.7 |
| Total | 30 | 100 |
| | STAIN | Valid Percent |
| Absent | 15 | 50 |
| 1+ | 10 | 33.3 |
| 2+ | 4 | 13.3 |
| 3+ | 1 | 3.3 |
| Total | 30 | 100 |
| | Hyperpigmentation | Valid Percent |
| Absent | 23 | 76.7 |
| Present | 7 | 23.3 |
| Total | 30 | 100 |

| | Salivation | Valid Percent |
|---|---|---|
| Increased | 1 | 3.3 |
| Normal | 28 | 93.3 |
| Reduced | 1 | 3.3 |
| Total | 30 | 100 |
| | **Burning sensation** | **Valid Percent** |
| Absent | 29 | 96.7 |
| Present | 1 | 3.3 |
| Total | 30 | 100 |
| | **Oral candidiasis** | **Valid Percent** |
| Absent | 29 | 96.7 |
| Present | 1 | 3.3 |
| Total | 30 | 100 |
| | **Keratotic lesion** | **Valid Percent** |
| Absent | 28 | 93.3 |
| Present | 2 | 6.7 |
| Total | 30 | 100 |

## Discussion

The mouth is the mirror of systemic health and the primary indicator of ill health. We aimed to determine if COPD status is associated with oral health as we are aware that the oral cavity harbors a lot of commensals, aerobic, and anaerobic potential respiratory pathogens like methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa[10,11]

Our study had the maximum number of cases in the age group of 61-70 years. This concurs with various other studies which also had a similar study group with a maximum number of cases were in the age group of 55-65 years.[12-14]

In our study, we also observed that the incidence of COPD was higher in males than females and this finding also coincides with other studies where they had around 80-93% males recruited in their study [15,16] Pulmonary function tests were performed and we had recruited only cases with a conclusive diagnosis of COPD.

Dental plaque was significantly more prevalent in the study group than controls when matched with age and gender ($p<0.01$) and this significant finding explains the number of sites exhibiting gingival bleeding, recession, and increased pocket depth in this population with COPD. The mean number of remaining teeth was significantly reduced in COPD compared with controls without COPD when matched

for age and gender.

Studies suggest a causal link between poor oral health and pulmonary disease as oral pathogens that harbor the gingival crevices can get aspirated into the pulmonary tissues which cause respiratory illness, mainly pneumonia, and COPD. In patients with neglected oral care, the composition of the oropharyngeal flora becomes heavily colonized by virulent gram-negative pathogens that have the potential to cause respiratory infections. In a few prospective controlled trials, when the sputum of patients with COPD was analyzed, the antibody levels against Fusobacterium nucleatum and Prevotella intermedia were elevated.[17] These are the anaerobic organisms that have been found in patients with chronic periodontitis. Studies have proved that the microbiome of the lungs resembles the oral microbiome and the chronic microaspiration of saliva also plays a role.[18,19] It is proved that saliva carries pathogenic bacteria, such as Haemophilus influenza from diseased teeth which worsens respiratory symptoms when aspirated.[20]

A study conducted by Kucukcoskun et al [21] clearly showed that regular periodontal treatment significantly reduced the frequency of COPD exacerbations. A partially edentulous state was more prevalent in the COPD group compared to the non COPD groups after matching for age. This can be explained by the fact that oral hygiene gets partially neglected among patients with systemic disease and also periodontal disease progresses faster among smokers than in patients with no habit history. Wang et al [22] also found fewer remaining teeth in patients with COPD than in a control group.

Tobacco used in any form induces oral mucosal changes in the form of which intra-oral mucosal pigmentation is a known fact and that explains the oral pigmentation that was seen in our COPD group. Various studies have stated that the heat generated during smoking triggers the melanocyte activity responsible for pigment changes in the oral cavity [23]. Labial mucosa showed a high degree of pigmentation followed by the buccal mucosa in our study.

Nonscrapable white lesions in the form of keratotic patches are common on the lips at the site of habitual cigarette smoking and were appreciated in our study group. This is more visible in people who have a habit of retaining cigarettes or cigars on their lips for lengthy periods. These lesions were appreciated on the mucosal surface of the lower and upper lips at the site at which the cigarette bud is held. They were flat or slightly elevated whitish areas with red striations and can be classified as benign with no premalignant potential. Similar findings were reported in other studies among smokers and cannot be correlated with the presence or absence of COPD[24]

Inhalational Corticosteroids (ICS) are prescribed as the mainstay of treatment in COPD and are usually administered in combination with long-acting β2-agonists especially in patients with severe airflow limitation [24-25]. Recent studies have further shown that ICS is being prescribed in COPD even more widely and frequently than expected, particularly among patients with reduced severity[26-27]. Oral candidiasis is a well-documented local side-effect associated with corticosteroids and is known to reduce local immune response which promotes the growth of oral Candida [28]. Candidiasis induces temporary symptoms like burning sensation in the oral cavity and intolerance to spicy food which can be clinically significant and may affect patient quality of life and therapy adherence [29-31].

In the present study, the oral changes among patients with COPD were compared with subjects who were smokers but without a history or any apparent clinical signs & symptoms of COPD. We found that participants with COPD have poor dental hygiene practices and compromised oral health.

These oral changes could have been due to factors like age, gender, habit associated, or because of COPD condition itself. It is well documented that there is a positive relationship between aging and oral mucosa diseases and acts as a confounding factor as we had few elderly in our study group [32]

## Conclusion

We conclude that there is an intense diversity in the pathogenesis of COPD, its association with smoking, and its oral manifestations. We theorize that the oral microbiota and oral inflammation play key roles in this relationship between dentition status and COPD and oral flora is a major source of the lung microbiota.

The cumulative evidence of our study suggests an association between oral and pulmonary disease and the incidence of the latter can be reduced by good and regular oral hygiene measures. Smaller sample sizes and single-center observational studies are limitations of the study and daily respiratory symptom data or change in symptoms during an exacerbation were not documented. These findings will need to be corroborated in larger study groups, but they do support the idea of potentially targeting oral health to improve COPD outcomes in future days.

**Ethical Clearance-** Taken from Institutional Ethics committee of MCODS, Mangalore

**Source of Funding-** Self

**Conflict of Interest** -nil.

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# A Comparative Evaluation of Dental Erosion Caused by Tetra-Packed and Aerated Beverages: An In-Vitro Study

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## Abstract

**Introduction:** Dental erosion is an evolving concern with the food market globalization. In a rapidly developing nation as India, beverage consuming behavior has significantly increased. There is free access to new foods and beverages even in the neighborhood grocery stores and they are perceived as modern and healthy. Extrinsic erosion appears to be a growing problem as a result of overconsumption of soft drinks or fruit juices or both**. Materials and Methods:** Freshly extracted premolars were treated with $H_20_2$ for 24 hours and weighed with 'Essae FB-200' with a readability of 0.001 gram. The beverages were grouped into 2 categories, 1- Natural or tetra-packed beverages, 2 - aerated beverages and a control. 20ml of each beverage was taken and the pH was measured. The tooth was immersed in each of the beverage and pH were noted 4$^{th}$ hourly for 48 hours accounting for sipping the beverage for 5 minutes every day for 1.5 years. The weight of the tooth was rechecked after 48 hours. **Results:** The mean pre-treatment pH of category 1 was 5±1.33 and that of category 2 was 4.66±1.32. There is no significant difference in the mean loss of weight between the two groups (p-value 0.94) there is no significant difference in the mean change of pH between the two groups (p-value 0.26) **Conclusion:** The objective erosive liability of tetra-packed and aerated beverages on the dental enamel was found to be similar. However, natural fruit juices showed nil to negligible objective signs of dental erosion.

***Keywords:*** *Beverage, Dental erosion, Enamel*

## Introduction

Enamel forms a protective layer over the entire surface of the crown. It consists of 96% inorganic material, 4% of organic substance & water. (1) It is constantly exposed to changing environments, and a significant portion of such changes is brought about by the type of diet consumed by the individual. (2) The dynamic nature of the oral environment is a major contributing factor for many of the prevalent dental conditions such as dental caries, periodontitis and of recent times, dental erosion. Erosion is a progressive dissolution of tooth substance by exposure to acids, without the involvement of bacteria which could be either intrinsic or extrinsic. Extrinsic dental erosion is an evolving concern with the food market globalization. In a rapidly developing nation as

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India, beverage consuming behavior has significantly increased. Juice & juice-based drinks are growing 2.5 times faster than aerated drinks. [3] Enamel present in the fully formed crown has no viable cells, hence enamel lost after a tooth has erupted cannot be formed again. [4] Accordingly dental erosion is irreversible which could further lead to hypersensitivity, the unaesthetic appearance of teeth, and can also lead to loss of vertical dimension. All of this could add up to the burden of oral diseases, decreasing the quality of life. [5] Hence an experimental study was conducted to compare the dissolution caused by tetra-packed beverages and aerated beverages on freshly extracted teeth.

## Materials and Methods

### Experimental design

An in-vitro study was designed to compare the erosive liability by measuring physiochemical properties like pH and calcium dissolution by measuring the weight of the tooth to assess the amount of dissolution of calcium into the beverage thereby leading to a loss in tooth structure.

### Sample size

Sample size was calculated to be 20 (10 in each group) by setting a Power of 80%, level of significance at 5% and an effect size of 1.2 using G*Power software version 3.1.9.7. [6]

### Beverage selection

The commonly consumed beverages in India were identified and categorized as

Category 1 - Natural or tetra-packed beverages

Category 2 - Aerated beverages and

The control was taken as distilled water.

The beverages under category 1 were fresh apple juice, fresh grape juice, tea, Amul Masti buttermilk, Amul lassi, B natural mixed, Tropicana mixed, Real orange, Nescafe and Frooti. The beverages under category 2 were Pepsi, Miranda, Slice, Bovonto, Eno, Limca, Pulpy orange, Coco-cola, and Maaza.

### Physiochemical properties

20 ml of each beverage was poured in a labelled plastic transparent cup. The pre-treatment pH before the placement of tooth was noted for each of the beverage with PSI universal full range pH (1-14) strips (A-70, MIDC Industrial Area, Amravati, Maharashtra 444607)

### Tooth Preparation

The selection criteria for the teeth are that they are freshly extracted permanent maxillary and mandibular premolars weighing between 4 to 4.85 grams. The teeth were treated with hydrogen peroxide for 24 hours. Each tooth is weighed before placement in the assigned beverage.

### Measurement

The freshly extracted teeth were immersed in the beverage for 48 hours. Immersing in the beverage for 48 hours would account for sipping the beverage every day for 5 minutes for 1 and a half years. The pH of the beverage was then measured at a 4-hour interval for 48 hours.

After 48 hours the tooth was removed from the beverage and rinsed with distilled water. The weight of the tooth was then measured on a calibrated weighing scale 'Essae FB-200' with a readability of 0.001 gram (# 410, 100ft Road 4th Block, Koramangala, Bengaluru, Karnataka – 560034)

## Statistical Analysis

Descriptive and inferential statistics were analyzed by IBM SPSS version 20.0. Throughout the study, a 'P' value of $<0.05$ was considered as a statistically significant difference. [7]

## Results

Table 1 shows that beverages B natural, Frooti, Pepsi, Miranda and Pulpy orange showed a low pre-treatment pH of 3 before placement of the tooth. The mean pre-treatment pH of category 1 was 5±1.33 and that of category 2 was 4.66±1.32. Immersing the freshly extracted teeth in the beverages for 48 hours showed an increase in mean pH from day 1 to day 2 as 3±0 to 4.1±0.4 in B natural, 6.5±0.54 to 7±0 in Amul Masti buttermilk and Amul lassi, 4±1.5 to 5.5±0.54 in Pepsi, 6.33±0.51 to 8.66±0.51 in Eno, 3.7±0.75 to 6±0 in Pulpy orange and 5±0 to 6±0 in Coco-Cola. The other beverages showed the following changes in pH between day 1 and day 2 – Tea 7±0 to 7.66±0.51, Tropicana mixed 3.66±0.51 to 3.33±0.51, Real orange 4.66±0.51 to 3.83±0.4, Nescafe 6.5±0.54 to 7±0, Frooti 3.5±0.54 to 4±0, Miranda 2.66±0. 51 to 3±0, Slice 5±0 to 5.33±0.51, Bovanto 4.66±0.51 to 3±0 and Limca 6.14±0.4 to 7.66±0.51. However, there was an almost negligible increase in pH in fresh apple juice (5±0 to 5±0), fresh grape juice (5±0 to 5.66±0.51) and Maaza (6±0 to 6±0). The control showed a constant pH of 7 throughout the 48 hours. Table 2 shows weight of the tooth before and after placement in each beverage for 48 hours. The mean weight of teeth considered for placing in the tetra-packed beverages and aerated beverages was 4.425±0.35 and 4.442±0.22 respectively. After 48 hours the mean weight of tooth in tetra-packed and aerated beverages was 4.311±0.33 and 4.326±0.23. Comparison of mean difference in weight before and after placement in the beverage for 48 hours revealed no significant difference between the two beverages (p-value = 0.9)

**TABLE 1: pH of Tetra-packed and Aerated beverages**

| Beverage | Pre pH | Post pH at 24 hrs | Post pH at 48 hrs |
|---|---|---|---|
| Fresh apple juice | 5 | 5±0 | 5±0 |
| Fresh grape juice | 5 | 5±0 | 5.66±0.51 |
| Tea | 7 | 7±0 | 7.66±0.51 |
| Amul masti | 6 | 6.5±0.54 | 7±0 |
| Amul lassi | 6 | 6.5±0.54 | 7±0 |
| B natural mixed | 3 | 3±0 | 4.16±0.4 |
| Tropicana mixed | 4 | 3.66±0.51 | 3.33±0.51 |
| Real orange | 5 | 4.66±0.51 | 3.83±0.4 |
| Nescafe | 6 | 6.5±0.54 | 7±0 |
| Frooti | 3 | 3.5±0.54 | 4±0 |
| Water | 7 | 7±0 | 7±0 |
| Pepsi | 3 | 4±1.54 | 5.5±0.54 |
| Miranda | 3 | 2.66±0.51 | 3±0 |
| Slice | 5 | 5±0 | 5.33±0.51 |
| Bovonto | 5 | 4.66±0.51 | 3±0 |
| Eno | 6 | 6.33±0.51 | 8.66±0.51 |
| Limca | 6 | 6.14±0.4 | 7.66± 0.51 |
| Pulpy orange | 3 | 3.71±0.75 | 6±0 |
| Coco-Cola | 5 | 5±0 | 6±0 |
| Maaza | 6 | 6±0 | 6±0 |

**TABLE 2: Weight of tooth before and after 48 hours**

| Beverage | Pre-weight | Post-weight |
|---|---|---|
| Fresh apple juice | 4.85 | 4.77 |
| Fresh grape juice | 4.33 | 4.26 |
| Tea | 4.9 | 4.71 |
| Amul masti | 4.53 | 4.43 |
| Amul lassi | 4.05 | 4 |
| B natural mixed | 4.02 | 3.84 |
| Tropicana mixed | 4 | 3.99 |
| Real orange | 4.79 | 4.65 |
| Nescafe | 4.57 | 4.45 |
| Frooti | 4.21 | 4.01 |
| Water | 4 | 3.89 |
| Pepsi | 4.63 | 4.49 |
| Miranda | 4.7 | 4.64 |
| Slice | 4.13 | 4.03 |
| Bovonto | 4.12 | 4 |
| Eno | 4.34 | 4.22 |
| Limca | 4.53 | 4.43 |
| Pulpy orange | 4.41 | 4.27 |
| Coco-Cola | 4.71 | 4.63 |
| Maaza | 4.41 | 4.23 |

**TABLE 3: Comparison of change in pH after the procedure, between the two groups**

| Group | Mean change of pH | SD | SEM | Mean difference | 95% Confidence Interval | | t | P-value |
|---|---|---|---|---|---|---|---|---|
| Tetra-packed beverages | .333 | 0.571 | .180 | -0.500 | -1.42 | 0.42 | 1.14 | 0.26 |
| Aerated beverages | .833 | 1.244 | .414 | | | | | |

**Test**: Mann-Whitney U test (Non-parametric equivalent of Student's t-test: Since SD is not less than half the mean value)

**Inference**: The test shows that there is no significant difference in the mean change of pH between the two groups

**TABLE 4: Comparison of loss of weight after the procedure, between the two groups**

| Group | Mean change | SD | SEM | Mean difference | 95% Confidence Interval | | t | P-value |
|---|---|---|---|---|---|---|---|---|
| Tetra-packed beverages | 0.114 | 0.064 | 0.020 | -.00156 | -.052 | .049 | 0.065 | 0.94 |
| Aerated beverages | 0.116 | 0.036 | 0.012 | | | | | |

**Test**: Mann-Whitney U test (Non-parametric equivalent of Student's t-test: Since SD is not less than half the mean value)

**Inference**: The test shows that there is no significant difference in the mean loss of weight between the two groups

## Discussion

Earlier studies reveal the effects of carbonated beverages and acidic dietary choices leading to an increased prevalence of dental erosion. A study by Al-Malik MI on the relationship between erosion, caries and rampant caries and dietary habits in preschool children in Saudi Arabia showed a direct relationship between carbonated beverage consumption and dental erosion. [8] However tetra-packed juices are consumed as a replacement to fresh fruit juices increasingly especially with the ease of availability. In the present study B natural, Frooti, Pepsi, Miranda and Pulpy orange showed a pre-treatment pH of 3 before placement in the beverage. Of which Pepsi showed a pH change from 4±1.5 to 5.5±0.54. A study by Avanija Reddy et al 2016 on the pH of beverages in the United States showed that pH <3 were considered extremely erosive. [9] A study by Rafey Ahmad Jameel et al 2016 showed that all beverages exhibited a positive erosive effect on the tooth enamel

surface on quantitative analysis of tooth erosion, micro hardness, surface hardness and surface height ($p<0.005$). [10] A study by Leonardo S Antunes et al 2017 on Sports drink consumption and dental erosion among amateur runners that there was no significance between the association of isotonic drinks & dental erosion. [11] In the present study beverages containing isotonic pH showed nil to negligible change in pH after 48h hours. A study by Yousef H Al-Dlaigan 2017 showed that Sixty per cent of the children regularly consumed juice drinks. Among daily consumers, 84% of children showed erosion prevalence with a strongly significant association ($p < 0.005$). Holding the drink in the mouth also showed a significant association with erosion ($p < 0.02$). [12] These results agree with the present study, as greater the exposure time, the greater is the erosion that is objectively identified by an increase in pH. A study by Palak Shroff, Shailesh M Gondivkar et al 2018 showed that that the mean titratable acidity values of the packaged fruit juices were higher than carbonated drink [13] which agrees with the present study as both tetra-packed and aerated beverages showed a mean pre-treatment pH of 5±1.33 and 4.66±1.32 respectively. Limitations to the present study are that rather than measuring the subjective signs of erosion, objective signs of erosion were measured. They are the change in weight and pH. A study by Brent L Gravelle et al 2015 on Soft drinks and invitro erosion showed there was a significant positive correlation between the amount of titratable acid and percentage of tooth erosion, while a significant negative correlation was revealed between the beverage pH and percentage of tooth erosion. [5] This study serves as a pilot showing that the difference in erosion caused by tetra-packed beverages and aerated beverages are not significant.

## Conclusion

The objective erosive liability of tetra-packed and aerated beverages on the dental enamel was found to be similar. The greater the exposure time of the beverage to the tooth surface, the greater is the erosion. However, natural fruit juices showed nil to negligible objective signs of dental erosion. Hence, although the tetra-packed beverages are consumed with the intent of an alternative substitute for natural fruit juices, they are as erosive as the aerated beverages.

**Ethical Clearance:** Taken from CARE IHEC committee, Tamilnadu.

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**Conflicts of Interest**: There is no conflicts of interest

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# Health Promotional Life Style Intervention on Knowledge and Practice Regarding Prevention of Co-Morbid Conditions and Complications of Chronic Renal Failure among Hemodialysis

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## Abstract

Hemodialysis is the most frequent treatment method for CRF. However, it has been argued that a number of restrictions and modifications accompany this treatment, which have a detrimental impact on the quality of patient's life and affect individuals' physical and psychological well-being. Objective of the study was to assess health promotional life style modification regarding prevention of comorbid condition and complications of chronic renal failure among hemodialysis patient. A Quantitative Research approach and Quasi experimental research design (Nonequivalent control group pretest post Design) was used for the present study. The sample consisted of 200 chronic renal failure patients undergoing hemodialysis were selected by convenient non probability sampling technique. Results The finding of the study revealed that in experimental group, pretest Knowledge mean score of the samples had 20.26 and in posttest knowledge mean score had 33.93 so the difference is 13.67, this difference was large and it is statistically significant. Whereas in control group, pretest, this difference was small and it was statistically not significant. In experimental group, mean pretest Practice score of the samples showed statistically significant. In experimental group 16.28% improvement in QOL score after intervention and in control group improvement score is 0.35%. This difference showed the effectiveness of the study. Considering correlation between experimental group posttest knowledge score and posttest practice score was (r=0.44 P≤01), correlation between experimental group posttest knowledge score and posttest QOL score was (r=0.45 P≤01) and correlation between posttest practice score and QOL score was (r=0.52 P≤01). It showed moderate positive correlation among them. The result of the present study suggest that health promotional life style modification is necessary to reduce complications and co morbidities which is occur due to CRF and enhance quality of life of chronic patients.

***Keywords:*** *Health promotional life style, co-morbid conditions; chronic renal failure patients and hemodialysis.*

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## Introduction

Lifestyle is a part of life that most people have control over it and such behaviors often are changeable. In fact, healthy lifestyle would help health promotion and bad life-style has negative effects on health.

Health status and quality of life are very important concepts for patients with chronic kidney disease (CKD) and those undergoing hemodialysis[1] At the end of the next decade, the number of patients with end-stage renal disease, who need dialysis therapy, may be doubled. Now a days End-stage renal disease is an important public health issue.[2]

The CKD burden is increasing rapidly worldwide. At the end of 2010, 1,78,3000 patients worldwide were receiving treatment for ESKD, of which 77% were on dialysis and 23% had a functioning renal transplant and this number is increasing at a rate of 7% every year. If the current situation prevails, the global ESRD population will exceed 2 million by 2020. The average incidence of ESKD in developing countries is 175 per million populations.2,3,4

Yaghmayi Thomas et al. quoted that" chronic renal failure has mutual effects on physical, psychological and functional status of individuals which causes types of deprivation and lifestyle changes including financial problems, unemployment, restriction in fluid intake and diet, change in familial roles and tasks and reduction in achieving long term goals" It is estimated that 7 out of 10 death can be prevented through changes in lifestyle Even in developed countries, there are some problems to cope with this increase. Therefore, there is an urgent need to highlight the importance of modifiable risk factors as a basis for treatment strategies to prevent complications and comorbidities through recent knowledge from a healthy lifestyle.

Hsiu LanTeng et al (2013) conducted randomized controlled trial study on Effects of Targeted Interventions on Lifestyle Modifications of Chronic Kidney Disease Patients. This study examined the effects of a targeted Lifestyle Modification Program based on the readiness to change health-promotion lifestyle behaviors, renal protection knowledge, and physical indicators of patients with early CKD. A repeated-measures design randomized 160 CKD patients from four southern Taiwan outpatient nephrology clinics into control and intervention groups. Data were collected five times over a year with a participant retention rate of 64.4%. The intervention group demonstrated significant improvement with regard to diet behavior modifications. Compared with the control group, the intervention group showed a significant improving trend of renal function protection knowledge, stress management, and interpersonal relations. Targeted interventions for patients in the early phases of CKD promotes adherence to proper diet, exercise behavior, and positive lifestyle modifications.8

**Objectives:**

- Assess the knowledge and practice regarding prevention of co-morbid conditions and complications among chronic renal disease patients undergoing hemodialysis in experimental and control group in pretest and posttest.

- Develop and implement the health promotional life style intervention on prevention of co-morbid conditions and complications of chronic renal disease among hemodialysis patients.

- Assess quality of life of hemodialysis patients in experimental and control group before and after administrating intervention.

- Compare the mean pretest and posttest knowledge, practice and QOL score regarding prevention of co-morbid conditions and complications of chronic renal disease among hemodialysis patients in experimental and control group.

- Evaluate the effectiveness of health promotional life style intervention on prevention of co-morbid conditions and complications of chronic renal disease among hemodialysis patients in experimental group after administrating intervention.

· Find out the correlation between posttest knowledge, practice and QOL score regarding prevention of co-morbid conditions and complications of chronic renal disease among hemodialysis patients in experimental and control group.

· Determine association between posttest knowledge, practice and QOL score regarding prevention of co-morbid conditions and complications of chronic kidney disease with selected socio-demographic variables in experimental and control group.

**Delimitation:**

The study was delimited only Chronic Kidney Disease patients undergoing for haemodialysis.

Delimited in South Gujarat region haemodialysis centre.

The study was delimited to patients who are undergoing Haemodialysis twice in a week.

## Research Methodology:

**Research Approach:**

Quantitative evaluative Research Approach was used to assess effectiveness of health promotional life style intervention.

**Research Design:**

Quasi experimental research design with Non-equivalent control group pretest post Design

**Research Setting:**

New Civil Hospital Surat, Gujarat and Manav Seva Sangh (chhayado) Surat, Gujarat

**Sampling Techniques:**

Convenient non probability sampling technique.

**Sample Size and Sample size determination:**

Total Sample Size is 200, 100 for experimental group and 100 for control group. Power analysis method used to determine sample size.

**Variables:**

Independent Variable:

Health promotional life style intervention

Dependent variables:

1. Knowledge of chronic renal failure patients undergoing hemodialysis regarding life style modification behavior.

2. Practice of chronic renal failure patients undergoing hemodialysis towards life style modification behavior.

3. Quality of Life of chronic renal failure patients

**Description of the tool:**

**Section: 1**: Socio demographic data:

It consists of demographical variables including age, sex, education, occupation, monthly income, marital status, sources of information, present associated illness, duration of receiving hemodialysis, frequency of receiving hemodialysis, and length of each hemodialysis session.

**Section:2:**

Self-administered questionnaire's regarding knowledge on health promotional life style modification on following points –definition, causes, risk factor, diagnosis, complications nutrition plan, stress management, medication, exercise, vascular care, prevention of complications and comorbid condition. Knowledge questionnaire has 50 questions. Each correct answer carries one score and total possible maximum score is 50.

**Section: 3:**

Assessment of quality of life by WHO QOL-BREF checklist. Obtained permission from WHO to use this tool. It is a standardized tool, given by WHO and has 26 items. It is five Point scale to measures four domains that is physical health, psychological, social relationships and environmental.

**Section: 4:**

Checklists to assess practice towards health promotional life style intervention among hemodialysis patients. It is a three-point scale and has 25 items.

Intervention:

Development of health promotional life style intervention for chronic renal failure patients undergoing hemodialysis.

## Data Analysis

**Table 1.1: Distribution of subjects in experimental and Control group as per their Socio-demographic Variables N=100+100**

| Sl. No. | Variables | Experimental group | | Control group | | Chi square test |
|---|---|---|---|---|---|---|
| | | F | % | f | % | |
| 1. | Age in years | | | | | |
| | <20 | 3 | 3 | 7 | 7 | c2=3.35 P=0.34 (NS) |
| | 21-30 | 13 | 13 | 10 | 10 | |
| | 31-40 | 44 | 44 | 36 | 36 | |
| | >40 | 40 | 40 | 47 | 47 | |
| 2. | Gender | | | | | |
| | Male | 62 | 62 | 60 | 60 | c2=0 P=1 (NS) |
| | Female | 38 | 38 | 40 | 40 | |
| | Other | 00 | 00 | 0 | 0 | |
| 3. | Religion | | | | | |
| | Hindu | 71 | 71 | 70 | 70 | c2=1.79 P=0.61 (NS) |
| | Muslim | 22 | 22 | 22 | 22 | |
| | Christian | 5 | 5 | 3 | 3 | |
| | Other | 2 | 2 | 5 | 5 | |
| 4. | Education | | | | | |
| | Illiterate | 20 | 20 | 10 | 10 | c2=7.07 P=0.07 (NS) |
| | Primary education | 41 | 41 | 34 | 34 | |
| | Higher secondary education | 28 | 28 | 39 | 39 | |
| | Graduate | 11 | 11 | 17 | 17 | |

***Cont...*** **Table 1.1: Distribution of subjects in experimental and Control group as per their Socio-demographic Variables N=100+100**

| | | | | | | |
|---|---|---|---|---|---|---|
| 5. | Income | | | | | |
| | <15000 | 42 | 42 | 38 | 38 | c2=1.04 P=0.79 (NS) |
| | 15000-25000 | 38 | 38 | 36 | 36 | |
| | 25001-35000 | 16 | 16 | 21 | 21 | |
| | >35000 | 4 | 4 | 5 | 5 | |
| 6. | Marital status | | | | | |
| | Unmarried | 15 | 15 | 8 | 8 | c2=6.83 P=0.08 (NS) |
| | Married | 76 | 76 | 86 | 86 | |
| | Widow | 5 | 5 | 6 | 6 | |
| | Divorced | 4 | 4 | 0 | 0 | |
| | | F | % | F | % | |
| 7. | Employment | | | | | |
| | Employed | 18 | 18 | 22 | 22 | c2=2.83 P=0.24 (NS) |
| | Unemployed | 69 | 69 | 58 | 58 | |
| | Retired | 13 | 13 | 20 | 20 | |
| 8. | Other Co-morbid disease | | | | | |
| | Diabetes | 21 | 21 | 28 | 28 | c2=2.22 P=0.52 (NS) |
| | Hypertension | 39 | 39 | 30 | 30 | |
| | A & B both | 22 | 22 | 23 | 23 | |
| | None of above | 18 | 18 | 19 | 19 | |
| 9. | Previous knowledge | | | | | |
| | Newspaper/journals/mag. | 30 | 30 | 23 | 23 | c2=5.77 P=0.12 (NS) |
| | T.V. | 15 | 15 | 23 | 23 | |
| | Radio | 0 | 0 | 3 | 3 | |
| | Health personnel | 55 | 55 | 51 | 51 | |
| 10. | Duration of treatment | | | | | |
| | <6 month | 18 | 18 | 30 | 30 | c2=0.99 P=0.80 (NS) |
| | 6 month- 3 years | 43 | 43 | 42 | 42 | |
| | 3-6 years | 29 | 29 | 34 | 34 | |
| | >6 years | 10 | 10 | 11 | 11 | |
| 11. | Treatment per week | | | | | |

***Cont...*** **Table 1.1: Distribution of subjects in experimental and Control group as per their Socio-demographic Variables N=100+100**

| | | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 9 | 9 | 11 | 11 | c2=2.31 P=0.31 (NS) |
| | 2 | 54 | 54 | 62 | 62 | |
| | 3 | 37 | 37 | 27 | 27 | |
| | >3 | 00 | 00 | 0 | 0 | |
| 12. | Length of session | | | | | |
| | 3 hours | 8 | 8 | 18 | 18 | c2=4.46 P=0.11 (NS) |
| | 3 hours 15 minutes | 52 | 52 | 45 | 45 | |
| | 3 hours 45 minutes | 40 | 40 | 37 | 37 | |
| | >4 hours | 00 | 00 | 0 | 0 | |

**Table: 1.2: Comparison of pretest and posttest knowledge and practice score in experimental and control group**

| Group | Variables | Pretest (n=100) | | Posttest (n=100) | | Mean Difference | Student paired t-test |
|---|---|---|---|---|---|---|---|
| | | Mean score | Standard Deviation | Mean score | Standard Deviation | | |
| Experiment | Knowledge | 20.26 | 2.37 | 33.93 | 4.70 | 13.67 | t=26.46 P=01*(S) |
| Control | | 20.38 | 2.27 | 20.65 | 2.35 | 0.27 | t=1.89 P=0.06(NS) |
| Experiment | Practice | 35.46 | 2.99 | 57.21 | 2.51 | 21.75 | t=56.36 P=01*(S) |
| Control | | 35.89 | 2.22 | 36.82 | 2.74 | 0.93 | t=1.91 P=0.06(NS) |

P≤01 very high significant S=significant

P>0.5 is not significant

Table 1.2 depicts comparison between pre and posttest knowledge and practice score. In experimental group pretest and posttest knowledge score difference was 13.67, this difference was large. Hence it was statistically significant. Whereas in control group pretest and posttest, knowledge score was 20.65, so the difference was 0.27, this difference was small and it was statistically not significant.

Experimental group pretest practice score and posttest practice score difference was 21.75, this difference was large. Hence it was statistically significant. Whereas In control group, pretest practice score and posttest practice score difference was 0.93, this difference was small and it was statistically not significant.

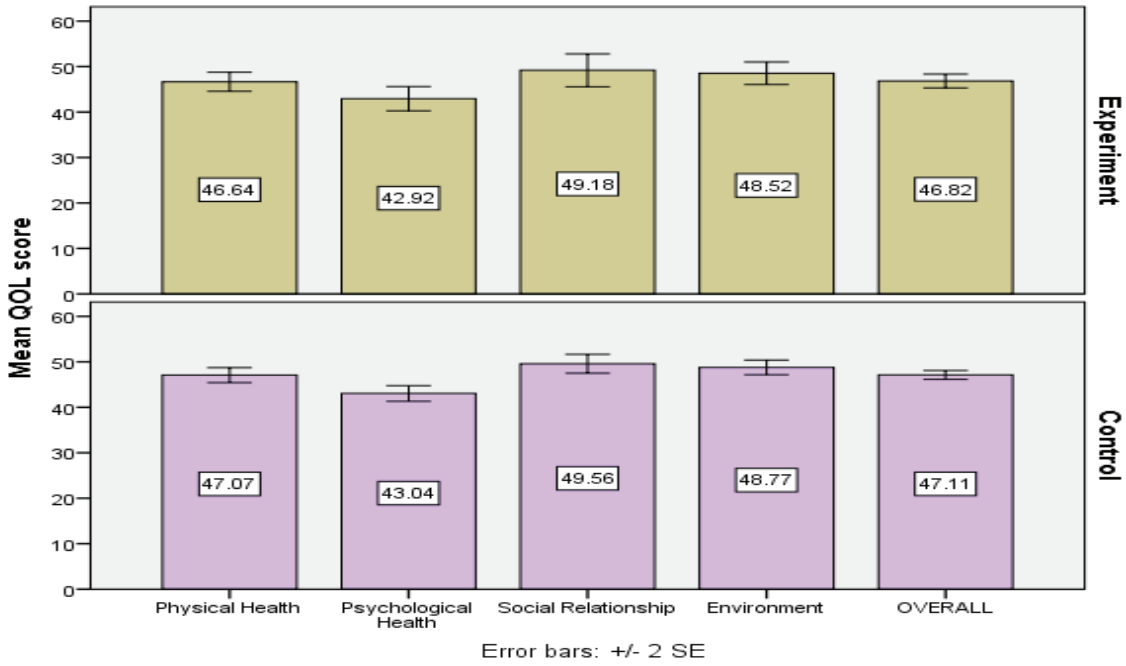


**Figure: 1:1: Comparison of pre-test quality of life scores in experimental and control group**

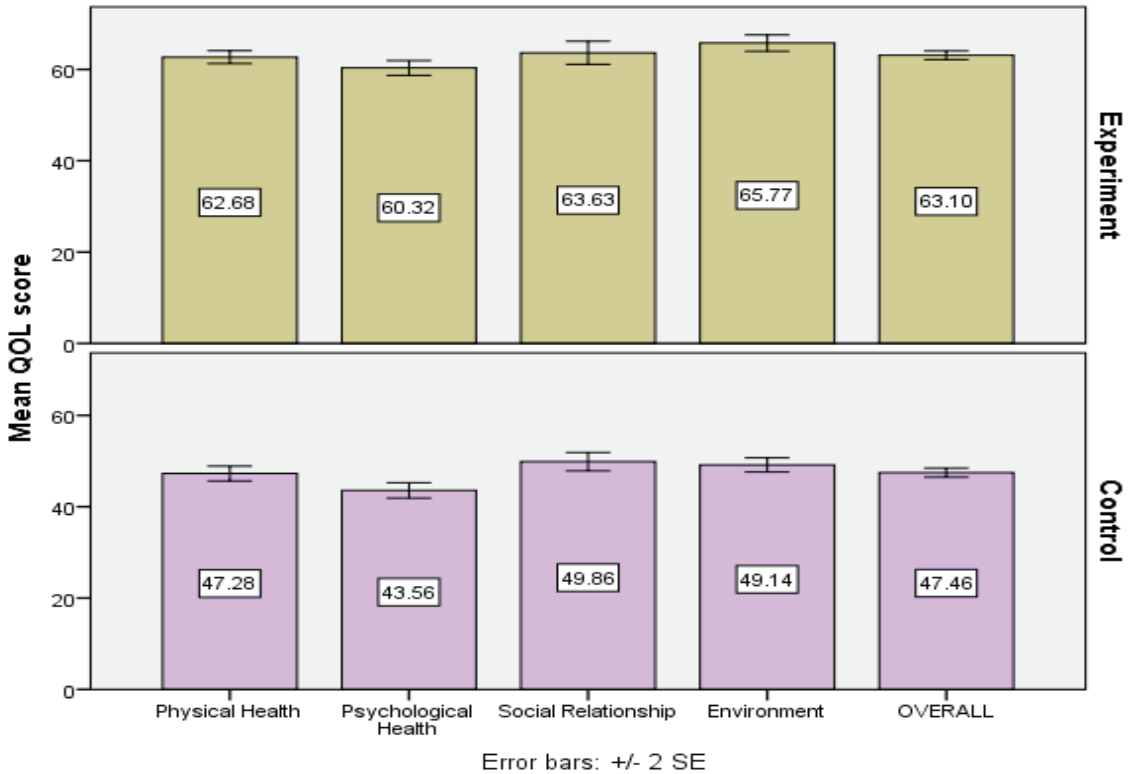


**Figure: 1:2: Comparison of post-test quality of life score in experimental and control group**

**Table 1.3: Effectiveness of health promotional life style intervention and generalization of QOL gain score**

| Group | Observation | Maximum score | Mean | % of mean score | mean difference with 95%CI | % of difference with 95%CI |
|---|---|---|---|---|---|---|
| Experiment | Pretest | 100 | 46.82 | 46.82% | 16.28 (14.58 -17.98) | 16.28% (14.58% -17.98%) |
| | Posttest | 100 | 63.10 | 63.10% | | |
| Control | Pretest | 100 | 47.11 | 47.11% | 0.35 (0.04 -0.74) | 0.35% (0.04% -0.74%) |
| | Posttest | 100 | 47.46 | 47.46% | | |

Table: 1.3 illustrates, in experiment group 16.28% improvement in QOL score after intervention and in control group improvement score was 0.35%. This difference shows the effectiveness of the study. Effectiveness of the study was given in mean with 95% confidence interval and percentage with 95% confidence interval.

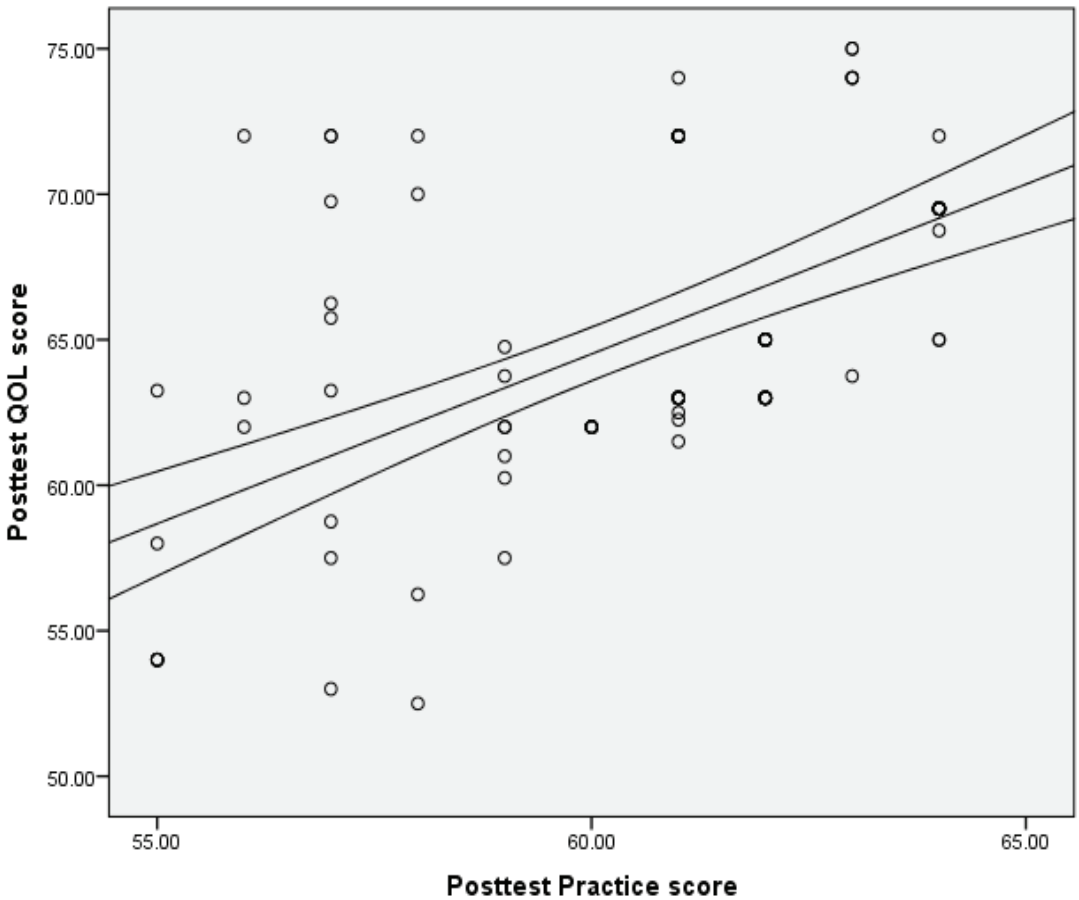


**Fig !:3: Scatter plot with regression estimate shows the moderate, positive correlation(r=0. 52 P≤01) between patients posttest practice score and posttest QOL score in experiment group.**

Considering correlation between patients posttest knowledge score and posttest practice score in experimental group there was a statistically significant, moderate positive correlation between them(r=0.44 P≤01.Considering correlation between patients posttest knowledge score and posttest QOL score, there was a statistically significant, moderate positive correlation between them(r=0.45 P≤01) and correlation between patients posttest practice score and posttest QOL score, there is a statistically significant, moderate positive correlation between them(r=0.52 P≤01).

The association between post-test knowledge score and demographic variables among experimental group are age, Gender, education and occupational status had association with their demographic data, and control group none of the variable is significantly associated with demographic variables.

The association between post-test practice score and demographic variables among experiment group and control group are age, education and other co-morbid conditions had associated with their demographic data and in control group none of the variable is significantly associated with demographic variables.

The association between post-test QOL score and demographic variables among experimental and control group. Age education and income of the patients had associated with their demographic data and control group none of the variables is significantly associated with demographic variables.

## Discussion

Hemodialysis is an expensive treatment modality for chronic renal failure patients, it is very essential to assess the outcome of therapy in terms of quality of life through life style modification. Nursing knowledge has been guided toward helping the individuals, family and society for the purpose of achieving maximum level of health. Hence the aim of the study to enhance quality of life is by reducing complications and co morbidities.

This finding coincides with the findings of **Dulal S L, Thakurathi MT, et.al** conducted study on dietary practice among the patients with end stage renal disease undergoing maintenance hemodialysis. The level of knowledge score found to be medium and practice score was even low. Considerable limited knowledge (medium) and practices (low) scores were found. Similarly showed in present study and after intervention and followed up increase knowledge and correct practice towards quality of life.

This finding also coincides with the findings of **Mukadder Mollaoğlu** on Quality of Life in Patients Undergoing Hemodialysis. The results of studies suggest that the QOL of hemodialysis patients is considerably impaired compared to that of the healthy subjects, especially with respect to the physical, psychological and social relationship domains. Studies have shown that patients on hemodialysis have a poor health-related quality of life (HRQoL) and present with complications such as depression, malnutrition, and inflammation. Many of them suffer from impaired cognitive 1a functioning such as memory loss and abnormally low concentration, as well as other unhealthy physical, mental, and social aspects of life that can, and do, affect even the simplest activities of daily life. Thus its essential to enhance quality of life of CKD patients with life style modification.

## Conclusion

The health promotional life style intervention on prevention of co-morbid condition and complications among chronic renal failure patients undergoing hemodialysis was effective in improving their knowledge, practice and quality of life. It helps the subjects to adhere to diet plan and exercise regularly. The health promotional life style intervention was

effective to helping them to control comorbid conditions and live quality of life.

**Ethical Clearance**: Ethical clearance taken from institutional ethical committee.

**Conflict of Interest**: Nil

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# A Study on Determinants of Irritant Contact Dermatitis in the Workers of a Slaughterhouse on Jalan Abu Bakar Lambogo, Makassar

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## Abstract

Contact dermatitis, in general, is a non-infection inflammatory condition because the skin has contact with both chemical and biological compounds. The aims of this study is to analyze determinants of irritant contact dermatitis in the workers of a slaughterhouse. This study was analytical observation using a cross-sectional approach. The population of this study was 93 workers of a broiler chicken slaughterhouse located on Jalan Abu Bakar Lambogo, Makassar. Data collection was by performing a direct observation and interview to the skin condition of the workers' hands who were suffering from the symptoms of irritant contact dermatitis, such as skin rash, dry skin, itchy and painful skin, swollen skin, and chapped, and observing the work process of chicken slaughtering. Data were analyzed using multiple logistic regression analysis. The result of this study shows that the use of PPE, personal hygiene, length of employment, personal history of diseases of the skin, and duration of contact are significantly correlated with ($p < 0.05$) the incidence of irritant contact dermatitis symptoms. Based on the result of the multiple logistic regression analysis, personal hygiene ($p = 0.003$) and duration of contact ($p = 0.004$) are the main determinants of irritant contact dermatitis symptoms in the workers of the broiler chicken slaughterhouse. It can be concluded that the personal hygiene and duration of contact are the main determinants of irritant contact dermatitis symptoms in the workers of the broiler chicken slaughterhouse.

***Keywords:*** *irritant contact dermatitis, personal hygiene, duration of contact*

## Introduction

Work-related diseases still become a health issue as a concern for stakeholders, the government, and entrepreneurs. Occupational diseases are the disease caused by an occupation and work environment. In Asia, work-related dermatitis contributes highly and significantly to occupational diseases felt by the majority of the workers in the formal and informal sectors[(1)]

Occupational skin disease with the highest proportion felt by people is contact dermatitis (92.5%), around 5.4% is caused by skin infection and 2.1% is caused by other factors. Globally, in more

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than two decades, the incidence rate of occupational dermatitis reaches around 1.3 to 8.1 per worker annually[2] Occupational contact dermatitis is a skin disease caused by work that is widely found in worker populations with a prevalence of 70 – 90 %[3]

Contact dermatitis, in general, is a non-infection inflammatory condition because the skin has contact with both chemical and biological compounds. Contact dermatitis is divided into two types. First, irritant contact dermatitis is a non-immunological response to substances or factors that worsen the condition, such as damp workplace, soap, or liquid triggering a hot condition in the skin. Second, allergic contact dermatitis is caused by specific immunological mechanisms[4]

The prevalence of dermatitis in Indonesia is 6.78%. An epidemiological study in Indonesia shows that 97% out of 339 cases is contact dermatitis, whereby 66.3% of them is irritant contact dermatitis([5]. A study conducted by Fath on the workers of a poultry slaughterhouse showed that contact dermatitis happened due to several factors, such as length of employment, personal hygiene, the use of personal protective equipment (PPE), and water contact[6]. Meanwhile, the result of a study conducted by Marwah on daily freelance workers at PT. Indojaya Agrinusa, Medan, showed a significant relationship between the length of employment (p=0.003), personal history of diseases of the skin (p=0.0001), and the use of PPE (p=0.033) against the symptoms of contact dermatitis[7]

One of the industries having a risk of contact dermatitis symptoms is a poultry slaughterhouse. The broiler chicken slaughterhouse industry located on *Jalan Abu Bakar Lambogo*, Makassar, is one of the traditional slaughtering areas (home industry). The flow of a simple work process starting from weighing to non-hygiene slaughtering has a risk of emerging irritant contact dermatitis in workers. The preliminary study showed that the majority of workers complained about feeling itchy and hot, especially in the space between fingers while working. Therefore, a study on determinants of contact dermatitis symptoms in the workers of a broiler chicken slaughterhouse located on *Jalan Abu Bakar Lambogo*, Makassar, needs to be conducted. The specific objective of this study was to know the relationship between the use of Personal Protective Equipment (PPE), personal hygiene, length of employment, personal history of diseases of the skin, and duration of contact with contact dermatitis symptoms in the workers of the broiler chicken slaughterhouse and to know the main determinants of irritant contact dermatitis symptoms in the workers of the broiler chicken slaughterhouse.

## Material and Methods

This study was an analytical observation using a cross-sectional approach in 93 workers of a broiler chicken slaughterhouse located on Jalan Abu Bakar Lambogo, Makassar. It was analyzed from the dependent variable, namely the symptoms of irritant contact dermatitis, and the independent variables in the form of the use of personal protective equipment (PPE), personal hygiene, length of employment, personal history of diseases of the skin, and duration of contact. This study was conducted from July to September 2021.

Data collection was by performing a direct observation to the skin condition of the workers' hands who were suffering from the symptoms of irritant contact dermatitis, such as skin rash, dry skin, itchy and painful skin, swollen skin, and chapped, and observing the work process of chicken slaughtering. In addition, an interview with the workers was done according to the questions provided in the questionnaire. The questionnaire contained several questions related to research variables, namely the questions related to the symptoms of irritant contact dermatitis, length of employment, personal history

of diseases of the skin, personal hygiene, duration of contact, and the use of personal protective equipment (PPE). To analyze the most influential variable as a determinant, multiple logistic regression analysis was used, whereby a p-value of less than 0.05 was considered as the most influential and significant variable statistically.

## Findings

**Table 1 The Relationship between Research Variables and the Symptoms of Irritant Contact Dermatitis in the Workers of a Broiler Chicken Slaughterhouse on *Jalan Abu Bakar Lambogo*, Makassar**

| Variable | Total (93) | % | Sig |
|---|---|---|---|
| The Use of PPE | | | |
| Poor | 87 | 93.5 | 0.018 |
| Good | 6 | 6.5 | |
| Personal Hygiene | | | |
| Poor | 62 | 66.7 | 0.000 |
| Good | 31 | 33.3 | |
| Length of Employment (years) | | | |
| ≥ 3 | 39 | 41.9 | 0.023 |
| < 3 | 54 | 58.1 | |
| Personal History of Diseases of the Skin | | | |
| Yes | 24 | 25.8 | 0.019 |
| No | 69 | 74.2 | |
| Duration of Contact (hours/day) | | | |
| ≥ 8 | 82 | 88.2 | 0.000 |
| < 8 | 11 | 11.8 | |
| The symptoms of Irritant Contact Dermatitis | | | |
| Experiencing | 73 | 78.5 | |
| Not Experiencing | 20 | 21.5 | |

The result of this study can be seen in table 1. Table 1 shows that 73 respondents have the symptoms of irritant contact dermatitis (78.5%). The use of PPE in the respondents is mostly poor APD of 87 respondents (93.5%), and 62 respondents (66.7%) have poor personal hygiene. The majority of the respondents' length of employment is less than 3 years with a total number of 54 respondents (58.1%). Most of the respondents do not have a personal history of diseases of the skin (74.2%), and 82 respondents (88.2%) have a duration of contact greater than or equal to 8 hours.

**Table 2. The Symptoms of Irritant Contact Dermatitis Perceived by the Workers of A Broiler Chicken Slaughterhouse on Jalan Abu Bakar Lambogo, Makassar**

| No | Symptoms | n | % |
|---|---|---|---|
| 1 | Skin Rash | 60 | 27.8 |
| 2 | Dry Skin | 23 | 10.7 |
| 3 | Itchy and painful skin | 72 | 33.3 |
| 4 | Swollen Skin | 18 | 8.3 |
| 5 | Chapped Skin | 43 | 19.9 |

The symptoms of irritant contact dermatitis was statistically and significantly correlated with the use of PPE (p = 0.018), personal hygiene (p = 0.000), length of employment (p = 0.023), personal history of diseases of the skin (p = 0.019), and duration of contact (p = 0.000) (See Table 1). Meanwhile, the symptoms of dermatitis mostly experienced by the workers were itchy and painful skin (33.3%), skin rash (2.8%), chapped skin (19.9%), dry skin (10.7%), and swollen skin (8.3%). (See Table 2).

**Table 3. The Result of the Multiple Logistic Regression Analysis**

| Variable | B | Wald | Df | Sig |
|---|---|---|---|---|
| The use of PPE | 2.731 | 1.274 | 1 | 0.032 |
| Personal Hygiene | 2.576 | 0.856 | 1 | 0.003 |
| Length of Employment | -1.179 | 0.739 | 1 | 0.110 |
| Personal History of Diseases of The Skin | 1.950 | 1.363 | 1 | 0.152 |
| Duration of Contact | 3.91 | 1. 357 | 1 | 0.003 |
| Constant | -14.261 | 10.546 | 1 | 0.001 |
| Nagelkerke R Square = 0.590<br>Hosmer-Lemeshow goodness of fit test = 0.605<br>Overall percentage = 0.785 | | | | |

Based on the result in Table 3, it can be seen that the Nagelkerke R Square value is 0.590, indicating that the ability of independent variables to explain the dependent variables is 0.590 or 59%, whereby 41% of them is the factor outside the equation model explaining the dependent variables. The Hosmer-Lemeshow goodness of fit test (GoF) value of 0.605 (p > 0.05) indicates that the equation model that has been performed is correct since there is no significant difference between the models and the observation value. The overall percentage of 0.785 indicates that the accuracy value of this research model is 78.5%.

Based on the multiple logistic regression analysis, three variables had a significant impact, namely personal hygiene, duration of contact, and the use of PPE. The contribution value of each independent variable can be seen from the B value. The values are as follows: personal hygiene of 2.576, duration

of contact of 3.92, and the use of PPE of 2.731 (See Table 3). From the result, it can be inferred that the variables with the highest contribution are the duration of contact, personal hygiene, and the use of PPE. The following is the regression equation model that can be calculated.

**"The symptoms of irritant contact dermatitis = 14.261+2.576 x personal hygiene + 3.92 x duration of contact + 2.731 x the use of PPE"**

## Discussion

The result of the study showed that 73 respondents (78.5%) suffered from irritant contact dermatitis and the symptoms frequently felt by the respondents were itchy and painful skin and skin rash on their hands. The industries with a high risk of being exposed to this disease are industries in the manufacturing sector, food production, construction and machine tool operations, printing, leather craftsmen, and agriculture. The workers in the informal sector, such as a chicken slaughterhouse, are prone to the symptoms of irritant contact dermatitis, whereby the production process has some dangers in the form of chemical exposure, such as chlorine, and biological exposure in the form of bacteria in a chicken carcass, namely *Staphylococcus* and *Salmonella sp.* The sources of bacterial contamination in chicken carcasses encompass feces, water, feathers, digestive system, workers, and equipment used for slaughtering. The stages of the process, such as scalding, chicken feather removal, evisceration, and cleaning, have a significant impact on bacterial contamination.

In this study, personal hygiene had a significant impact on the symptoms of irritant contact dermatitis. The workers' behavior of the chicken slaughterhouse that became the factor causing the symptoms of irritant contact dermatitis was not washing hands using flowing water and soap; instead, they only washed their hands using water in the provided tub or container. Therefore, the bacteria in the container of dirty water penetrate the workers' skin and cause the symptoms of irritant contact dermatitis. Proper and correct hand-washing is performed by using soap since using only water is proven ineffective [8]. The result of this study is in line with a study conducted by Callahan et al. that hand-washing behavior (≥ 10 times a day) is correlated with irritant contact dermatitis affecting the hands [9].

The duration of contact statistically and significantly influenced the symptoms of irritant contact dermatitis. The result of the observation toward some workers with the symptoms of irritant contact dermatitis showed that it was caused by having direct contact with irritant substances and most workers had direct contact with irritant substances for around ≥ 8 hours/day resulting in inflammation to the skin and the workers suffered from irritant contact dermatitis. A study conducted by Behroozy & Keefel stated that working in a damp/wet workplace (dipping hands in the water) for around > 2 hours per work hour is the main risk factor of suffering irritant contact dermatitis that affected the hands [2]. The workers with a duration of contact of ≥ 8 hours were potentially infected with irritant contact dermatitis due to the exposure of irritant substances that continuously come into direct contact with the workers' skin causing inflammation and stimulating action in the skin making the workers' hands feel itchy, skin rash, thickening of the skin, swollen skin, and blisters or vesicles (small bumps filled with clear liquid) on the skin; all of them are the symptoms of irritant contact dermatitis.

The result of this study showed a significant impact of the use of PPE on the symptoms of irritant contact dermatitis. Based on the observation, the majority of the workers did not use the PPE in the form of a pair of rubber gloves. This happened due to several reasons. Based on the result of the direct interview with the respondents, the first reason was

that the respondents did not feel comfortable when wearing PPE while slaughtering chickens. They felt bothered if something was obstructing their hands during the process of slaughtering poultries. The second reason was that their actions were limited and they could not move freely when performing the job. The result of this study is in line with a study conducted by Birawida et al. that the use of PPE is significantly correlated with the incidence of contact dermatitis symptoms in fishermen in Spermonde Island, Makassar [10]

The multivariate analysis in the multiple logistic regression test showed a result that personal hygiene and duration of contact became the highest determinant variable for the symptoms of irritant contact dermatitis in the workers of a chicken slaughterhouse on *Jalan Abu Bakar Lambogo*. This result is in line with a study conducted by Iwan et al. that personal hygiene and duration of contact are the most influential variable with a significant impact on the incidence of irritant contact dermatitis caused by a job in ship workers in Samarinda, Indonesia[11]

The result of this study shows that the use of PPE, personal hygiene, length of employment, personal history of diseases of the skin, and duration of contact are significantly correlated with the incidence of irritant contact dermatitis symptoms. Based on the result of the multiple logistic regression analysis, personal hygiene and duration of contact are the main determinants of irritant contact dermatitis symptoms in the workers of the broiler chicken slaughterhouse located on Jalan Abu Bakar Lambogo, Makassar. The workers are expected to always apply good personal hygiene, especially when they have had contact with irritant substances for a long term. Moreover, the owner of a chicken slaughterhouse business is expected to provide complete PPE and supervises the use of PPE by the workers while performing their job.

**Conflict of Interest:** None

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**Ethical Clearance:** None

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# *myc* Gene and Cancer Variant Analysis and Network Interaction: An In-Silico Analysis

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## Abstract

The *myc* is a proto-oncogene that regulates the cellular process like cell growth, proliferation, metabolism, apoptosis and malignancy pathologies. *myc* deregulation occurs in several cancers and plays a significant role in disease development, metastatic potential, and therapeutic resistance. Drug targeting *myc* in cancer remains highly desirable with substantial challenges, delaying the direct *myc* targeted drugs's development. These drugs target the *myc* transcriptions deregulations in tumours cell by compound inhibiting the inducing of epigenetic silencing or regulation of G-quadruplex structures within the *myc* promoter. Proteins involved in *myc*'s post-translational modulation have been identified as significant surrogate targets for lowering *myc* activity downstream of aberrant cell stimulatory signals.

***Keywords:*** *myc gene, in-silico analysis, gene expression, transcription*

## Introduction

*Myc* gene is located on chromosome 8 with a size of ~6000 bases and an isomer of ~ 7500 bases, and it is considered as a proto-oncogene that translated into a nuclear phosphoprotein. This gene belongs to a gene family that consists of three related human genes: c-myc (MYC), l-myc (MYCL), and n-myc (MYCN) [1]. The discovery of the *myc* gene to recognize protein expression and mRNA is associated with cell proliferation rates. Around 15% of gene expressions are regulated by transcription factors, *myc* genes [2]. It also regulates cellular functions like normal cell proliferation, differentiation, growth, metabolism, apoptosis and malignancy pathogenesis. The proliferation and cell growth increase due to *myc* overexpression by regional gene amplification or chromosomal translocation. Finally, intense *myc* enhances the efficacy of induced pluripotent stem cell generation [3]. A ubiquitin-proteasome pathway regulates the degradation of *myc* gene levels in normal cells [4]. However, cellular *myc* is activated and overexpressed in breast cancer, colon cancer, prostate carcinoma, ovarian tumours, lung cancer and other hematopoietic malignancy. The overexpression and amplification of cellular *myc* provide poor clinical outcomes in 20% of human cancer that subsequently aggravates metastatic phenotype and increased relapsed risk [3,5,6].

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The enhanced transcriptional amplification by overexpression of *myc* cause increased protein and mRNA level by a diverse set of genes [7]. The *c-myc* regulates the cell cycle at the G1/S phase checkpoint, and it also produces ROS in mitochondria that leads to genetic instability and DNA damage. The uniform quantity of *c-myc* protein regulates the production of the c- *myc*. The life span of *c-myc* lasts around 20-30 minutes [8]. Lie et al. studied the significant effect of an ideal *c-myc* amount to prevent tumorigenesis [9]. The P1 promoter regulates 80-90% of transcription of normal *c-myc* and the rest of oncogenic *c-myc* oncogene. However, nuclease hypersensitivity element III1 (NHE III1) regulates the P1 promoter. The cancer therapy is achieved by targeting the G-quadruplex of NHE III1 regions of the *c-myc* promoter. Thus, ration drug development requires a virtual screening method that targets triplex-binding ligands and G-quadruplex binding ligands [10]. The proto-oncogene *myc* product is a heterodimeric transcription factor categorized under a subunit of basic helix-loop-helix and leucine zipper (bHLHZip). The complex is the result of the association of bHLHZip with *max* that blocks or activates transcription. The *myc* and *max* gene association with their receptor binding results in the *myc* transcription network [11].

The genome-wide analysis demonstrated that *myc* bind with gene promoter with different enhance affinities. The *myc* expression levels determine the number and functional class of the gene. The low proliferating cell has a lower affinity and *myc* protein number, while the tumours cell has higher affinity *myc* proteins regulated by *myc* inductions. Instead, additional *myc* increment regulates the gene with lower affinity at the binding cell where the cells are already saturated with higher affinity binding sites with moderate-high *myc* concentrations. Transcriptional amplification is achieved at a higher *myc* level [12,13].

Genome advanced technology has introduced the process of identifying the molecular mechanism of genes in all the areas of cancer clinical research. A unique type of fingerprints in all cancer is due to intertumoral heterogeneity, dynamics genome changes and genetic abbreviations [14]. Risk stratification and prognostic subtypes of cancer better understand gene expression profiling, an equivalent way for prognosis and therapy sensitivity in cancer. Thus, gene expression profiling is the best method to foresee drug sensitivity [15,16]. The International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) are the catalogues that determine the genetic alteration in different cancer types. This catalogue provides protein expression, gene expression, DNA mutations, clinical data and DNA methylation and assists the researcher in validating hypothesis that eventually helps in the development of diagnostic methods, preventative methods and novel cancer therapy [17,18]. However, researchers without the knowledge of computational programming and insufficient guidance face challenges for the integration, exploration and analysis of a large volume of complex data. However, these open-access databases facilities limited studies of complex data analysis but may be limited when exploring the fails to provide much deeper analysis. Practical data analysis and subset genetic analysis can be performed with data visualization tools (Table1) [19].

**Table 1: Summary of open access portal with a visualization tool and subset genomics analysis.**

| **Analysis of genomics data using the open-access portal and visualization tools** | |
|---|---|
| Source | Visualizing tool. |
| https://cgwb.nci.nih.gov/ | Cancer Genome Workbench (CGWB) |
| https://gdac.broadinstitute.org/ | The Broad GDAC Firehose |
| https://xenabrowser.net/ | UCSC Xena |
| https://portal.gdc.cancer.gov/ | Genomic Data Commons Data Portal |
| https://canevolve.org/ | Can Evolve |
| http://cbioportal.org/ | The cBio Portal for Cancer Genomics |
| Overview of a profiling data type with genomics data | |
| Data | Genomic expression type |
| RNA-seq Tumor RNA | Gene expression |
| Reverse-phase protein array | Protein expression |
| Methylation | DNA methylation |
| Category of biomarker-based on the risk of cancer | |
| Penetrance level | The relative risk of cancer development |
| Low | 1.00 ≤1.5 |
| Moderate | 1.5 ≤ 5.0 |
| High | >5.0 |

The graph plot between gene expression regulators and target gene nodes and interaction edges is referred to as gene regulatory network (GRN). However, establishing such a relation is a real challenge in experiment and computational biological studies. Gene regulation expression is governed by transcription factors (TF). The biological process of cell growth and proliferation to development and differentiation leads to understanding by determining the regulatory relationship between transcriptional regulators and their target. GRNs reconstruction determines complex heritable disease and cancer due to dysregulation of gene expressions. The gene regulatory relationship is effectively identified based on genome-scale technique. The continuous effort of the last 20 years has led to the development of computational and experimental processes to support the technical and machine learning advancement for designing GRNs. This method describes the observational gene expression level by the relationship existing between TF and its target with the inputs of gene expression data [20].

Population frequency and penetrance are the essential criteria for categorizing genetic biomarkers for cancer risk. Penetrance is the presence of a specific

genotype that can develop cancer, which considers a cancer predictive biomarker that is classified into several levels (Table 1); also, it can be cancer-specific (e.g., colon or breast cancer). However, one risk may contribute to numerous cancer developments [19].

Gene mutation of the specific gene may lead to high penetrance among the population [21,22]. For instance, the most widely recognized high penetrance susceptibility gene, *brca1* and *brca2*, is mutated and inherited to increase the relative risk for ovary and breast cancer. Additionally, the lifetime risk of developing breast and ovarian cancers increases from 45% to 85% and from 20% to 55%, respectively, due to the mutation of *brca1* [21-23]. Genes like *chek2*, *atm*, *brip1* and *palb2* have been identified to moderate breast cancer risk. The *chek2**1100delC is a mutated gene with twice the frequency for the general population to develop breast cancer (odds ratio 2.34, 95% CI 1.72-3.20), which is more common in women with a family history of breast cancer [24,25]. However, this mutated gene's overall performance does not diversify with the outcome of a meta-analysis demonstrating that lifetime breast cancer risk is 37% (95% CI 26-56%) by age 70 [26]. The *myc* is a proto-oncogene encoding nuclear phosphoprotein and forms a heterodimer associated with transcription factors *max*. This complex is associated with the E-box DNA sequence regulating specific target gene transcription. Moreover, gene amplification and gene translocation may lead to several human cancer like multiple myeloma and Burkitt lymphoma.

For these reasons, the current study aimed to use In-silico computational analysis to understand the role of the *myc* gene in cancer by evaluating gene expression level, mutation, and genes and proteins interaction. Also, it aimed to determine a list of drugs and components that can be used as targeted *myc* gene therapy for cancer patients.

## Materials and Methods

The GRCh38.p13 long promoter sequence of human gene chr8:127,735,434-127,742,951 (GRCh38/hg38); chr8:128,747,680-128,755,197 (GRCh37/hg19 by Entrez Gene); and chr8:128,747,680-128,753,674 (GRCh37/hg19 by Ensembl) were obtained using the human genes card human database, which provides detailed information about myc genomic, proteomic, transcriptomic, genetic, and functional. The major aim of this step is to obtain further information about myc phenotypes that can be incorporated in gene expression and function .

The promoter sequence was analysed using six databases COSMIC, Ensembl, gene cards, GTEx portal SAP HANA proteomics and Gepia, to provide comprehensive and updated information on predicted transcription factors (TFs) experimentally. Also, these databases help in studying various features of *myc* overexpression.

The current research also provides a comprehensive overview of the cellular cell's genomic location, the distribution of *myc* gene and its expression at the primary normal tissue and various cancer showing association of *myc* mRNA expression predicted novel therapy targeting *myc* gene function. Additionally, an overview of the types of mutation observed in cancer was determined using the Somatic Mutations in Cancer (COSMIC) database (release version 94; http://cancer.sanger.ac.uk/).

Highly complex *myc* gene interaction with other genes was evaluated using STRING, a web-based tool for retrieving interacting genes (http://string-db.org/). Additionally, myc cellular functions were conducted by tightly interacted groups of proteins encoded by genes using Cytoscape (http://cytoscape.org), the protein-protein interaction (PPI) networks of myc were constructed.

List of Drugs for *myc* gene were also determined using online databases including DrugBank, ClinicalTrials, ApexBio, DGIdb, and Novoseek

The current study did not require ethical approval from the institutional review board as it utilizes publicly available dates.

## Results and Discussion

Around 10% of human gene expressions are affected by *myc* transcriptions that interfere with both the proliferation and cell growth sequentially could lead to cancer. The mRNA expression level of *myc* resulting from GeneCard indicated that the overexpression of the *myc* gene was observed in blood tissue followed by the bone marrow, brain, head, intestine, jaw, lymph nodes, mandible, ovary, pelvis, spinal cord, and WBC while least tentative organs are the wrist, vertebra, urinary bladder, ulna, teeth, toes. However, normal gene expression across the various organ. Similarly, the most confident systems for overexpression of *myc* are digestive, immune, lymphatic, nervous, reproductive, and skeleton, while the least tentative systems are urinary, skeletal muscle, respiratory, integumentary, endocrine, and cardiovascular. The most confident body regions with higher expression of the *myc* gene are the abdomen, pelvis, head, and neck, while limbs and thorax have the lowest tentative *myc* expression. However, ectoderm, mesoderm and endoderm have the confident *myc* overexpression [27-30].

The *myc* gene is one of the first identified oncogenes and the most altered one in a cancer cell that acts as a proto-oncogene and governs gene expressions over several targets [31,32]. The differential RNA expression screen human HT29 colorectal cancer cell-line poses mutant adenomatous polyposis coli alleles demonstrated Wnt/β-catenin target gene, *c-myc* [33]. The cellular behaviour changes with abnormal *myc* expression were studied in the transgenic animals and cultured cells and found that it regulates several metabolic pathways, genome instability and DNA damage induction [34,35]. In-vivo studies of *myc* gene demonstrated that tumorigenesis is promoted with angiogenesis and tumour-cell invasiveness [36,37]. Despite, *myc* damage results in cell growth limitation, impairment in apoptosis and/or proliferation [38]. *myc* is transcription factors that associated with bHLHLZ and Max with DNA direct binding to E-box. Also, binding to the CACGTG DNA sequence is responsible for activating or repressing the *myc* gene expression. However, the functional interference with Miz-1, a transcriptional factor, cause gene repression.

The first recognized human oncogene and altered gene in tumours was myc, which controls cellular biology like cell growth, apoptosis, proliferation, and metabolism with several target genes [39]. Its cellular function controls both the transcriptional and post-transcription regulatory mechanism that ensures the regulation of the protein level in proliferating cells. Several genetic mutations or epigenetic changes control its expression, which may lead to gene overexpression and promote oncogenesis in different cells, cell lines, and a transgenic model. Additionally, the *myc* cause overstimulation of cell division and cellular metabolism, which may lead to genetic instability by increasing DNA damage with enhanced mutation, gross chromosomal reorganization. This persuades inappropriate cell division that finally endorses tumorigenesis [40,41].

Generally, *myc* regulates cell proliferation and genomic instability in both cancer and normal cells. However, DNA replication and its initiation are regulated at late mitosis/early G1 phase, the transition of G1 to S phase and associated with epigenetic modification and altering adjacent chromatin. Any modification during these steps results in a significant alteration in the cell cycle and the fate of the cell. The unscheduled DNA synthesis, genome

instability and dysregulated checkpoint activation results due to alteration of the previously mentioned steps. Thus, *myc* regulates the critical gene for cell division and consider as a mastermind controlling cell proliferation. Additionally, *myc* gene directly unexpectedly regulates DNA replication but is evidently based on the non-transcription process [42,43], especially by regulating the mechanism the control the overexpression of *myc* gene at the cell division phase. First, DNA replication should undergo once per cell division. Secondly, DNA replication errors should be regulated to check the degree of DNA damage and stop such replication until the error gets corrected. Such regulatory mechanism includes cell process as "checkpoint response". The checkpoints like "ATM/Chk2 and ATR/Chk1 kinase pathways" immediately activate to avoid the errored cell progression and provide sufficient time to repair its DNA. The upstream regulation of checkpoints like ATM and ATR and their function depends on upstream kinase and proteins like p53, Chk1, and Chk2 [44-46].

However, DNA replication should be continued without intrusion with checkpoints for the normal cells to differentiate as cancer cells following genomic instability. The proteins are linked with the *myc* gene and expression of other genes in quiescent cells, for an instant, the association of *myc* with p300 and CBP. This association characterised nuclear phosphoproteins that function as transcriptional co-activators in various cell lines and brings innate enzymatic activity to acetylate histone proteins with chromatin remodelling by histone acetyltransferase. Additionally, CBP and p300 suppression induce *myc* for the DNA synthesis despite the deficiency of serum cell line culture or lack of growth factors stimulation. In addition, adenovirus E1A or SV40 are convention DNA tumours suppression virus that causes cellular transformation by direct binding and inducing *myc* overexpression and deactivates p300/CBP. Thus, these DNA viruses induce the S phase in the resting cell despite the provision from external mitogens[47,48]. The early DNA replication in p300 suppressed the cell to aid the cell division towards the S phase from the G1 phase. This leads to the accumulation of such cells at S phase without proceeding forward to G2 results in apoptosis. However, elevated *myc* synthesis is the result of serum addition without p300/CBP cause blockage of such proliferation at the S phase and inhibiting proceeding to the M phase [49]. Thus, it is summarised as an S phase block with a lack of G/M phase, replication stress due to inappropriate stimulated action of DNA replication by *myc* cause DNA damage responses, signifying the role of p300 proteins in maintaining genome stability.

Besides *myc* gene expression in all tissue (as in figure 1) inducing transcription, *myc* interacts and blocks several genes (figure 3) that are involved in cell-cycle progression and cell adhesion [50]. Also, *myc* block the transcription by interacting with co-repressor and transcription factors such as, SP1 and/or MIZ-1 at the gene promoter regions. The two transcriptions individually activated transcription where it inhibits the transcription when combined with *myc*. The *myc* -MIZ-1 complex in association with DNA methyltransferase blocks transcription [51].

Moreover, *Myc* stimulates cell division by the repression of cell cycle inhibitors. Serine/threonine-protein kinase regulated cell cycle progression that is composed of a catalytic subunit and regulatory subunit cyclin, CDK (cyclin-dependent protein kinase). The major regulators of the mammalian cell cycle are CDK 1,2,4 and 6 with cyclin types A, B, D, and E. The release of E2F transcription factor and retinoblastoma protein phosphorylation occurs at the early cell cycle G1 phase due to binding and activation of CDK4 and CDK6 by cyclins-D. The retinoblastoma protein phosphorylated at late G1 phase with cyclin E1/2-CDK2 complex required for S phase transition due to expression of the E2F target gene. Cyclin A complex

with CDK2 is required for DNA replication that is expressed through S and G2 phases. Cyclin B activates CDK1 that regulating M phase.[52,53] The regulation of the cell cycle accomplishes CDK inhibitory proteins (CKIs). The CDK4 and CDK6 kinase is inhibited by the binding INK4 family that impair the binding to cyclins D. Each cell-cycle progression is inhibited by CIP when binding to the cyclin-CDK complex [54].

The cell cycle is arrested at G1 and G2 phases by ARF. Similarly, ARF/MDM2/p53 regulates apoptosis through the induction of the P53 apoptotic pathway mediated by ARF. The programmed cell death mechanism is retracted for the proliferation in the absence of growth factors where *myc* overexpression is subjected to selective high pressure. The MDM2 inactivation at the mRNA cause ARF induction to regulate *myc* induced apoptosis. This cause activation and stabilization of p53 that in turn results induction of p21 and other protein for p53 dependent apoptosis [55].

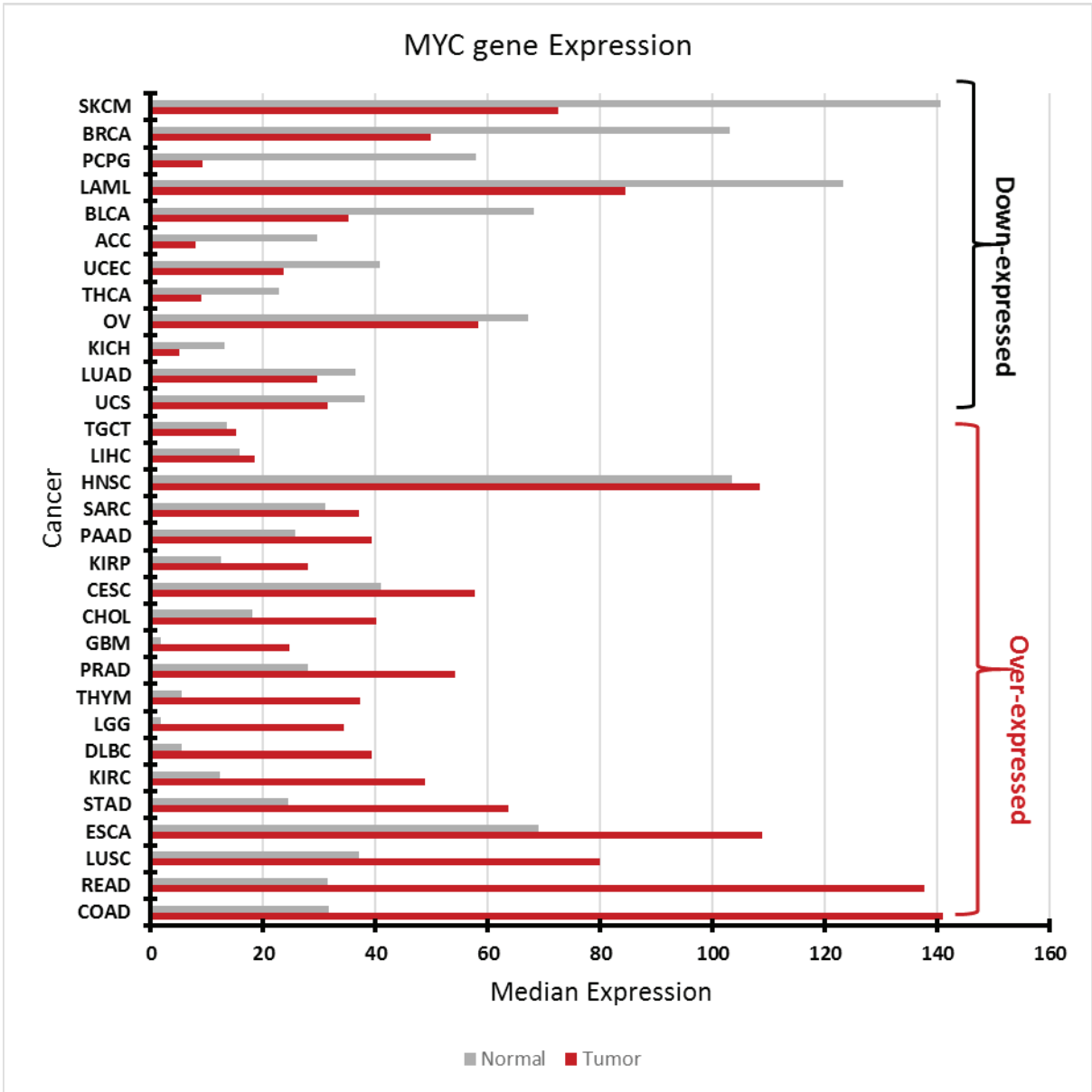


**Figure 1: The *myc* gene expression profile across all tumour samples and paired normal tissues, Data is generated from GEPIA database. The height of bar represents the median expression of the gene.**

Human genome sequence measures mapped gene position and demonstrated the genetic variation among individuals. Single nucleotide polymorphism (SNPs) is available in the genome with more than 106 DNA sequence variations between 2 different individuals that make genome-wide association studies (GWAS) to find the map linkage among all single nucleotide polymorphism and trait for cancer risk. Amundadottir *et al.* identified a complete genome as an inherited sequence variant that has having a chromosomal band 8q24 with a higher risk of prostate cancer [56]. Numerous GWAS publications recognized SNPs linking to Hodgkin's lymphoma, chronic lymphatic leukaemia, breast cancer, ovarian cancer, bladder cancer, prostate cancer, and colon cancer. Genome 8q24 have higher tumour risk related SNPs with a 1.25-1.5 odd ratio. SNPs are linked with several cancer types. Breast cancer risk is linked with rs13281615 polymorphism [57], and bladder cancer is linked with rs9642880 [58]. However, rs6983267 like SNPs, alter the risk for multiple cancer and is related to the risk for colon and prostate cancer and more [59]. Therefore, not all *myc* variants were discovered; the impact of tumour-derived mutations on *myc* expression and function has been controversial.

Additionally, not all types of *myc* variants occur with equal probability. Figure 2 displays the percentage of variants and their distinct distribution of the *myc* gene. Moreover, it indicated that the highest genetic alteration is missense variants (39%), in which a single base pair substitution alters the genetic code. Results indicated that further research is required to fully understand the impact of these mutations on cancer patient management and survival.

The gene that encodes a protein is a multifactorial and nuclear phosphoprotein that has a significant role in drivers for the cell cycle, cellular transformation, and programmed cell death. This functioned as TFs controlling transcription of the target gene. Several hematopoietic tumours, leukaemia and lymphomas are associated with *myc* gene overexpression, mutations, translocation, and rearrangements. Substitute upstream translation initiations, in-frame non-AUG (CUG), and a downstream AUG start site create two isoforms with distinct N-termini, as per evidence. Non-AUG initiated protein synthesis is suppressed in Burkitt's lymphomas, implying that it is necessary for the gene's normal activity [60,61]. Similarly, survival analysis of gene expression profiles across the various tumours and normal tissue was described in different databases (cBioPortal, KMPLOT, and GEPIA) using Kaplan-Meier plotter. However, as discussed earlier further studies were needed to investigate the impact of mutation in patient survival.

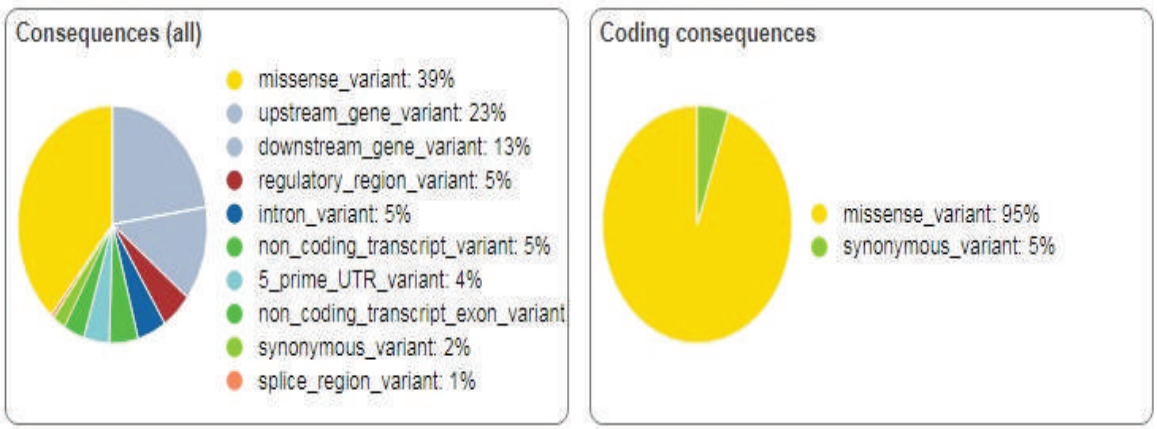


**Figure 2: Pie chart showing the percentage of *myc* variants and their distinct distribution in cancer, based on polymorphism.**

To understand the molecular interactions and state measurements of *myc* in a common framework with other biological attributes, an investigation was conducted using Cytoscape and STRING, network analyser programs. The visualizing of *myc* biological pathways, molecular interaction networks and integrating these networks with annotations, gene expression profiles and other state data (figure 3). Results showed that Importin α, MAX, Aurora-A, and WDR5 are the four *myc* complex crystal structures. Also, the C-terminal of *myc* produces essential DNA-binding with the bHLHZ domain in the MAX complex. The *myc* box acts as a docking site for protein-protein interaction. Furthermore, the selection of functional protein genes based on previous literature and data was made. Hence the following 11 predicted proteins including cyclin-dependent kinase inhibitor 2A (CDKN2A), Cyclin-Dependent Kinase 2 (CDK2), Cyclin-Dependent Kinase Inhibitor 1A (CDKN1A), Jun Proto-Oncogene, AP-1 Transcription Factor Subunit (JUN), Mediator Complex Subunit 1(MED1), myc-associated factor X (MAX), lysine acetyltransferase 2A (KAT2A), SUPT3H, MED16, AKT1 and *myc*. These interaction findings suggested that *myc* activity may directly link the gene's ability to promote tumorigenesis and cell cycle progression. Therefore, understanding the complete interaction process would help to produce more targeted therapy for cancers.

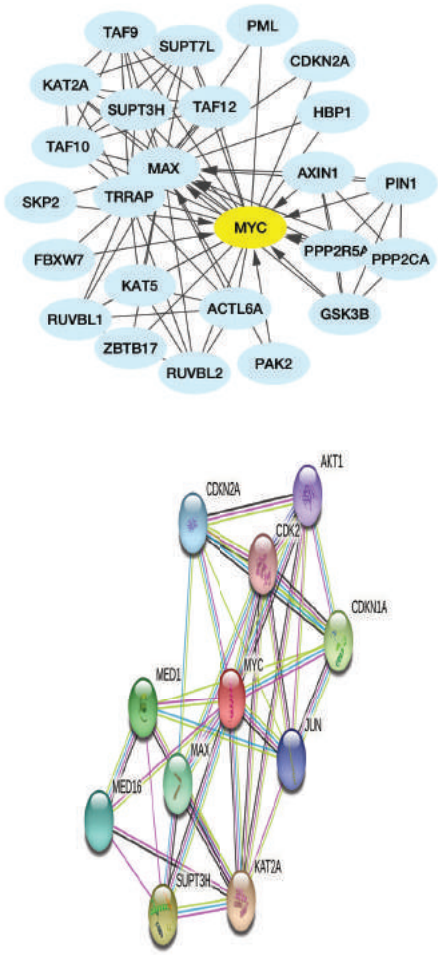


**Figure 3:** *myc* gene and protein interaction. (A) *c-myc* gene interaction with other genes generated with STRING. (B) myc protein modes network nodes interaction with other proteins generated by Cytoscape.

The most direct therapeutic strategy relies on disrupting expression, DNA binding or Protein-protein interactions with the inherent flexibility of transcription factors. The therapeutic targeting includes MYC transcription inhibition, dimerization of partner protein and activating post-translational modification. The epigenetic silencing reduces *myc* expression and activity. The inhibition of several enzymes and motifs have demonstrated efficacy against the *myc* gene, such as, histone demethylases, histone methyltransferases, histone deacetylase, DNA methyltransferases, and bromodomain and extra-terminal motif (BET). BET inhibitor decreases tumour cell survival both in vivo and in vitro. The PI3 K–histone deacetylase inhibitor decrease transcription of *myc* gene and protein stability in vitro studies of lymphoma xenografts [62]. G-quadruplexes inhibits *myc* transcription while BRD4 inhibitors *myc* transcription indirectly. Bouvard *et al.* proved that stauprimide not only inhibited TFs, but also stabilised the MYC G-quadruplex, and decreased the MYC transcriptional gene [63].

Some compounds, such as 10058-F4, 10074-G5, inactivates *myc* downstream proved to have better efficacy and less acquired resistance and target bHLHZ as it is needed for the dimerization of MAX and *myc* at E-box sequencing. However, few compounds have low potency and half maximal inhibitory concentration ranging from 20-40 μM [64]. The *myc* DNA binding and the transcriptional process is reduced by disrupting *myc* /MAX heterodimers by peptides against *myc*. The short half-life and low bioavailability of peptides have been challenging for clinical applicability. Moreover, *myc* function in cancer is reduced by therapeutic targeting of a post-translational modification. The mechanism modifying post-translation are 1) kinase phosphorylating S62-MYC 2) phosphatases dephosphorylating S62-MYC 3) the PIN1 proline isomerase, and (4) enzymes affecting *myc* ubiquitin-dependent proteolysis. The drug targeting *myc* gene expression is summarized in the following table 2. These registered drugs and components have a different mode of action where some inhibit DNA synthesis, activate caspase-3, and potent pro-apoptotic anticancer agents. Also, some of them block ribonucleotide reductase, AURKA Inhibitors and Kinase. These drugs also block the mechanisms of CDK, CDK2 and kinases.

**Table 2: Drug targeting *myc* gene expression from: DrugBank, ClinicalTrials, ApexBio, DGIdb, and Novosee**

| Mechanism | Target | Compounds | Pre-clinical/clinical stage | References |
|---|---|---|---|---|
| Epigenetic silencing | BET inhibitor | JQ1/TEN-010 | Pre-clinical with in vivo efficacy; phase I/II | 65 |
| | | BI 894999 | Phase I | 66 |
| | PI3 K-BRD4 inhibitor | SF2523 | Pre-clinical with in vivo efficacy | 67 |
| | PI3 K-HDAC inhibitor | CUDC-907 | Multiple phases I/II | 62 |
| G-quadruplexes | myc | GQC-05 | Pre-clinical | 68 |
| myc: MAX dimerization | myc | Omo myc | Pre-clinical with in vivo efficacy | 69 |
| | MAX | KI-MS2-008 | Pre-clinical with in vivo efficacy | 70 |
| PP2A activation | SET inhibitor | OP449 | Pre-clinical with in vivo efficacy | 71 |
| | CIP2A inhibitor | Celastrol | Pre-clinical with in vivo efficacy | 72 |
| | Protease/CIP2A inhibitor | Bortezomib | FDA approved for multiple myeloma, multiple phase I/II/III/IV | 73 |

## Conclusion

The *myc* gene is a proto-oncogene that regulates cellular function like normal cell proliferation, differentiation, growth, metabolism, and apoptosis. The proliferation and cell growth increase due to overexpression by regional gene amplification or chromosomal translocation. The current research demonstrated that the *myc* gene is expressed in all tissue and has different expression patterns based on tissue type. Although it is clear from the Insilco results that the expression profile of the gene changes in cancerous tissues, each cancer has a distinguished profile, as seen in the results. Furthermore, the current analysis demonstrated that the *myc* gene and protein interact with different genes and proteins inside the tissue. The results also demonstrated several ways of *myc* deregulation in existing tumours, and *myc* reregulation is not the same.

Nevertheless, the Insilco analysis of drugs that targeted *myc* function or mechanism of activation has different and favourable outcomes. The current finding suggested that the significant biomarker differentiating the pathologies of *myc* leads to the development of diverse therapeutic drugs targeting specific parts of *myc* oncogenicity. Nevertheless, *myc* and its variants may further be investigated for their role in many other disease associations. Further study can be conducted on predicted variants and their interaction with other proteins to predict the type of diseases *myc* may cause and the adverse effects of this on cancer patients.

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### Data Availability Statement

Research data are not shared. The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflicts of Interest**: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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**Ethical Clearance** : The current study was not required ethical approval form the institutional review board as it utilizes publicly available date.

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# Isolation and Identification of Multi-Drug Resistant "*Pseudomonas Aeruginosa*" from Burn Wound Infection Iraq

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## Abstract

*P. aeruginosa"* is considered as ubiquitous bacteria that can rapidly obtain resistance against various wide spectrum antibiotics It can rapidly obtain resistance against various wide spectrum antibiotics which lead to problematic conditions**..** In current study, 120 samples were collected from 120 patients suffering from contaminated burns. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients 'consent. Samples are collected from patients after they have stopped using antibiotics for 48 hours. After the swabs had been cultured on different media, conventional biochemical tests to identify bacterial isolates and antimicrobial sensitivity to the most common antibiotics were performed by viteck 2 compact .The results showed that the highest percentage of bacterial species was of *P. aeruginosa* was 17.78%. Isolation No. 7 was the most resistant of the diagnosed isolates . The lowest Percentage other bacterial species example *Proteus , Staphylococcus , Klebsiella* .

***Key word:*** *Burn, Wound , Antibiotic, Multidrug resistant, P. aeruginosa.*

## Introduction

Burn wound infections are most commonly caused by bacteria, fungi, or viruses. However, bacteria cause the majority of infections in most burn care centers. Almost all burn wound infections caused by bacteria are due to aerobic microorganisms. Microorganisms are transmitted from the patient's surroundings at the time of injury due to implantation or can be acquired endogenously from the patient or exogenously from hands of medical personnel, as well as fomites [1].

*Pseudomonas aeruginosa* is a ubiquitous gamma proteobacterium found in different environmental niches such as soil and water. As an opportunistic pathogen, it also causes severe infections in mammals and other animals and in plants [2]. The pathogenicity of *P. aeruginosa* is mediated by its capacity to produce a large range of virulence factors and is strengthened by its intrinsic resistance to environmental stresses and xenobiotic agents such as antibiotics, disinfectants, and heavy metals [3]. Taking the data together, it has been shown that these factors allow the pathogen to establish efficient invasion, colonization, and persistence inside the host organism [4,5].

Burn wounds are complex microenvironments where infections by bacterial pathogens such as *P. aeruginosa* or *Staphylococcus aureus* represent major concerns in patient treatment [6, 7]. The understanding and characterization of the bacterial physiology in relation to burn wound exudate composition are of high interest for the development of novel strategies to

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prevent and cure bacterial infections. In this study, we focused on the analysis of the pathogenic traits of *P. aeruginosa* PAO1 in BWE and linked these findings to an understanding of the physicochemical and biological properties of those exudates to eventually propose an artificial burn wound exudate medium for the establishment of an in vitro system to analyze the initial steps of burn wound infections.

Antibiotics have not only saved patients' lives , they have played a pivotal role in achieving major advances in medicine and surgery. They have successfully prevented or treated infections that can occur in patients who are receiving chemotherapy treatments; who have chronic diseases such as diabetes, end-stage renal disease, or rheumatoid arthritis; or who have had complex surgeries such as organ transplants, joint replacements, or cardiac surgery. Antibiotics have also helped to extend expected life spans by changing the outcome of bacterial infections. In 1920, people in the U.S. were expected to live to be only 56.4 years old; now, however, the average U.S. life span is nearly 80 years. Antibiotics have had similar beneficial effects worldwide. In developing countries where sanitation is still poor, antibiotics decrease the morbidity and mortality caused by food-borne and other poverty-related infections [8].

Specimen collection

In current study, 120 samples were collected from 120 patients suffering from contaminated burns. Samples were collected under sterile conditions using sterile swabs. The samples were immediately transported to the laboratory to be implanted in the appropriate media.

The average age of the patients was 42.6 ± 5.8 years. The number of males was 72 and the number of females 48. The study was conducted after obtaining ethical approvals from the ethics committee in the Department of Biology, College of Science, University of Baghdad as well after obtaining the patients 'consent. Samples are collected from

**Bacterial isolation**

The collected samples were cultured on MacConkey agar under aerobic and sterile conditions. To diagnose the isolated bacteria the select colonies were re-cultured on Cetrimide agar For further identification of isolated bacteria catalase test, oxidase test and Gram stain were used to identify the pure isolated bacteria [9].

**Microscopic Examination**

The morphological identification of the isolates as bacilli was confirmed microscopically by performing Gram staining, for which single colony of each isolate was picked up and stained as per the standard protocol and viewed under oil immersion for similar type of cells.

Catalase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions**,** use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide then Place a drop of 3% H2O2 in the glass slide the result observed for the evolution of oxygen bubbles [10].

Oxidase test

The collected samples were cultured on MacConkey agar, Blood agar under aerobic condition and sterile conditions**,** strip of Whatman's No. 1 filter paper are soaked in a freshly prepared 1% solution of tertramethyl-p-phenylene-diamine dihydrochloride, After draining for about 30 seconds, the strips are freeze dried and stored in a dark bottle tightly sealed with a screw cap, for use, a strip is removed, laid in a petri dish and moistened with distilled water. The colony to be tested is picked up with a platinum loop

and smeared over the moist area. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds, a "delayed positive" reaction by colouration in 10-60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds [9].

Identification using the VITEK 2 fluorescent system (ID-GNB card)

The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) (ID-GBB card and ID- GNB card) includes 43 non enterobacterial gram-negative taxa and gram positive. Testing was performed according to the instructions of the manufacturer. Briefly, strains were cultured on nutrient agar for 18 to 24 h at 37°C before the isolate was subjected to analysis. A bacterial suspension was adjusted to a McFarland standard of 0.50 to 0.63 in a solution of 0.45% sodium chloride using the VITEK 2 DensiCheck instrument (bioMe´rieux). The time between preparation of the solution and filling of the card was always less than 1 h. Analysis was done using the identification card for gram-negative and gram positive bacteria (ID-GNB card) and (ID-GBB) containing 41 fluorescent biochemical tests. Cards are automatically read every 15 min. Data were analyzed using the VITEK 2 software version VT2- R03.1 [10].

**Antibiotic susceptibility**

The standard method of Mazzariol *et al.* (2008) was followed to test the susceptibility of identified bacteria to the several antibiotics (Cefotaxime, Ampicillin, amoxicillin/Clavulanic acid, ampicillin/ Sulbactam, Piperacillin/ Tazobactam, cefazolin, ceftazidime, ceftriaxone, cefepime, imipenem, gentamicin, tobramycin, ciprofloxacin, levofloxacin, nitrofurantoin, trimethoprim/sulfamethoxazole, tricarcillin, amikacin). VITEK 2 DensiCheck instrument (bioMe´rieux) was used to check the supportability of isolated and identified bacteria [11]

## Results and Discussion

Isolation and identification of bacterial species

5896In present study, 120 swabs were collected from infected burns. The samples were collected from 120 patients. The swabs were inoculated onto number of culture media (Blood agar, MacConkey agar) for growing and isolating and then for pre-identification on cetrimide agar visual examination may also reveal the typical yellow-green to blue color which indicates the production of pyocyanin. Both pyocyanin and fluorescein are typically produced by strains of *P. aeruginosa*. Most of isolates were grown on blood agar with different shape according to the genera of isolates.. The results showed that the highest percentage of bacterial species was The percentage of isolation of *P. aeruginosa* was 17.78%. The lowest percentage of bacterial isolates that isolated from infected wound was found in case of *Proteus , Staphylococcus , Klebsiella* .

Previous study of Forson *et al.* (2017) mentioned that *P. aeruginosa* represented the highest percentage of bacterial species that isolated from burn wound, while Church *et al.* (2006) reported that the highest percentage of bacterial species that isolated from infected burn wound was *P. aeruginosa* followed by *E. coli* and the lowest percentage was found in case of *Acinetobacter spp and Bacteroides* spp. Similar finding was reported by other investigators [16].

**Table 1:- Number and percentage of bacterial species that isolated from 120 clinical samples.**

| | Bacteria | Number | Percentage (%) |
|---|---|---|---|
| 1 | Proteus mirabilis | 14 (Pm1, Pm2, Pm3, Pm4, Pm5, Pm6, Pm7, Pm8, Pm9, Pm10, Pm11, Pm12, Pm13, Pm 14). | 31.1 |
| | | | |
| 3 | Pseudomonas aeruginosa | 8 (Pa1, Pa2, Pa3, Pa4, Pa5, Pa6, Pa7, Pa8). | 17.78 |
| 4 | Klebsiella Pneumoniae | 2 (Kp1,Kp2). | 4.4 |
| 7 | Staphylococcus aureus | 1 (Sa1) | 2.2 |
| 8 | Pseudomonas fluorescens | 1 (Pf1) | 2.2 |
| 10 | Burkholderia cepacia | 1 (Bc1) | 2.2 |

The diversity of bacteria species isolated from infected wounds was one of the features that distinguished the present study. Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora [13]. Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers [12]. Immediately following injury, gram-positive bacteria from the patient's endogenous skin flora or the external environment predominantly colonize the burn wound [14]. Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury [12]. Microorganisms transmitted from the hospital environment tend to be more resistant to antimicrobial agents than those originating from the patient's normal flora [15].

Previous study, 185 (61.87%) bacteria were isolated from the wounds of burnt patients. Among the culture positive samples, 112 (60.54%) were from female patients and 73 (39.46%) were from male patients. The most commonly isolated organisms were Pseudomonas species (43%). *K. pneumoniae* and *A. baumannii* were second and third predominant bacterial pathogen with a prevalence of 28% and 14.83% respectively. Similar finding with *P. aeruginosa* a predominant isolate followed by *K. pneumoniae* and *A. baumanniiin* atertiary care hospital in India were also reported [16]. High prevalence of these pathogens is associated with their ability to flourish well in a moist environment and persistence in hospital environment [16]. In present study, *P. aeruginosa* was reported as one of domain species that isolated from burn wound infection.

**Antibiotic susceptibility**

The susceptibility of 45 isolates to different antibiotics was done by VITIK 2 DensiCheck instrument. The antibiotics that used were different according to the group of species of bacteria because the routinely antibiotic that used clinically was different according to the clinical cases and species [17] that covered in the study.

The current results showed that the effect of antibiotics varies greatly according to the species

of bacterial used and the type of antibiotics. Where, many types of antibiotics were used in present study. Through an overview of the results, it can be confirmed that there are no bacterial species sensitive to all antibiotics used, and no bacterial isolate that resists to all antibiotics . Figure (1) shows that the *P. mirabilis* gave the highest percentage of resistance to different kinds of antibiotic, followed by the *P. aeruginosa*. While, the lowest percentage of sensitivity to different kind of antibiotics was shared among *S. paucimobilis: C. testosterone and B. mallei.* The present study showed that the highest percentage of intermediate response of bacteria to antibiotics was seen in case of *S. paucimobilis* followed by *P. fluorescens*.

Nosocomial infection in the burnt patients is major challenge for a clinician. It has been estimated that 75% of all deaths in burnt patients were associated with infections. Prolonged use of antibiotic leads to the development as well as selection of multidrug resistant (MDR) bacteria which results in treatment failure and intensifies the complications. Thus, the information of microbial flora and the current antibiotic susceptibility patterns are important for the clinician treating burn sepsis [18].

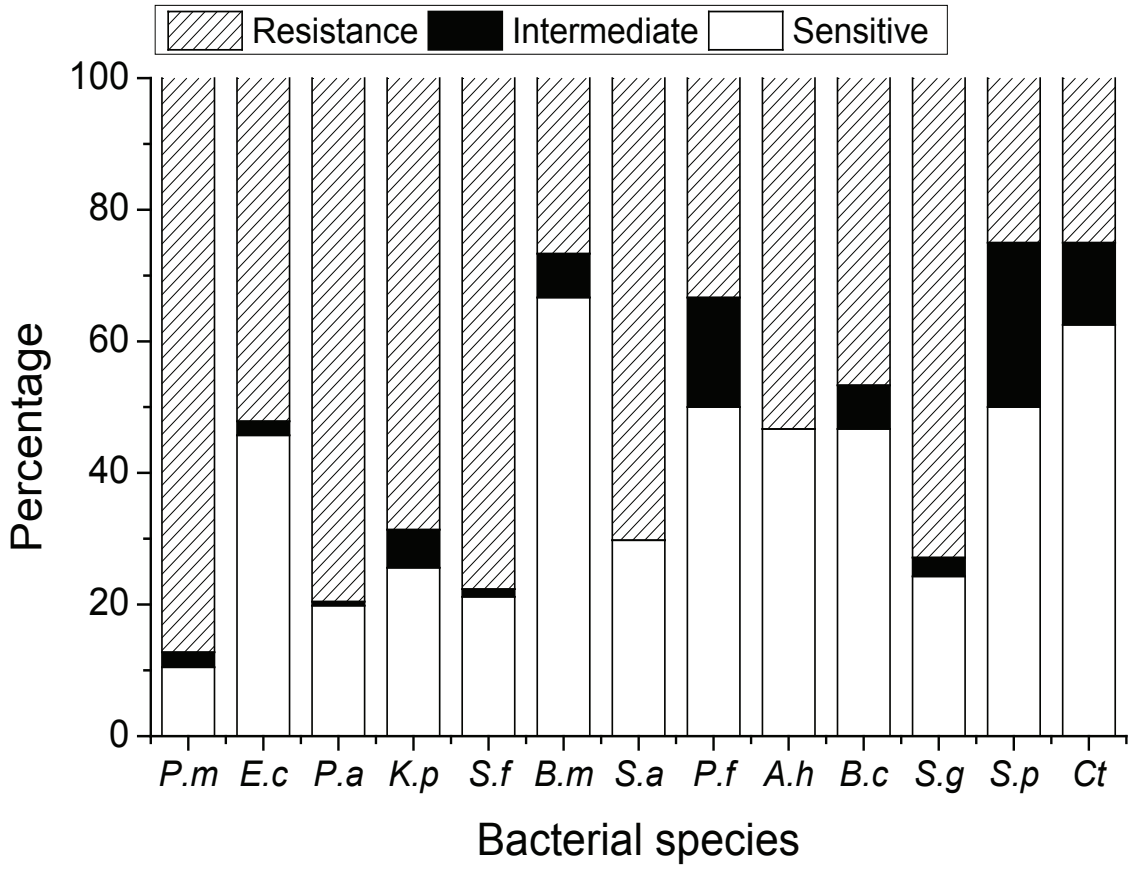


**Figure 1:- The percentages of susceptibility of different species of bacteria to different kinds of antibiotics (P.m: P. mirabilis, E.c: E. coli, P.a: P. aeruginosa, K.p: K. pneumonia, S.f: S. ficaria; B.m: B. mallei, S.a: S. aureus, P.f: P. fluorescens, A.h:A. haemolyticus, B.c: B. cepacia, S.g:S. gallinarum, S.p: S. paucimobilis, C.t: C. testosterone.**

In present study, *P. aeruginosa* (P.a. 7) (Table 1) was used for further studies to identify the efficiency of using of different method to increase the efficiency of antimicrobial treatment against this isolate of bacteria. This isolate was used because it is resistance to the high important antibiotic (cefotaxime). The importance of this antibiotic was coming from routinely using of this antibiotic for several years in treating of several wound infection (burn infection) especially in Iraq [19].

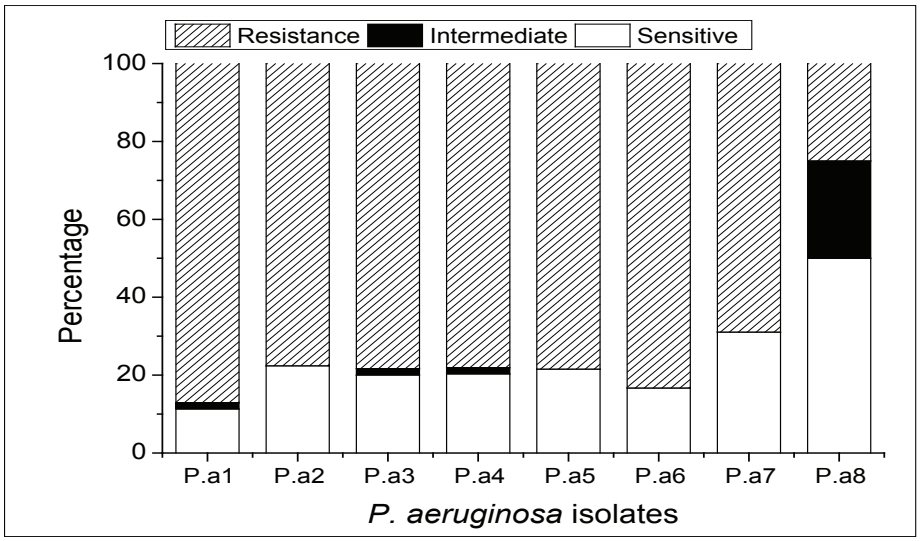


**Figure 2: The percentages of susceptibility of different isolates of P. aeruginosa that used in present study.**

**Table 1:- Minimum inhibition concentration (MIC) and interpreting of different antibiotics against P. aeruginosa (P a 7). R, resistance, S, sensitive.**

| Susceptibility Information | Analysis Time: 11.63 hours | | | Status: | Final |
|---|---|---|---|---|---|
| Antimicrobial | MIC | Interpretation | Antimicrobial | MIC | Interpretation |
| +Mecillinam | | R | +Cefixime | | R |
| +Ternocillin | | R | +Cefpodoxime | | R |
| +Amoxicillin | | R | +Cefteram | | R |
| +Ampicillin | | R | +Ceftibuten | | R |
| +Amoxicillin/Clavulanic Acid | | R | +Cefotaxime | | R |
| +Ampicillin/Sulbactam | | R | Ceftazidime | 8 | S |
| +Carbenicillin | | R | +Ceftizoxime | | R |
| Ticarcillin | | *R | +Ceftriaxone | | R |
| Ticarcillin/Clavulanic Acid | 64 | S | +Ceftazidime/Avibactam | | S |
| +Azlocillin | | R | +Ceftolozane/Tazobactam | | S |
| +Mezlocillin | | R | Cefepime | 4 | S |
| Piperacillin | >= 128 | R | +Doripenem | | S |
| Piperacillin/Tazobactam | >= 128 | R | +Ertapenem | | R |
| +Cefaclor | | R | +Faropenem | | R |
| +Cefadroxil | | R | Imipenem | 2 | S |
| +Cefalexin | | R | Meropenem | 0.5 | S |
| +Cefalotin | | R | Amikacin | 4 | S |
| +Cefazolin | | R | Gentamicin | <= 1 | S |
| +Cefonicid | | R | +Isepamicin | | S |
| +Cefprozil | | R | +Netilmicin | | S |
| +Cefradine | | R | Tobramycin | <= 1 | S |
| +Loracarbef | | R | +Cinoxacin | | R |
| +Cefamandole | | R | +Nalidixic Acid | | R |
| +Cefotiam | | R | +Pipemidic Acid | | R |
| +Cefuroxime | | R | Ciprofloxacin | <= 0.25 | S |
| +Cefmetazole | | R | +Levofloxacin | | S |
| +Cefotetan | | R | +Norfloxacin | | S |
| +Cefoxitin | | R | +Ofloxacin | | S |
| +Cefcapene | | R | +Prulifloxacin | | S |

A number of other studies in various institutions all over the world dealt with the species of bacteria that cause burn wound infection and are responsible for their contamination. As well as, there were number of studies that dealt with the types of antibiotics that are used in the treatment of burn wound infection with different species of bacteria. [20] highlighted on the different types of treatments that can be used in the treatment of burns wound infection, as they showed that the beneficial and harmful side of each treatment and that the response to treatment is related to the type of burns and the type of bacteria that cause them this finding agree particularly with this study in terms of the response of bacteria to antibiotic is highly [21,22] found that high-resistance antimicrobials included penicillin, ampicillin, amoxicillin, cefazolin, and cefotaxime.

Low-resistance antimicrobials included teicoplanin, tigecycline, vancomycin, and linezolid, this finding is similar to the present results that showed the isolated bacteria were resistance to the common antibiotics and moderate sensitive to the new routinely antibiotics used for the burn wound infection cases. Other studies of several investigator from different institution is agree with the results of present study [23,24,25].

**Conflict of Interest:** The authors declare that there is no conflict of interest regarding this study.

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# Impact of Pregnant Adolescents' Knowledge about Preventive Health Behaviors during Pregnancy upon Pregnancy Outcomes in AL- Diwaniyah City

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## Abstract

**Objective(s):** The aim of the study is to evaluate the impact of pregnant adolescents' knowledge about preventive health behaviors during pregnancy upon their pregnancy outcomes .

**Methodology**: A quasi-experimental design, using the evaluation approach for the determination the impact of pregnant adolescents' knowledge about preventive behaviors during pregnancy upon pregnancy outcomes in Al-Diwaniyah city from the period 17th January 2020 to 1st June 2021. Non- probability, purposive sample of (35) adolescent pregnant are selected from those who visit Al-Diwanyiah Maternity and Pediatric Teaching Hospital. Data are collected through the use of the study instruments (questionnaire) in a form of Google format and through video calls as means of data collection. Data were analyzed through the use of descriptive statistical data analysis approach and inferential statistical data analysis approach.

**Results:** Results of this study indicate that most of the pregnant are between (16-19) year old (57.1%), (34.3%) are primary school graduates, (71.4%) are housewives, (77.1%) are living in rural area and (80%) are earning an income of (300-600) thousands ID. The overall evaluation of pregnant adolescents' have a lack of knowledge about preventive health behaviors during pregnancy without significant relationship between pregnant adolescents' knowledge and their demographic characteristics. As well as, pregnant adolescents' knowledge about preventive behaviors during pregnancy does not impose any effect upon their pregnancy outcome.

**Conclusions:** The study's unique finding is that pregnant adolescents have a lack of knowledge about preventive health behaviors during pregnancy, and this knowledge does not have any effect on their pregnancy outcome.

**Recommendations**: Improving pregnant adolescents' health literacy is the responsibility of healthcare systems and healthcare professionals through emphasis by the Ministry of Health role through the antenatal care units to take a part and dissemination of education about the preventive health behaviors among pregnant adolescents especially primigravida, and particularly with each specific trimester.

***Key word** : Impact , Pregnant Adolescents' ,Knowledge ,Preventive Health Behaviors ,Pregnancy outcomes*

## Introduction

Adolescent pregnancy it is one of the most important challenges affecting adolescent's reproductive health, not just in impoverished nations but also in wealthy countries. Every year, 13 million children are born to women under the age of 20 around the world, with more than 90% of them in developing countries [(1)].

According to a new World Health Organization (WHO) prediction, the rate of teen pregnancy will rise by 2030. Due to pregnancy and childbirth complications, mothers aged 10 to 14 years were five times more likely to die than mothers aged 20 to 24, accounting for more than 70,000 adolescent girls' deaths each year [(2)].

Unhealthy behaviors and lifestyles are two of the leading causes of death around the world. Pregnant adolescent's healthy habits have an impact on the outcome of their pregnancy [(3)].

It is estimated that every year about 16 million girls aged between 15–19 years give birth in low income countries and 70,000 die of complications during pregnancy and childbirth [(4)].

There are several health habits that women are expected to initiate, maintain, or adjust before, during, and after pregnancy. These behaviors include supplementation (e.g., folic acid, various vitamins) diet and healthy eating (e.g., fiber intake, hydration) Other habits, like smoking, will have to be abandoned [(5)].

Pregnancies in adolescence are associated with a high risk of maternal and neonatal complications. These are associated with a high number of preterm births, caesarean sections, and increased morbidity in mothers during both the antenatal and postnatal periods. Extremely low birthweight, and the need for neonatal hospitalization (NICU) admission are all common [(6)]. Thus, the current study aims to evaluate the impact of pregnant adolescents' knowledge about preventive health behaviors during pregnancy upon pregnancy outcomes.

## Methodology

A quasi-experimental design, is carried out in order to achieve the objectives of the current study using the evaluation approach for the determination of pregnant adolescents' knowledge about preventive behaviors during pregnancy in Al-Diwaniyah city from the period 17th January 2020 to 1st June 2021 .Non- probability , purposive sample of (35) pregnant adolescents' has been selected for the present study. The data are collected through the utilization of a constructed questionnaire as a Google format and video calls as means of data collection (Arabic version).

The questionnaire is composed of two main parts as follows: Part I: Pregnant Adolescent Sociodemographic Characteristics : It is concerned with the identification of the socio demographic characteristics of the study group, which include (age , education level for adolescent pregnant, occupation for adolescent pregnant, residency, and monthly family income). Part II: Pregnant Adolescent's Knowledge about preventive Health Behaviors during pregnancy: This part consists of three domains and they are responded by answering the multiple choice questions (MCQ) with correct answer that represent of four answers (one of them is correct answer, scored 2 and the three others answers are incorrect answer, scored 1). This part is comprised of (50) item that measure pregnant adolescent's knowledge about preventive health behaviors during pregnancy. It is measured as (50-66) = poor level of knowledge, (67-83) = fair level of knowledge and (84-100) = good level of knowledge. Content validity and Pearson correlation coefficient reliability are determined through a pilot study. The data of the present study are analyzed through the use of the Statistical Package of Social Sciences (SPSS) version 20.throughdescriptive statistics (frequency, percentage, mean, mean of scores, total of scores, range and standard deviation) and statistical inferential (T-test, multiple linear regressions, person

correlation coefficient, Chi Square test and analysis of variance ANOVA). Results were determined as highly significant at (P≤0.01) significant at (P≤0.05) and non-significant at (P>0.05)

## Results

**Table (1): Pregnant Adolescents' Demographic Characteristics**

| Characteristics | Frequency | Percent |
|---|---|---|
| 1. Pregnant Age (Years) | | |
| 13 - 15 Year | 15 | 42.9 |
| 16 - 19 Year | 20 | 57.1 |
| Total | 35 | 100.0 |
| 2. Pregnant Education | | |
| Read and write | 9 | 25.7 |
| Primary | 12 | 34.3 |
| Intermediate | 5 | 14.3 |
| Secondary | 3 | 8.6 |
| Institute/ University | 6 | 17.1 |
| Total | 35 | 100.0 |
| 3. Pregnant Occupation | | |
| Student | 9 | 25.7 |
| House wife /unemployed | 25 | 71.4 |
| Other | 1 | 2.9 |
| Total | 35 | 100.0 |
| 4. Residency | | |
| Urban | 8 | 22.9 |
| Rural | 27 | 77.1 |
| Total | 35 | 100.0 |
| 5. Monthly Income | | |
| 300 thousand -600 thousand | 28 | 80.0 |
| 600 thousand and one dinar - 900 thousand | 5 | 14.3 |
| 900 thousand and one dinar - one million and 200 thousand | 2 | 5.7 |
| Total | 35 | 100.0 |

Results out of this table indicate that most of the pregnant are (16-19) year old (57.1%), (34.3%) of them are primary school graduates, (71.4%) of them are housewives, living in rural area (77.1%) and earning an income of (300-600) ID (80%).

**Table (2): Overall Evaluation of Pregnant Adolescents' Knowledge About Preventive Health Behaviors during Pregnancy**

| Overall Evaluation | Poor (50-66) | Fair (67-83) | Good (84-100) |
|---|---|---|---|
| Pre-test | 31 (88.57%) | 4 (11.43%) | 0 (0%) |

Results out of this table depict that pregnant adolescents' have a fair of knowledge about preventive health behaviors during pregnancy.

**Table (3): The Impact of Pregnant Adolescents' Knowledge about Preventive Behaviors during Pregnancy upon Their Pregnancy Outcome**

| Model | | Sum of Squares | Degree of Freedom | Mean Square | F-Statistics | Significance |
|---|---|---|---|---|---|---|
| | **Regression** | **0.071** | **1** | **0.071** | **0.033** | **0.856** |
| | Residual | 69.929 | 33 | 2.119 | | |
| | Total | 70.000 | 34 | | | |
| a. Dependent Variable: Pregnancy Outcome | | | | | | |
| b. Predictors: (Constant), Pregnant Adolescents' Knowledge | | | | | | |

Results, out of this table, depict that pregnant adolescents' knowledge about preventive behaviors during pregnancy does not impose any effect upon their pregnancy outcome.

## Discussion

The distribution of the sociodemographic characteristics as shown in(Table -1) revealed that the highest percentage (57.1%) percent of the study sample were within age 16-19 years old that age represent a between middle and late adolescence and consider a common age for marriage according to our culture in Al-Diwanyiah city. According to the United Nations Children's Emergency Fund, there is an increase in the rates of early marriage in Iraq; one in every five women in high school, or 21%, is married [7]. Regarding the participants' educational level, the study revealed that the majority of them are either primary school graduates or can only read and write, indicating that early marriage is considered a reason for leaving school early. This finding is consistent with a study conducted in Basra City [8], as well as a study conducted in Baghdad Cit [9] on teenage pregnant with significantly lower levels of education.

Regarding the occupation of the participants the study revealed that the highest percentage (71.4%) of

them is housewives. This finding occurs due to low education of the participants leading to minimize their employment possibilities. This findings is supported with a study that reported that teenage mothers more likely to be housewives maybe related to the low education degree which not helping if they want to have a job or an employment [10]. Regarding the residential area of the participants the study revealed that the highest percentage (77.1%) of them are living in rural area , which reflects the social and cultural factors in our country towards early marriage among rural population which may have adverse effects on maternal outcome. What is common to every region, however, is that girls who are poor, live in rural or remote areas and who are illiterate or have little education are more likely to become pregnant than their wealthier, urban, educated counterparts [11]. Regarding the monthly family income of the participants the study revealed that the majority of the study sample (80%) is within considering to insufficient monthly family income as monthly earning (300-600) thousand ID. Most of teenage mothers are not in a good socio economic condition so transition to motherhood becomes problematic for them [12]. Analysis of data related the pregnant adolescents' knowledge about preventive health behaviors during pregnancy reveals that, the majority of them have poor level of knowledge (Table 2). Pregnant adolescents typically have the fewest resources because they have not had the time to obtain the necessary education or experience to be self-sufficient, or even to best identify their own outside supports [13].

A lack of knowledge among pregnant adolescents can have a negative impact on their lives as well as the lives of their unborn children [14]. Limited knowledge about high-risk pregnancies and the dangers will also increase the incidence of high-risk pregnancy [15].

Concerning the impact of pregnant adolescents' knowledge about preventive health behaviors during pregnancy on their pregnancy outcomes; no effect has been imposed on their pregnancy outcomes (Table 3). This result occurs due to the lack of pregnant adolescents' knowledge about preventive health behaviors during pregnancy important information that can help pregnant to prevent complications during pregnancy.

The age and maturity level of pregnant women may impact their susceptibility to education and their ability to identify danger signs associated with their pregnancy [16].

Maternal education is an important factor for safe delivery which proved that women with no education had 2.7 times the risk of maternal mortality than women with more than 12 years of education [17]. Lack of knowledge of leads to multiple undesirable health outcomes, such as unintentional or unplanned pregnancy in a marriage, and unsafe abortion among unmarried adolescences [18].

Cultural factors rather than economic factors seem to be related to early age at marriage and adolescent childbearing, which are associated with poor birth outcomes [19] .

Pregnancy outcomes rank among the most pressing reproductive health problems in the world. Factors influencing pregnancy outcomes may include poor nutrition of the woman, child spacing, maternal age, inadequate prenatal care, lifestyle behaviors , such as; smoking, alcohol consumption, drug abuse, overweight, obesity and poverty.

## Conclusions

The study's unique conclusion was that pregnant adolescents lacked knowledge about preventive health behaviors during pregnancy, and although the results of this study showed no effect of the knowledge factor on pregnancy outcome in adolescent girls, the knowledge factor may have an indirect role because it is related to many other factors that may influence the outcome of adolescent pregnancy, such as level of education, poverty, and behavior of unhealthy lifestyle.

**Recommendations:**

1- Improving pregnant adolescents' health literacy is the responsibility of healthcare systems and healthcare professionals through emphasis by the Ministry of Health role in through the antenatal care units to take a part and dissemination of education about the preventive health behaviors among pregnant adolescents especially primigravida, and particularly with each specific trimester.

2- Improving pregnant adolescents' knowledge regarding preventive health behaviors during pregnancy through education programs, booklet, educating sessions, mass media, and articles….etc.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# Molecular detection of *Brucella canis* in Blood of Dogs

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## Abstract

The aims of this study was to evaluate a PCR for detecting *Brucella canis* in the blood of dogs, using a primer pair designed for *Brucella* spp.

A study was conducted on 150 blood sample collected from dogs suspected to Veterinary Hospital in Baghdad / Aden Square. All blood samples (150) were tested by PCR technique using a common primer of the 23S ribosomal RNA (23s RNA) gene and specific primer for *brucella canis* (B0548). The genomic DNA was extracted and PCR was applied. Our study recorded 5.3% of brucellosis in common primer and 3.3% in specific primer for *brucella canis* in dog in Baghdad city, the sequences of *Brucella canis* in dog in different isolates in our study recorded 99% compatibility recording to National Center Biotechnology Information (NCBI). Following correspondence from National Center for Biotechnology Information, the 23S ribosomal RNA gene was registered, given an agreement number, and became a resource for Iraq and Middle East, as well as the rest of the world. As more type strains are published, this set will grow, and it can be download from NCBI at: https://www.ncbi.nlm.nih.gov/nuccore/.

From this study we can conclude that, the percentage of Brucellosis in dogs in Baghdad city is 5.3% and 3.3% in a common and specific primer, respectively and the molecular method (PCR), is a good idea for confirmation of diagnosis of *Brucella canis* infection in dog.

***Keywords:*** *Brucella canis, dogs, Iraq*

## Introduction

Brucellosis is a disease of animals and humans caused by Gram negative, facultative intracellular bacteria of the genus *Brucella.* Of the 12 currently recognized species, four are considered zoonotic pathogens: *Brucella melitensis, Brucella suis, Brucella abortus, and Brucella canis* in decreasing order of pathogenicity. Canine brucellosis, caused by Brucella canis, is a worldwide disease of dogs that primarily results in reproductive disease and may be transmitted to humans[1,2] . Canine brucellosis were first recognized in 1966[3,4].

In bitches, this manifests as abortion and stillbirths and in males, predominant symptoms include prostatitis and epididymitis[5] . Like all *Brucella* species, *Brucella canis* invades via the conjunctival, oronasal, or venereal route and distributes to organs of the reticuloendothelial system, resulting in a chronic, persistent infection[6]. Clinical signs may not become apparent in infected dogs for months to years after infection, making it difficult to implement control measures and avoid spread of disease to other dogs and humans[7].

## Materials and Methods

### Methods

**Clinical examination**

Clinical examination was carried out to all animals prior to sample collection, which includes pulse rate, rectal temperature and respiratory rate, The case history was taken which include appetite and other signs, while age and Sex was recorded for each animal.

**Animals and Data collection:**

The number of sick cases of domestic and police dogs admitted to the Baghdad veterinary hospital were 150 animals in different Ages, sex and breed with variable cases, the study period extend from November 2019 to July 2020 by two visits weekly.

**Blood sample collection:**

Two milliliter of Whole blood samples with EDTA anticoagulants tubes were collected from 150 dog from cephalic vein. All sample was stored in deep freeze until used for DNA extraction. All samples were transferred in a cooling box to department of internal and preventive veterinary medicine/ college of veterinary medicine/ university of Baghdad.

## Results and Discussion

**Molecular identification of *Brucella Canis* in dogs**

*Brucella canis* is a Gram-negative organism infecting, mainly, the genital organs of both sexes and resulted in several reproductive problems[8].

All blood samples (150) were tested by PCR technique using a specific primer of the 23S ribosomal RNA (23s RNA) gene. The genomic DNA extraction was given concentrated DNA range of 1.5 µl of genomic DNA were used for each PCR reaction, with purity reached at 260/280 nm and read by a Nanodrop.

Table (1) has shown that the percentage of Brucella Canis in dogs by PCR in Baghdad Governorate. This table show that from 150 samples, 8 (5.3%) Positives and 142 (94.7%) Negatives which found locally in Iraq in common primer, while 5 (3.3%) positive and 145 (96.7%) negative as shown in table (2)

**Table (1): percentage of *Brucella Canis* in dogs by PCR (common primer)**

| No. | Number of samples | Positive (%) | Negative (%) |
|---|---|---|---|
| 1. | 150 | 8 (5.3%) | 142 (94.7%) |

**Table (2): percentage of *Brucella Canis* in dogs by PCR (specific primer)**

| No. | Number of samples | Positive (%) | Negative (%) |
|---|---|---|---|
| 1. | 150 | 5 (3.3%) | 145 (96.7%) |

In recent years, several studies were carried out to evaluation of PCR in diagnosis a specific DNA for *B. canis* in depending on different samples[9,10]. Aras and Uçan, [11] demonstrated that PCR technique had a detectable effectiveness equally for bacteriological culture in diagnosis of brucellosis with extra advantages including the fastness, speediness in performance, absence of riskiness with the highly sensitivity and specificity.

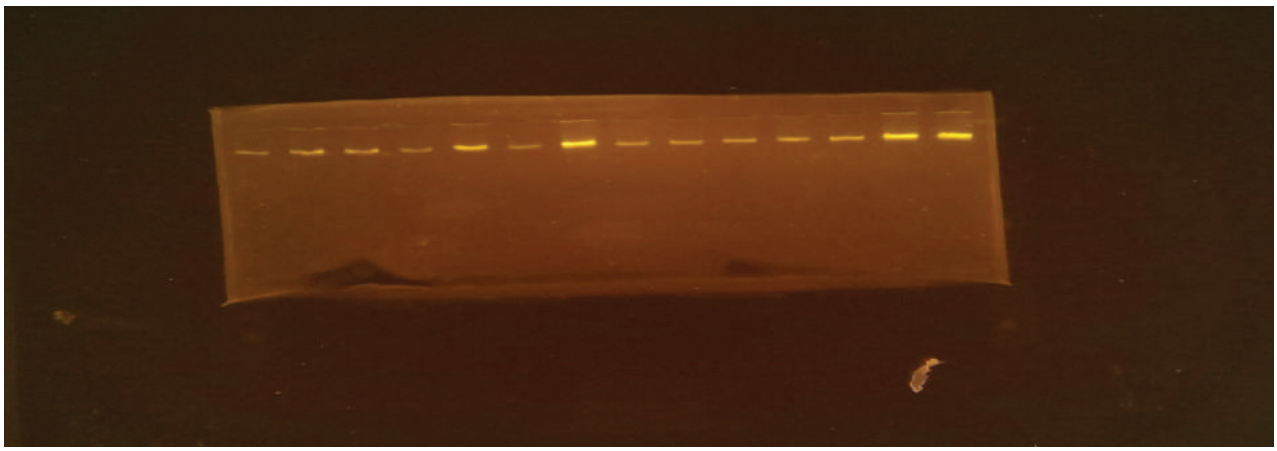
The current results showed that the prevalence of B. canis was 5.3%, this result was slightly agreed with a result of Alfattli [8] who found that the prevalence of B.

canis in Iraq by using PCR was 3.05%. Also, Tamimi and Wali [12] showed that prevalence of canine Brucellosis in Iraq was 2.7%.

The present result was also in agreement with results of Kang et al. [13] who reported that the prevalence of B. canis in dogs was 8.5%.

**DNA Extraction:**

The wizard genomic DNA purification Kit (Promega/ USA) is used to DNA isolation from the blood in a short time. All isolates showed bands, which indicated the genomic DNA on Agarose Gel Electrophoresis. As seen in figure (1).



**Figure(1): Agarose gel electrophoresis of genomic DNA on 1%agarose gel, (5 Volt/30 minute).**

***23S rRNA* Genetic Polymorphism.**

One and half µl of genomic DNA were used for each PCR reaction. A conventional PCR protocol was used to analyze simultaneously the presence of *23S ribosomal RNA (23s RNA) gene* of *Brucella Canis*. The presence of the *23s RNA* gene was identified by 214 bp, as shown in figure (4.2).

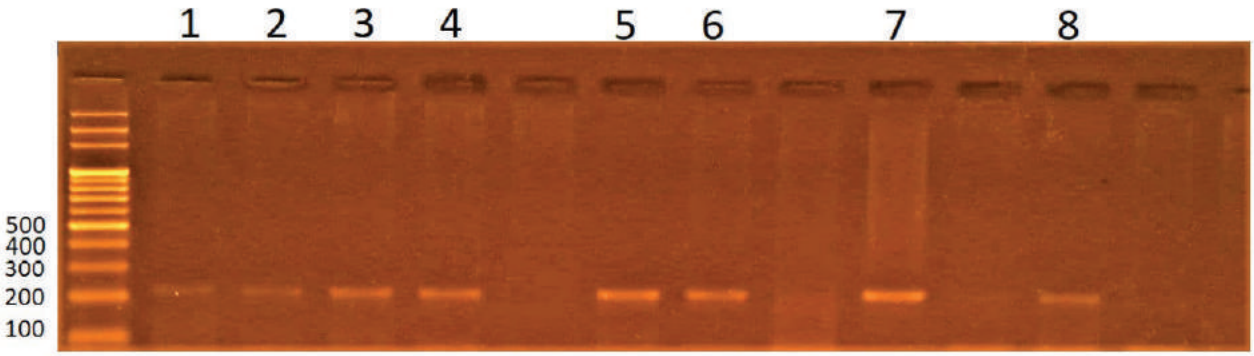



**Figure (2):- Agarose gel electrophoresis for *23S ribosomal RNA* gene (214bp) of *Brucella Canis*. Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5 volt/cm2, 1X TBE) and visualized under U.V light after staining with Ethidium bromide stain. Lane M: DNA ladder (100bp).**

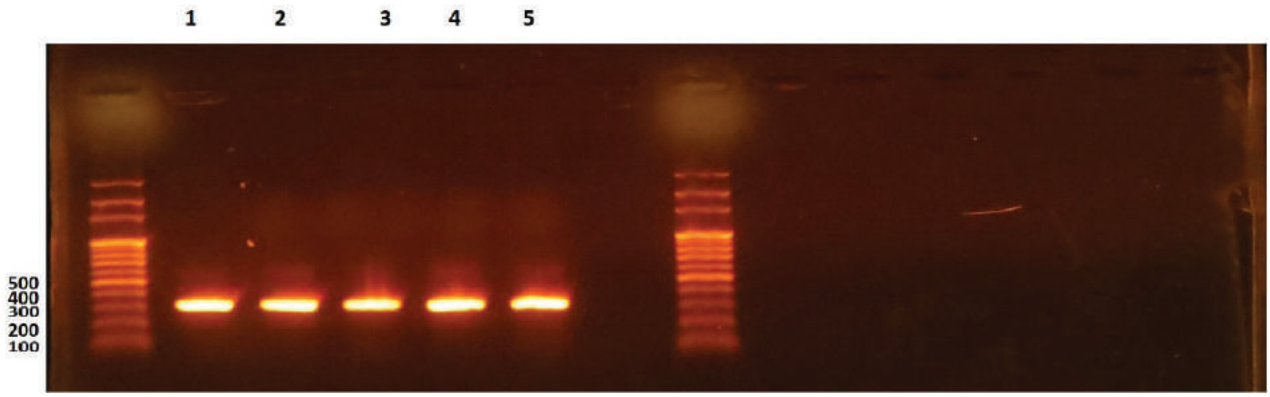


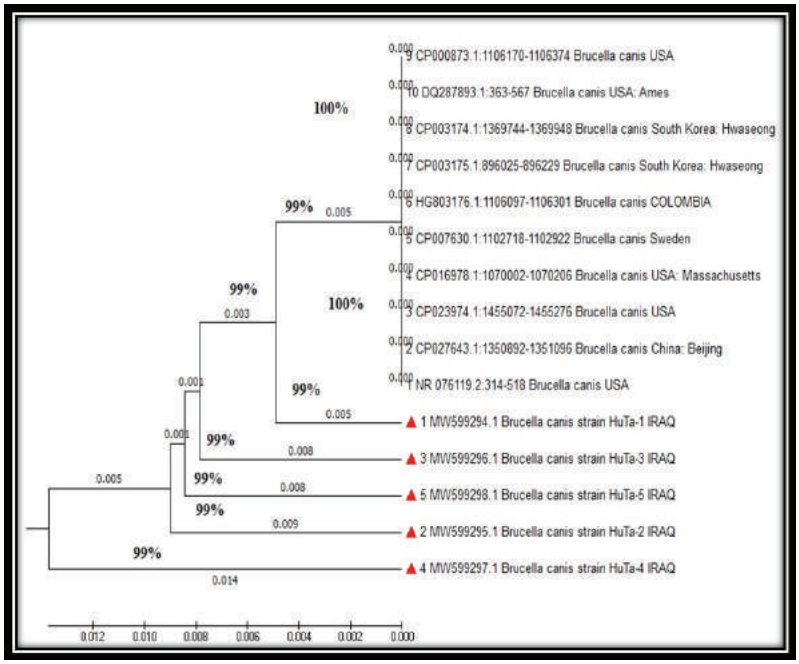

**Figure (3):- Agarose gel electrophoresis for *B0548 ribosomal RNA* gene (300bp) of *Brucella Canis*. Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5 volt/cm2, 1X TBE) and visualized under U.V light after staining with Ethidium bromide stain. Lane M: DNA ladder (100bp).**

Keid *et al*. (2007) using of 23S ribosomal RNA gene for detection of *B. canis* by using PCR and they found that the percentage was 30.5%.

It has been found that polymerase chain reaction is positive for *B. canis* DNA indicated by only amplification of 214 bp product using of 23S ribosomal RNA gene (ARAS *et al*., 2015).

**Phylogenetic tree structuring of Brucella Canis**

When comparison between Brucella Canis isolated from dogs. recorded in the National Center Biotechnology Information (NCBI) and isolated from different source have under sequence (ID: NR_076119.2, MW599295.1, CP023974.1, CP016978.1, CP007630.1, HG803176.1, CP003175.1, CP003174.1, CP000873.1, DQ287893.1) respectively with source of isolation and showed compatibility the highest identity (99%). As seen in figure (4).




**Figure(4.): Neighbor-joining tree of Brucella Canis 23S rRNA gene.**

**Submission of local Iraq isolate in NCBI.**

The 23S ribosomal RNA gene were registered after the correspondence of the National Center for Biotechnology Information and obtained accession number and became a reference to Iraq and the Middle East and the world. Ongoing work will add to this set as more type strains are published and it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/ MW599294.1, MW599295.1, MW599296.1, MW599297.1, MW599298.1 of Brucella Canis.

## Conclusions

1. The prevalence of Brucellosis in dog in Baghdad city must be taken into consideration because the samples were collected randomly.

2. Molecular method (PCR), is a good idea for confirmation of diagnosis of Brucellosis in dog.

3. 99% compatibility in the sequences of *Brucella canis* in dog in different isolates in our study recording to the National Center Biotechnology Information (NCBI).

4. Our results about 23S ribosomal RNA gene will adds as more strain types are published and it becomes available for downloading at NCBI.

**Ethical Clearance::** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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# Extraction of Outer Membrane Proteins of *Proteus Mirabilis* Isolated From Urinary Tract Infections and their Immunological Effect *In Vitro*

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## Abstract

300 urine samples were collected from patients visiting Fallujah city hospitals (consulting clinics of Fallujah Teaching Hospital and Women and Children Hospital) for the period from 1/9/2020 to 30/12/2020, of different ages and for both sexes. The results showed that out of 300 urine samples were 244(81.33%) A sample with a positive result for bacterial culture, and it was found there were high significant differences between the positive and negative cultures. Females and males (64.70) (35.29), respectively.

Isolates of *Proteus mirabilis* were diagnosed by observing the cultivar (colonies) and microscopic (bacterial cells) characteristics, biochemical tests and diagnostics with Vitek device. Antibiotic sensitivity were done at 12 types, Antibiotic resistance wre vary from Cefotaxime, Rifampicin, Ceftriaxon (100%), Cefixime, Ciprofloxacin (76.4%), Levofloxacin (64.7%), Nitrofurantoin (58.8%), Gentamicin (52.9%), Amikacin, Nalidixic acid (35.2%) (17.6%) Imipenem (11.7%).

Extraction and partial purification of outer membrane proteins (OMPs) from the most antibiotic-resistant isolate using lysozyme, DNase, RNase, and N-Lauroyl-Sarcosinate enzymes. associated with the ion exchanger.

To study the effect of the antigen of outer membrane proteins, some immunoassays were performed *in vitro* and the following results appeared: The use of concentrations (100, 50, 25) of OMPs antigen led to low significant of lymphocyte viability percentage (96.66, 97.66,98.0), respectively compared to With the negative control (99.66), the concentrations (100,50)% significantly reduced the percentage of survival of PMNs cells (97.66,98.33), respectively, compared with the control (99.66), while the concentration of 25 did not affect the survival of PMNs cells (99.66). The above concentrations showed a low significant in the diameter of the migration circuit of PMNs cells (8.830,10.59,12.21) compared with the negative control (15.21), while these concentrations led to a high significant in the percentage of formazone-forming PMNs cells (64.66,58.0,47.0) compared with the control (36.66). ) and a high significant in the sensitivity of lymphocytes (4.0,3.39,1.87), as the absorption spectrum values for concentrations (100,50,25) reached (0.92,0.74,0.41), respectively, compared with the negative control (0.22) and the positive control (1.53). The concentrations of the used proteins increased the phagocytic index (PI) of PMN cells ,The phagocytosis coefficient was high significantly with time until it reached its maximum at 90 minutes, and from the statistical analysis it was found that the phagocytosis coefficient increases with increasing concentration of proteins.

*Keywords: Proteus mirabilis, urinary tract infections*

## Introduction

*Proteus* spp. Part of the natural flora in the intestines of humans and animals and spreads in water and soil as a result of pollution [1]. They are Gram-negative bacilli and part of the family Enterobacteriaceae, It is moved by flagella and is characterized by the phenomenon of swarming. There are more than one species belonging to this genus, the most important of which is *P. mirabilis*, which is a common cause of urinary tract infections. These bacteria possess many virulence factors, fimbriae, motility, enzymes such as urease, toxins such as hemolysin, and factors of avoidance or immune evasion, and they are among the opportunistic pathogens responsible for many infections and nosocomial infections, especially in immunocompromised patients [2].

The outer membrane (OM) is found in gram-negative bacteria and is composed of an asymmetric bilayer, with phospholipids inward and lipopolysaccharide (LPS) outward. This asymmetry is important to maintain a tight permeable barrier. Almost half of the outer membrane mass is protein. It consists of integral OMPs and inwardly anchored lipoproteins [3]. Outer membrane proteins perform essential functions such as adhesion and nutrient uptake, are essential for maintaining the integrity and permeability of bacterial membranes, play an important role in bacterial virulence, and are powerful immune components. They are highly immunogenic and therefore would be an effective materials for vaccine development [4]. Activation of cellular and humoral immunity depends on the nature of antigens, and complex proteins such as bacterial outer membrane proteins can successfully activate both cellular and humoral immunity [5].

## Materials and Methods

### Sample collection and culture

300 urine samples were collected from clinical cases during the period from 1/9/2020 to 30/12/2020 from patients with urinary tract infection or suspected cases, according to the diagnosis of the specialist doctor from Fallujah General Hospital, Women's Hospital and private laboratories for different age groups and for both sexes. Collection of samples The first drops of urine are neglected and the mid -stream urine of it is taken in special sterile tubes, then the urine samples were transferred to the laboratory for the purpose of culture and diagnosis, t was cultured on petri dishes containing MacConkey medium as well as solid blood agar medium and incubated at 37 °C for a period of 18-24 hours. Diagnosis of bacteria as well as the using of the Vitek 2 compact device for the purpose of confirming the diagnosis of genus and species .

### Antibiotic sensitivity test

The sensitivity of the isolates was tested by the disc method using twelve antibiotics including (Amikacin, Cefixim, Levofloxacin, Ciprofloxacin, Imipenem, Nitrofurantoin, Cefotaxime, Ceftriaxone, Norfloxacin, Gentamicin, Nalidixic acid, Rifampicin) according to [6].

### Extraction and purification of outer membrane proteins

The method was followed by [7] in extracting outer membrane proteins (OMP), which includes the use of enzymes DNase, RNase, Lysozyme and N-Lauryl sarcosinate, and the cells were broken down using an sonicator, after which several centrifugation steps were performed, and the protein solution was concentrated Using polyethylene glycol, then membrane sorting was carried out against the same solution, then membrane filtration of the protein solution was carried out using membrane filters with holes with a diameter of 0.22 μm, and then proteins were purified by ion exchange chromatography, DEAE-Cellulose column was prepared according to

[8], the proteins that were extracted in the previous step were added to a DEAE-cellulose column that was titrated using Tris-HCl buffer 0.05M, then the column was washed with an equal volume of the same buffer, and the proteins were gradually filtered using gradual concentrations of sodium chloride( 0.9-0.7-0.5-0.3-0.1) NaCl, the flow rate in the column was 4 ml/part and the absorbance of each fraction was measured at 280 nm, the protein concentration was estimated according to Bradford method [9].

### Immunological experiments

The method of [10] was adopted to isolate PMNs from blood and method of [11] to isolate lymphocytes from blood, and the method of [12] was used to study the effect of outer membrane proteins on nitro blue tetrazolium reduction test (NBT test). In order to study the effect of outer membrane proteins on the viability of lymphocytes and the viability and migration of PMNs under Agarose the method of [13] and method of [10] was adopted to study the effect of outer membrane proteins onThe external phagocytosis of the heat-killed Candida yeast. The method of [14] was also based on the effect of outer membrane proteins (OMPs) on lymphocyte transformation assay.

## Results and Discussion

17 *Proteus mirabilis* isolates were identified from among the 300 urine samples. The isolates were diagnosed by their culture characteristics, as they were characterized by an odor resembling the smell of rotting fish, and they grew on MacConky agar medium. Blood Agar in order to observe the diagnostic character of bacterial colony, which is the phenomenon of swarming phenomena, as diagnosed based on the results of biochemical tests. All of these isolates were positive for urease, catalase, methyl red, citrate consumption and H2S gas production, and negative for the oxidase, indole and vogas – proskaur tests as shown in Table(1)

**Table (1): Results of Biochemical Tests for Diagnosing *Proteus* spp.**

| Biochemical tests | Results |
|---|---|
| Catalase | + |
| Oxidase | – |
| Urease | + |
| TSI | Acid butt/Alkaline slant<br>H2S + |
| Indole | – |
| Methyl red | + |
| Vogas – Proskaur | – |
| Citrate utilization test | + |

The Vitek2-Compact System was used to confirm the diagnosis of bacterial isolates isolated from clinical samples (urinary tract infections patients). This device performs 64 biochemical tests necessary for diagnosing bacterial isolates.

**Antibiotic sensitivity test**

An antibiotic sensitivity test was conducted for 17 isolates of *P. mirabilis* bacteria that were isolated from UTI patients, 12 types of antibiotics were used. The results in the in figure(1), which shows the percentage of resistance, sensitive and intermediate sensitivity by Measure the zone of inhibition around each disc used.

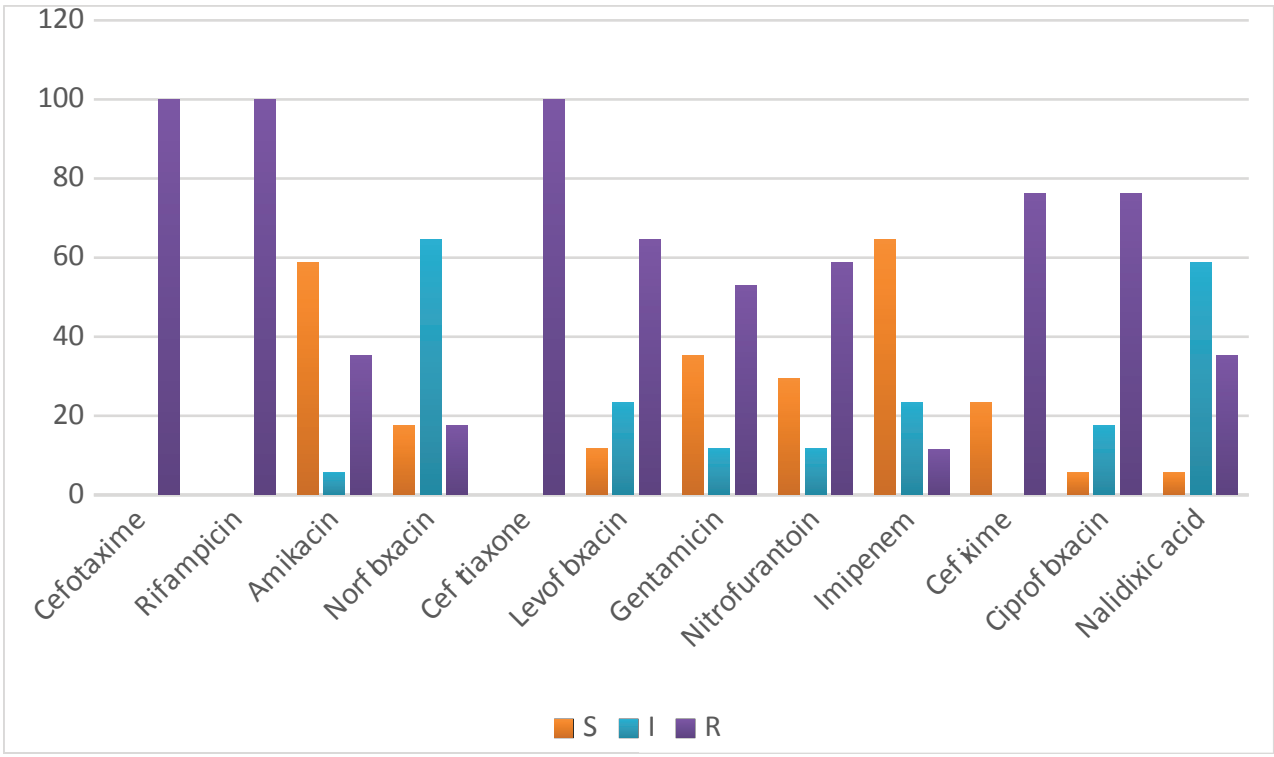


**Figure (1), which shows the percentage of resistance, sensitive and intermediate sensitivity of *P. mirabilis* isolates ( S : Sensitive, I : Intermediate , R: Resistant)**

The results of this study showed that all isolates were resistant to the Cefotaxime and Ceftriaxone (100%), which is of the third generation of cephalosporins, while Cefixime, which is also of the third generation of cephalosporins, had a resistance rate of (76.4%) and the isolates also showed resistance (100%) For Rifampicin, as for Ciprofloxacin, the resistance was (76.4%), and the resistance to Levofloxacin reached (64.7%), , and the resistance to Nalidixic acid, , was also 35.2% while the resistance to Norfloxacin was (17.6%). As for the anti-Nitrofurantoin, the results of the current study showed a resistance (58.8%). As for the resistance to Gentamicin, a group of aminoglycosides, it was (52.9%), and the resistance to Amikacin, which It also belongs to the group of aminoglycosides (35.2%) .Imipenem, which is from the Carbapenem group Most of the isolates are sensitive to it, reached (11.7%).

Extraction of the outer membrane proteins of *Proteus mirabilis*

Outer membrane protein antigen was extracted from isolate PM8, DNase, RNase enzymes were added to reduce the viscosity of the solution by breaking DNA, RNA [15]. On it (peptidoglycan layer), where it works to cleave the murine, thus weakening the binding of peptidoglycan to the proteins of the outer membrane, and this makes the exposure greater to the detergent that was used in the extraction process. Triton X100 detergent also does not dissolve outer membrane proteins and selectively dissolves the inner-membrane protein [16].

**Ion exchange chromatography**

The resin used in this step is Diethylaminoethylcellulose (DEAE-cellulose). The nature of this resin allows anionic proteins to bind and cationic proteins to cross. The binding of the protein to the resin depends on the PI value of the protein and the PH of the buffer solution that has been created. Used for equilibrated resin, DEAE-cellulose resin has many advantages including ease of handling, good separation, high accuracy and the ability to be reused several times and the principle on which it is based is charge difference [17]. Use a column with dimensions (2X20) cm for ion exchange chromatography of the extract of outer membrane proteins of *Proteus mirabilis*, where washing was carried out by means of a buffer and the fractions were collected at a rate of 4 ml per part at a flow rate of 30 ml / hour, then the proteins bound to the exchanger were eluted using gradual concentrations of sodium chloride (0.9- 0.7- 0.5-0.3-0.1) NaCl, and the amounts of protein in the fractions were followed by a . reading Absorption at a wavelength of 280 nm using a UV – Visible spectrophotometer, where a peak of proteins not bonded with the ion exchanger appeared as shown in Figure (2). The protein concentration was estimated according to the Bradford method [9] drawing the standard curve between the concentrations of BSA versus the absorbance at 595 nm, and the results showed that the concentration of the extracted protein was 0.6 mg/ml.

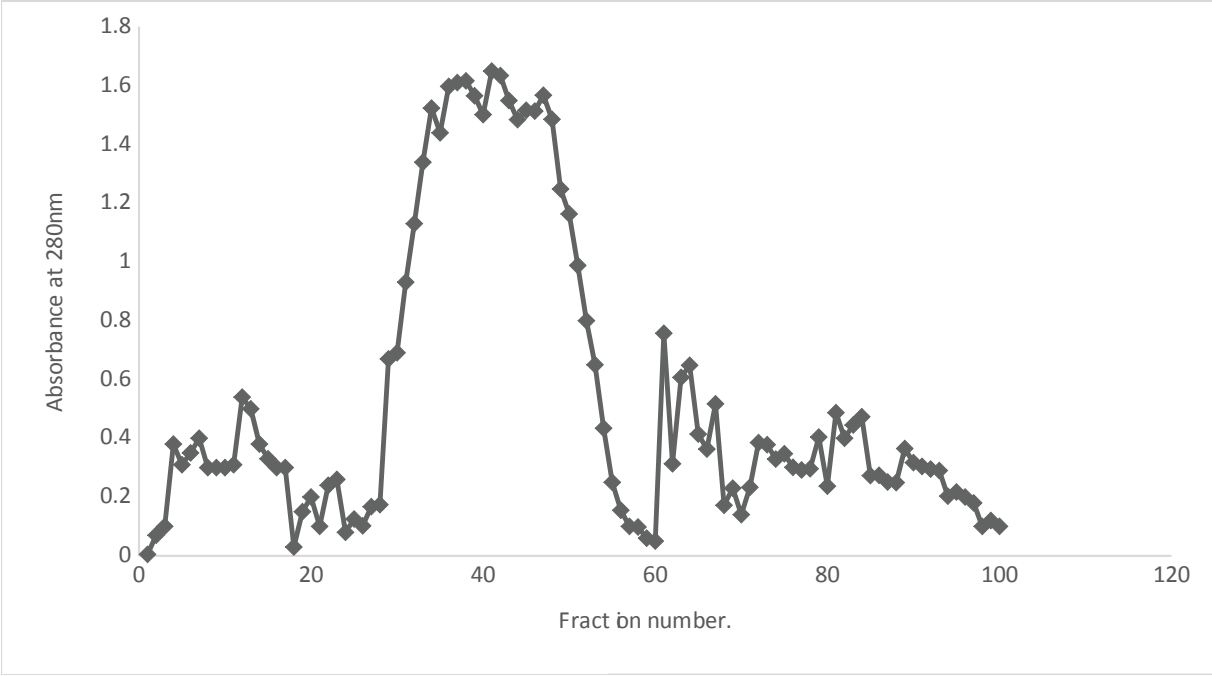


**Figure (2) Ion exchange chromatography of *P. mirabilis* outer membrane protein extract by DEAE-Cellulose column equilibrated by Tris-HCl buffer 0.05M and elution by salt gradient 0.1-0.9 NaCl in a 20X2 column at 30 ml flow rate. /hour.**

**Effect of OMPs antigen on the viability of PMNs and lymphocytes**

The results of the statistical analysis showed that the 25% concentration had no significant effect on the viability of PMNs cells. As for the concentrations (100,50), it was found that there is a low significant effect compared to the negative control, as the viability of phagocytic cells decreased from (99.667) in the treatment of Control to (97.667, 98.333) when using concentrations (100,50), respectively, as shown in Table (2).

**Table (2) Effect of OMP antigen on the viability of PMNs . macrophages**

| Concentration of OMP (%) | Percentage of survival of PMNs (average ± standard deviation) |
|---|---|
| Zero ( control) | 99.667b ± 0.5774 |
| 25 | 99.667b ± 0.5774 |
| 50 | 98.333a ± 0.5774 |
| 100 | 97.667a ± 0.5774 |

* Different letters within the column indicate significant differences at (P<0.05) level.

* Similar letters within the column indicate that there are no significant differences at the level (P<0.05).

As for Lymphocyte cells, the results of the statistical analysis in Table (3) showed that all the concentrations used led to a low significant in the level of viability of lymphocytes, as rate reached (96.66, 97.66, 98) compared to the viability of the control (99.66). Al-Dahan [18] found it, showing that the OMPs antigen extracted from *Moraxella catarrhalis* led to a low significant in the survival rate of both lymphocyte and phagocytic polymorphonuclear cells (PMNs). Cells lose their selective permeability and consequently cell death [19].

**Table (3) Effect of OMP antigen on the viability of lymphocytes**

| Concentration of OMP (%) | Percentage of survival of lymphocytes (average ± standard deviation) |
|---|---|
| Zero ( control) | 99.667c ± 0.5774 |
| 25 | 98.000b± 0.5774 |
| 50 | 97.667b ±0.5774 |
| 100 | ± 96.667 a 0.5774 |

* Different letters within the column indicate significant differences at (P<0.05) level.

* Similar letters within the column indicate that there are no significant differences at the level (P<0.05).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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# Impact of Psychosocial Domestic Violence upon Reproductive Health during Corona Virus Pandemic among Women Attending Primary Health Care Centers in Baghdad City

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## Abstract

**Objectives:** To assess Psychosocial domestic violence among women and assess the impact of Psychosocial domestic violence on women's reproductive health during the COVID-19 pandemic

**Methodology:** A descriptive analytical study was conducted on women subjected to psychosocial domestic violence and impacts of psychosocial domestic violence on women during the Corona pandemic period. A deliberate sample of (150) women exposed to violence was selected by a member of her family.

**Results:** The result of the study showed that the highest percentage (79.4%) of the study sample members ranged in age from (20 to 34) years, most of whom were housewives, three quarters of the study sample had a low educational level. Psychosocial domestic violence was at a higher level of relative sufficiency.

**Conclusions:** The results indicated that all study sample suffer psychosocial domestic violence during childbearing age, but most of the study sample considered psychosocial violence to be the most influential on their lives than other effects.

**Recommendations:** The study recommends that women be screened for psychosocial domestic violence during childbearing age. psychosocial domestic violence topics into education curricula, using social media, the availability of health services, and supporting the strengthening of cooperation between social agencies, justice and the police through law enforcement and research to promote and protect women's rights.

***Keywords****: Impact, Psychosocial Domestic Violence, Reproductive Health and Corona Virus Pandemic.*

## Introduction

Domestic violence is defined as physical, sexual, emotional, financial, or psychic harm or threats directed towards another person. It encompasses any act that isolates, frightens, terrorizes, coerces, threatens, harms, injures, or wounds another person, or even negatively controls them[1]. Women suffer substantial short and long-term physical, mental, sexual, and reproductive health difficulties as a result of intimate relationship abuse (physical, sexual, emotional, and sexual violence). They also have an impact on their children and result in significant social and economic costs for women, their families, and society [2].

Psychosocial Violence (also known as psychological or mental abuse) is a pattern of behavior that threatens, intimidates, or undermines the victim's self-worth or self-esteem while also restricting the victim's independence. Emotional abuse is a tough sort of domestic violence for many people to comprehend because it appears to be rather frequent in unstable relationships on the surface. Preventing the victim from conversing to anyone unless they have "permission"; preventing the victim from leaving the house are just a few instances. Threatening the victim with violence or using emotional blackmail to get the victim to do something the abusive partner doesn't like[3].

Some types of violence have been linked to a greater risk of adverse mental health outcomes among women. The most prevalent include depression, suicide attempts, post-traumatic stress disorder, other stress and anxiety disorders, sleeping or eating disorders and psychosomatic disorders[4].

During the covid-19 epidemic, there have been reports of growing domestic violence rates all around the world. Because ordinary people are forced to stay at home around the world, women and girls are more vulnerable to intimate partner abuse, domestic violence, and other forms of gender-based violence. As a result, domestic violence is still a severe threat to Iraqi women and girls [5].

## Methodology

### Design of the Study

A descriptive-analytical research design was carried out through interview women of their reproductive age who suffering from psychosocial domestic violence. It was conducted in the primary health care centers in order to determine the effects of psychosocial domestic violence during the Coronavirus pandemic from the period 26th Jan to 28Feb 2021.

### Ethical Consideration

1- the Approval was obtained from the Ethical Committee for Scientific Research of the University of Baghdad/ College of nursing.

2-The researcher provided verbal and written consent to obtain consent from each woman to participate in this study before the questionnaire was conducted.

### Setting of the Study

The study was conducted at ten primary health care centers (five health centers out of eighty-seven major health centers in Al-Karkh district), and (five health centers out of one hundred and four major health center in al-Rusafa district) in Baghdad city. These health centers were chosen randomly (Simple random sampling

### Sample of the Study

The non-probability sample (purposeful sample) consisted of one hundred and fifty (150) women who were victims of pychosocial domestic violence who entered primary health care centers in Baghdad City during the period from 26th Jan. to 28th Feb 2021 as shown in the table (3-2).

### Study Instrument (Tools)

The instrument was designed and constructed by the investigator after reviewing related literature, clinical background and previous studies which were consisted of sis parts.

### Methods of Data Collection

Data were collected through the use of questionnaires and individuals interviewing technique from during 26th Jan – 28th Feb 2021. All study samples were interviewed by the investigator, the interviews were carried out in private setting (empty rooms) in the absence of their husband or their relative

especially their mothers-in-law.

## Data Analysis

In order to determine whether the objectives of the study have met or not, The statistical procedures were used under the application of statistical package of social sciences(SPSS) version 23 in the analysis of the data of the study

## Results and Discussion

**Part I. Demographic characteristics (Table 1):-**

**1. Age**

The present study revealed that the most of the study sample (79.4%) were childbearing age with in (20-34) years. The mean age and standard deviation of the study sample was (28.9∓5.7) years This data is line with study conducted by Gebrewahd (2020) who reported that 682 women more than (84%) of respondent who suffered domestic violence were below 30 years of age with mean age and standard deviation of the respondents was (29.7± 5.7)[6].

**2. Level of education of study sample.**

Educationally, three-quarter of the study sample who suffered from domestic violence had graduated from intermediate school graduate or less.

**3. Occupation**

Regarding employee most (88.6%) of the study sample were housewife (that means they have not jobs), while (8.7%) of them were government employed and (2.7%) of them were self-employed, This study was consistent with study conducted by Yaya and others (2019) who stated that (81%) they do not have a jobs and (19%) they have a jobs [7].

**4. Monthly income**

Regarding the monthly income, more than half (55.3%) of the study sample had low monthly income while (34.2%) of the study sample had moderate monthly income and (10.7%) their monthly income was good This result is consistent with the study conducted by Gebrewahd (2020) who stated that (52%) of the study sample, had a low monthly income, while (31.2%) of them had moderate monthly income and (15.8%) of them had a good monthly income[6].

**5. Type of family**

Regarding the family type, (46%) of the study sample were nuclear families, while (54%) of them and were lived in the extended and sharing family This finding is in agreement with the study was done by Hussain and others (2020) who stated that (48.8%) of study sample were nuclear families, while (51.3%) were joint families. Among 160 married women in Pakistan suffered from domestic violence [8].

6. Domestic violence has increased in light of the corona pandemic

The finding of the study concerning domestic violence has increased in light of the corona pandemic (74.7%) three-quarters of the sample experienced increased violence in light of the corona pandemic, while (25.3%) of study sample did not increase violence against them This finding is in agreement with study was done by Boserup and other (2020) In the wake of the COVID-19 pandemic trends regarding domestic violence. Reports during the COVID-19 outbreak in China's Hubei province, indicate that domestic violence tripled during February 2020 compared to February 2019[9].

**Part II. Reproductive characteristics(Table2):-**

**1. Duration of marriage**

The highest percentage (66.7%) of the study sample their duration of marriage were ranged between (5-9) years, while the lowest percentage (5.3%) their duration of marriage was fifteen years and above and also there was a statistically significant association

between impacts of psychosocial domestic violence with the duration of the marriage .This finding is in agreement with the study was done Avanigadda (2021) who stated that more than two-thirds of the study sample were married between one and nine years facing one type of domestic violence[10].

**2. Gravidity**

The highest percentage (60.6%)of the study sample range between two and five pregnancies , while the lowest percentage (10.7%) of them were primigravida .The study sample had large family which may increase the financial burden on their husbands.

This finding is in agreement with study done by Aolymat (2020) a cross-sectional study was conducted in Jordan the sample consisted of 200 women stated that The mean and standard deviation number of pregnancies of the study sample was 2.9∓1.9 pregnancy[11].

**3. Parity**

The result of the study showed that (80.7%) of the study sample have children between two to more than five children .This finding is in agreement with the study was conducted by Dadras and other (2020) who stated that (81.1%) have children between two to more than five children among 424 Afghan women suffered from domestic violence[12].

**4. Number of abortion**

Regarding abortion, half (52.4%) of the study sample did not have any previous history of abortion during their childbearing life, while (47.6%) of the study sample which considered a high percentage had a previous history of abortion range between one to three and more .The previous history of abortion was due to domestic violence by their husbands. It had been reported that women who abuse during pregnancy are at risk for miscarriage. This finding is in agreement with the study done by Defilipo and others (2020) who stated that three-quarters of the study sample was no history of abortion, while the rest have a history of abortion among 771 women who participated in the study[13].

**5. Number of stillbirth**

Regarding the number of stillbirths, (85.3%) of study sample did not have a history of stillbirth, while (14.7%) had one stillbirth This finding is in agreement with the study was done by Dadras and others (2020) who stated that (92.5%) of the study sample did not a have history of stillbirth[12].

**6. Number of a live child**

Regarding number of a live child more than three quarters (78.6%) of the study sample have from one to four live children, while (6.1%) of study sample did not have a child This result is consistent with study was conducted by Gebrewahd (2020) who stated that (89.6%) of the study sample have live children one to four live children. Every additional child above the average of two children was associated with a higher risk of unwanted pregnancy which was significantly related to intimate partner violence[6].

**Discussion Psychosocial violence(Table 3):-**

The grand mean score of psychosocial domestic violence was (2.249) with relative sufficiency (78%), as while the percentage of the total research is (30%), which is the highest value than the rest of the types of violence shown in table (4-7).

This finding is consistent with a study conducted by Hameed and others (2020) the number of participants was (323 women) who stated that the prevalence of lifetime intimate partner violence against women was 58.5% and emotional violence is the commonest type. Verbal violence also included insulting a person alone or in front of others, threats of divorce, questioning, the use of arbitrary speech to

address women, demanding a dowry, and insulting the woman's personality[14].

**Discussion Impacts of Psychosocial Violence (Table 4):-**

Violence against women is a public health problem with an epidemic proportion, which permeates the whole world, putting women's health at risk, limiting their participation in society and causing great human suffering [13].

**1. Physical impacts**

The grand mean score of physical impacts of domestic violence was (1.937) with relative sufficiency (64.5%), while the percentage of the total research is (16%)

WHO (2021) who stated that physical violence health effects include headaches, pain syndromes (back pain, abdominal pain, chronic pelvic pain) gastrointestinal disorders, limited mobility and poor overall health[2].

**2. Psychosocial impacts**

The grand mean score of psychosocial impacts of domestic violence was (2.093) with relative sufficiency (70%), while the percentage of the total research is (18%) %), which is the highest value than the rest of the impacts of violence

This result is consistent with a cross-sectional survey was conducted by Al-Jawhara Alquaiz and others (2021) in Saudi Arabia, and the total sample was 1883 married Saudi women. The lifetime prevalence of any type of violence was 43%. The most common type was the control of woman's behavior (36.8%), trying to distance her from friends (13.7%), neglecting and treating indifferently (20.7%) trying to restrict contact with family (10.3%), taking permission before seeking health care (21.8%), insults and making feel bad (17.6%), intimidation or intimidation (14%), and threats to harm (4.3%). Social conditions and social relationships are largely related to domestic violence against women[15].

**3. Sexual impacts**

The grand mean score of sexual impacts of domestic violence was (1.982) with relative sufficiency (66%), while the percentage of the total research is (17%)

A study was conducted by Shahali and others (2020) who stated that sexual violence direct (physical) and indirect (psychological) has consequences against women. Sexual violence, itself, has many physical and emotional consequences Physical consequences include trauma, somatic problems, serious injuries, pregnancy, sexually transmitted infections, social isolation behavior, and sexual victimization. While psychological or mental consequences include the attempted suicide, depression, post-traumatic stress disorder (PTSD), stress, anxiety, sleep disorders, eating disorders, substance abuse, self-harm, panic attacks, quality of life, and self-esteem. and also it has negative impacts on society [16].

**4. impacts related to menstruation**

The grand mean score of menstruation impacts of domestic violence was (1.903) with relative sufficiency (63%), while the percentage of the total research is (16%)

This result is consistent with the study was by Aolymat (2020) who stated that (20.5%) of the participants suffered from increased domestic abuse during the COVID-19 pandemic. The average weight of the participants was significantly increased during the total curfew compared with the 6 months before COVID-19 (70.1% versus 68.6%). Before the pandemic, 17.5% of the women had menstrual aberrations; however, during the curfew, this proportion was decreased to 10.5%. Indicating

that the total curfew was associated with decreased reproductive tract infections, And that is due to the increase in personal hygiene for fear of COVID-19, which reduced disease[11].

**5. impacts related to pregnancy and labor**

The grand mean score of pregnancy and labor impacts of domestic violence was (1.847) with relative sufficiency (61%), while the percentage of the total research is (16%)

WHO (2021) stated that Intimate partner violence in pregnancy increases the likelihood of miscarriage, stillbirth, pre-term delivery and low birth weight babies. Where study showed that women who experienced intimate partner violence were (16%) more likely to suffer a miscarriage and (41%) more likely to have a pre-term birth. these forms of violence can lead to depression, post-traumatic stress and other anxiety disorders, sleep difficulties, eating disorders, and suicide attempts[2].

**6 . impacts related to postpartum**

The grand mean score of postpartum impacts of domestic violence was (1.958) with relative sufficiency (65%), while the percentage of the total research is (17%). A study was conducted by Bhatta and others (2018) surveyed respondents (165 women) reported that the prevalence of domestic violence against women during the postpartum period was 20%. Domestic violence can happen to any woman, regardless of age, economic status, race, and educational background [17].

**Conclusions:** The results indicated that three quarter study sample suffers from the impact of psychosocial domestic violence on them during childbearing age

**Recommendations:** The study recommends that women be screened for any type of domestic violence during childbearing age. Incorporating domestic violence topics into education curricula, using social media, the availability of health services, and supporting the strengthening of cooperation between social agencies, justice and the police through law enforcement and research to promote and protect women's rights.

**Table (1): Distribution of the total sample according to the socio-demographical characteristics (N = 150).**

| Socio-demographic variables | Groups | No. | % |
|---|---|---|---|
| women's age groups $\bar{x} = 28.9 \mp 5.7$ | 20-24 | 39 | 26 |
| | 25-29 | 57 | 38.1 |
| | 30-34 | 23 | 15.3 |
| | 35-39 | 26 | 17.3 |
| | 40-44 | 5 | 3.3 |
| women's level of education | Illiterate | 7 | 4.7 |
| | Read & write | 15 | 10 |
| | Primary school graduate | 58 | 38.7 |
| | Intermediate school graduate | 34 | 22.6 |
| | Secondary school graduate | 3 | 2 |
| | Institution graduate | 18 | 12 |
| | University graduate and above | 15 | 10 |

***Cont...*** **Table (1): Distribution of the total sample according to the socio-demographical characteristics (N = 150).**

| | | | |
|---|---|---|---|
| Wife's occupation | Housewife | 133 | 88.6 |
| | Government employed | 13 | 8.7 |
| | Self employed | 4 | 2.7 |
| Monthly Income | Enough | 16 | 10.7 |
| | Almost enough | 51 | 34 |
| | Not enough | 83 | 55.3 |
| Family Types | Nuclear family | 69 | 46 |
| | Extended family | 54 | 36 |
| | Sharing family | 27 | 18 |
| Domestic violence has increased in light of the corona pandemic | Yes | 112 | 74.7 |
| | No | 38 | 25.3 |

**Table (2): Distribution of the study sample according to reproductive characteristics (N= 150)**

| Psychosocial domestic violence | Responses | No. | % | MS | RS% | Ass. |
|---|---|---|---|---|---|---|
| It makes you feel like an unwanted person | Always | 95 | 63.3 | 2.37 | **79 | M |
| | Sometime | 16 | 10.7 | | | |
| | Never | 39 | 26 | | | |
| Ignores her and treats indifferently | Always | 102 | 68 | 2.50 | **83 | M |
| | Sometime | 21 | 14 | | | |
| | Never | 27 | 18 | | | |
| Anger when talking to another person and suspicious of betrayal | Always | 106 | 70.7 | 2.54 | **85 | M |
| | Sometime | 19 | 12.6 | | | |
| | Never | 25 | 16.7 | | | |
| Bullying the body | Always | 64 | 42.7 | 2.19 | *73 | L |
| | Sometime | 50 | 33.3 | | | |
| | Never | 36 | 24 | | | |
| Insults and talks badly about her family | Always | 93 | 62 | 2.45 | **82 | M |
| | Sometime | 32 | 21.3 | | | |
| | Never | 25 | 16.7 | | | |
| Blames her for violent behavior or tells her that her deserve | Always | 95 | 63.3 | 2.49 | **83 | M |
| | Sometime | 33 | 22 | | | |
| | Never | 22 | 14.7 | | | |
| Tries to insult her in front of others | Always | 33 | 22 | 1.62 | 54 | NR |
| | Sometime | 27 | 18 | | | |
| | Never | 90 | 60 | | | |
| Prevents her from communicating with your family or friends | Always | 102 | 68 | 2.54 | **85 | M |
| | Sometime | 27 | 18 | | | |
| | Never | 21 | 14 | | | |

***Cont...*** **Table (2): Distribution of the study sample according to reproductive characteristics (N= 150)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Did not sharing with family decisions | Always | 107 | 71.4 | 2.56 | **85 | M |
| | Sometime | 20 | 13.3 | | | |
| | Never | 23 | 15.3 | | | |
| Refuses to meet the basic necessities of the family | Always | 77 | 51.3 | 2.28 | *76 | L |
| | Sometime | 38 | 25.3 | | | |
| | Never | 35 | 23.4 | | | |
| Behavior control | Always | 120 | 80 | 2.69 | ***90 | H |
| | Sometime | 14 | 9.3 | | | |
| | Never | 16 | 10.7 | | | |
| Does not help her to visit a doctor when needed | Always | 28 | 18.7 | 1.76 | 59 | NR |
| | Sometime | 58 | 38.6 | | | |
| | Never | 64 | 42.7 | | | |
| Overall Evaluation Psychosocial violence: MS(2.249), RS(78), %(30) | | | | | | |

Table (3): Distribution of the study sample according to Psychosocial domestic violence

MS: Mean for total score, RS: Relative sufficiency, Ass: Assessment, NR: No response , Cut of point = 2 *Low= 66.67 - 77.78, **Moderate= 77.78 - 88.89, ***High= 88.89 – 100

**Table (4): Distribution of the study sample according to all impacts of psychosocial domestic violence (N=150).**

| Domains | MS | RS | % |
|---|---|---|---|
| Physical impacts | 1.937 | 64.5 | 16 |
| Psychosocial impacts | 2.093 | 70 | 18 |
| Sexual impacts | 1.982 | 66 | 17 |
| Menstruation impacts | 1.903 | 63 | 16 |
| Pregnancy and labor impacts | 1.847 | 61 | 16 |
| Postpartum impacts | 1.958 | 65 | 17 |
| Overall Evaluation | 1.953 | 65 | 100 |

**MS: Mean for total score, RS: Relative sufficiency**

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# Thiopurine S-Methyltransferase Genotyping in Iraqi Childhood Acute Lymphoblastic Leukemia Patients ; A Single Institute Study

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## Abstract

**Background:** Acute lymphoblastic leukemia (ALL) is the most common cancer seen in children worldwide and is the most common cancer in children under 14 years of age. Although there have been major advances in treatment approaches for childhood ALL, serious toxicities such as profound leukopenia frequently affect treatment and lead to life threatening consequences such as severe infections and even death. There has been a lot of interest in inter-individual differences in drug metabolizing enzymes in order to better adjust drug dosage and therapy. In this regards, Thiopurine S-Methyltransferase *(TMPT)* was the first pharmacogene that showed a substantial association with Mercaptopurine (6-MP) maximum tolerated dose and 6-MP related toxicities leading to the implementation of *TPMT* genotyping before drug administration **Aim**: To identify the most common TPMT polymorphism (TPMT*3A, TPMT*3B and TPMT*3C) and its frequencies in a sample of Iraqi ALL paediatric patients. **Methods**: A cross sectional study was performed for 79 patients with Acute lymphoblastic leukemia . Genotyping for (*3A , *3B , *3C ) the TPMT gene was performed by the allele-specific multiplex-PCR analysis method. **Results:** The TPMT*3A mutant allele was found in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of 6.3% . But TPMT*3B mutant allele was not detected in whole sample ALL patients .The correlation between gender and the polymorphism was not statically significant as p-value 0.23.. **Conclusion:** TMPT genotyping is an essential tool to reduce the cytotoxic effects of the anti-cancer drug 6-MP in Iraqi paediatric patients with ALL .

***Keywords:*** *6-Mercaptopurine* **,** *Acute lymphoblastic leukemia,* *neutropenia ,drug -toxicity*

## Introduction

Acute lymphoblastic leukemia (ALL) ,is a malignant disease in bone marrow where the early lymphoid predecessors proliferate and exchange the normal hematopoietic cells of the marrow. It's cancer of blood cells owing to an increase in the number of white blood cells, they are gathering out the red blood cells and platelets that is usually needed for the healthy body, and all those additional white blood cells cause problems . ALL is the most common type of cancer, e.g. in the United States about 2,400 children and adolescents diagnosed with ALL every year[1]. In a child with this type of leukemia, leukemia cells split up to make copies of themselves. These copies split over, again and again, producing more and more cells . Not like normal blood cells, leukemia cells don't die as they become old or damaged [2]. The 6-Mercaptopurine (6-MP) - is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug[2]. It is also known as 6-Methylmercaptopurine

or Thiopurine S-methyl .The 6-MP molecular formula is C5H4N4S and molecular weight of 152.175 g/ mol [3]. Thiopurine-S-methyltransferase (TPMT), is an enzyme that inactivates the drug. For instance, testing for specific decreased enzyme function polymorphisms prior to therapy, mainly *TPMT*2*, **3A*, **3B,* and **3C* has been included in several clinical guidelines and drug labels (PharmGKB, 2016; PharmGKB, 2018). (TPMT) catalyzes thiopurine and thiopyrimidine *S*-methylation, an important metabolic pathway for thiopurine drugs, such as *6*-t*hioguanine* (6-TG), and azathioprine, currently used to treat childhood acute lymphoblastic leukemia, .[4]

## Materials and Methods

A cross sectional study in Children Welfare Teaching Hospital, Medical City, Baghdad, including pediatric patients, aged less than 14, being treated for Acute Lymphoblastic Leukemia during Induction, consolidation and delayed intensification phases and before starting the continuation (maintenance) phase of treatment which involve the use of 6-MP drug as the backbone for a duration of 1-2 years. The study include 79 subjects was conducted to study the association of (**3A*, **3B,* and **3C* ) SNP of TPMT with Acute lymphoblastic leukemia. The patient population included 79 subjects( 30 girl and 49 boys ) with Acute lymphoblastic leukemia. Inclusion criteria constituted all patients with Acute lymphoblastic leukemia on chemotherapy (induction and consolidation phases) before starting maintenance phase of treatment, in which the 6-MP represent the back bone of treatment. Exclusion criteria represent any patient whose treatment was postponed due to Jaundice and / or impaired liver function due to hepatitis infection . Additionally, any patient who was recently received blood (within the last fourweeks) was excluded from the study

### Genotypic data

Peripheral blood samples of ALL were collected in EDTA-anticoagulant tube and DNA was extracted from whole-blood samples using the Reliaprep genomic DNA extraction Kit (Promega, U.S.A). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (Bio Drop, U. K.). Genotyping was performed using allele-specific multiplex-PCR analysis method. For TPMT gene using thermocycler (Biometra, Germany) was in use . The primer sequences were obtained from [5]: TPMT*1 F- 5′GTATGA TTT TAT GCA GGT TTG 3′ and R- 5′TAA ATA GGA ACC ATC GGA CAC 3 TPMT*3B F-5`-GGGACGCTGCTCATCTTCT-3` and

R-5`-GCCTTACACCCAGGTCTCTG-3` TPMT*3C F- 5`-AAGTGTTGGGATTACAGGTG-3`and R- 5`-TCCTCAAAAACATGTCAGTGTG-3` Amplification was performed in a total volume of 23 µlwhich contained 12.5 µl of Go Taq Green Master Mix, (Promega Corporation, Madison, WI), 1µl of each primer (One Alpha, U.S.A.),3.5 µl of nuclease free water and 5 µl of DNA template. PCR amplification consisted of initial-denaturation step at 95°C for five minutes followed by 35 cycles of denaturation at 95°C for one minute, annealing at the specified annealing temperature for two minutes, followed by extension at 72°C for one minute and the final extension step at 72°C for five minutes. The PCR amplicons were separated on 2% agarose gel through electrophoresis. The bands were visualized with an Ultraviolet (UV) transilluminator at 365 nm (Cleaver Scientific, UK). The TPMT*1 allele was analyzed by allele-specific PCR with an annealing temperature of 53°C for two minutes**.**Genotype analysis for G460 A point mutation at exon 7 was carried out by PCR assay with an annealing temperature of 59°C for two minutes .

## Statistical Analysis

Statistical analyses were done by using the SPSS version 22.0 for Windows, (SPSS Inc., Chicago, IL) [6].Genotype frequencies were tested using a Two-Sample Student's t-test to determine the difference in allele frequencies proportion in TPMT

## Results

The study included 79 ALL patients newly diagnosed including (30 girls , 38.0%), and (49 boys ,( 63.0%) .The three most common inactive alleles of the TPMT gene (TPMT*3A, TPMT*3B, and TPMT*3C) were sought. The TPMT*3A mutant allele was found in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of 6.3% . But TPMT*3B mutant allele was not detected in the sample population as shown in table 1

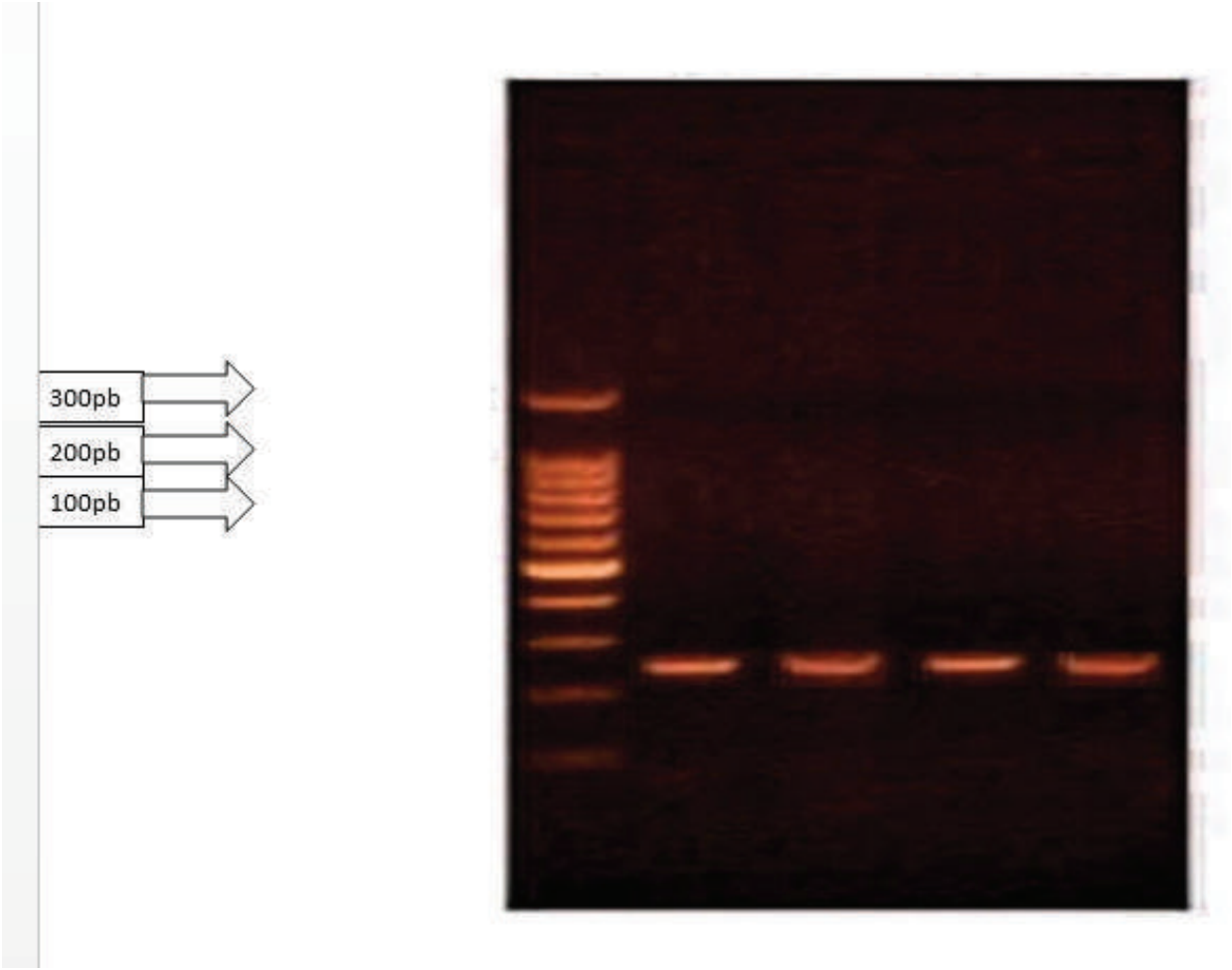
**Table 1: Genotype frequencies of TPMT variation in a sample of 79 ALL patients**

| Allele | SNP Position | rs | Amino acid Substitution | N | Frequency % |
|---|---|---|---|---|---|
| TPMT*1 | Wild-type | | | 56 | 70.9% |
| TPMT*3A | G460A<br>A719G | 1800460<br>1142345 | Ala 154 Thr<br>Tyr 240 Cyc | 18 | 22.8% |
| TPMT*3B | G460A | 1800460 | Ala 154 Thr | 0 | 0.00 |
| TPMT*3C | A719G | 1142345 | Tyr 240 Cyc | 5 | 6.3% |
| Total 79 | | | | | |

Result of digestion with allele-specific multiplex-PCR analysis for TPMT gene (TPMT*3A, TPMT*3B and TPMT*3C) ) including 245

bp band for the wild-type of TPMT*1 as shown in fig 1 , for the (TPMT*3C ) genotype 273 pb and two bands 273 and 338 pb for TPMT*3A as shown in fig.2.

**Figure 1 product of TPMT gene polymorphism analyzed by agarose gel electrophoresis.**



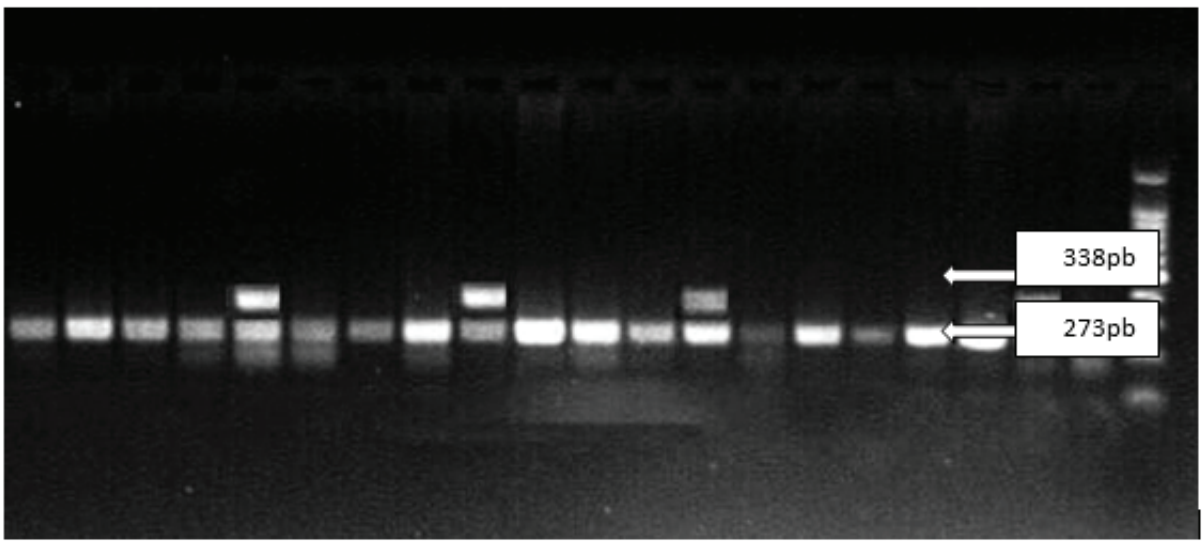




**Figure 1. product of TPMT gene polymorphism analyzed by agarose gel electrophoresis .two fragments of 338 and 273 bp corresponds to TPMT*3A polymorphism, a single fragment of 273 bp corresponds to TPMT*3C polymorphism**

## Discussion

TPMT is one of the best examples of the application of pharmacogenetics to clinical practice involving the genetic polymorphism. Treating TPMT-deficient patients with standard doses of mercaptopurine (6MP), thioguanine or azathioprine can be fatal [6] , [7] such patients can be successfully treated, without severe toxicity, if the dose is properly adjusted [8]. For this reason this study dealt with ALL patient which are newly diagnosed. Clinically it is well-known that patients with TPMT wild-type of non genetic factors (are still risk for thiopurine related hematotoxicity [9]. and even after consideration of non-genetic factors (e.g. concomitant medication with the XO inhibitor allopurinol or viral infection ) underlying mechanisms are so far unknown to completely explain thiopurine-related hematotoxicity [10]. This study revealed that the prevalence of TMPT genetic polymorphism was higher in boys than in girls. There were 23 patients carrying TPMT mutant alleles (3A,3C) ; 10 out of 30 girl (33.3%) and 8 out of 49 (16.3%) have TPMT*3A and 1out of 30 (3.3%) and 4 out of 49 (8.1%) have TPMT*3C , the correlation between gender and the polymorphism was not statistically significant as p-value 0.23. This result is similar to the Korean study and a study on Bulgarian population which showed that there was a significant gender-related difference in TPMT enzyme activity [11, 12].

The most common variants are reported were *3A,*3B, and *3C all involving G460A and/or A719G .[13, 14] So, the present study focused on the detection of TPMT*3A and TPMT*3B and TPMT*3C alleles were detected in 23 patient out of 79ALL pediatric patients .TPMT*3A was detected in 18 patients with allele frequency of (22.8 %), while TPMT*3C mutant allele was detected in 5 children with allele frequency of (6.3%) . on other hand TPMT*3B mutant allele was not detected in the sample population , these findings were similar to studies study in Libyan [15] and other study in Iraq [16] . This differs from the findings among black Africans such as the Ghanaian and Kenyan populations, among whom only TPMT*3C was detected [9]

Furthermore, In Iraqi population, it was found that TPMT*3A mutant allele had a higher frequency than TPMT*3C mutant allele contribution to the overall frequency, 22.8% and 6.3%respectively. These outcomes are similar to other researches on the Caucasian population in which the TPMT*3A is the dominant allele as compared with TPMT*3C mutant allele [11].

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# The Association of Low Taurine Levels with Diabetic Neuropathy in Iraqi Patients

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## Abstract

**Aim of this Study**: this study has been carried out to evaluate the role of taurine, and lipid profile in progression of diabetic complication.

**Methods:** this work included 81 Iraqi people (age ranged 40-65). Healthy control subjects (G1 group=28), type 2 diabetes mellitus without any complications (G2 group=26), and type 2 diabetes mellitus with peripheral neuropathy (DPN) (G3 group=27). Diagnosis of the disease was based on several symptoms and questions under the supervision of the consultant supervisor at the center. Serum taurine, lipid profile (Cholesterol, triglycerides, HDL, LDL, and VLDL) besides fasting blood glucose and plasma HbA1c were determined for each participant. **Results:** The results of taurine revealed that there was a highly significant decrease in G2 and G3, as compared to G1. The mean values of serum cholesterol showed no significant difference between G2 and G3 as compared with G1while serum triglycerides (TG), LDL and VLDL showed a high significant increase in G2 and G3 as compared to G1. The results of serum HDL levels showed that there was a high significant decrease in G2 and G3 as compared to G1. No significant differences were found in body mass indices in all the studied groups while WC, HC, WHR, and WHtR showed high significant increase in G2and G3 as compared to G1. Systolic blood pressure significantly increased in G2 and G3 while diastolic blood pressure significantly increased in G3 only.

**Conclusion:** Serum taurine levels were decreased in patients with T2DM and DPN .The reducing in taurine synthesis was not related to FBG, HbA1c, and dyslipidemia. We also found that taurine levels may be more important in the development of diabetes complications.

***Keywords:*** *Type 2 DM, diabetic peripheral neuropathy, taurine, dyslipidemia.*

## Introduction

Diabetes mellitus is a heterogeneous metabolic disorder that is characterized by the presence of hyperglycemia that happened due to impairment of defective insulin action, insulin secretion, or may be both. The chronic hyperglycemia of diabetes mellitus is correlated with long term microvascular complications affecting the nerves, eyes, kidneys, as well as an increased risk for cardiovascular disease (CVD)[1].Type 2 diabetes is a serious and common chronic endocrine disorder with risk factors such as obesity and sedentary lifestyle that is constitute a major worldwide public health [2]. The interaction between some of genetic and environmental factors results in a heterogeneous and progressive disorder with variable degrees of beta cell dysfunctions and insulin resistance. The β cells when have no longer ability to secrete sufficient insulin to overcome

insulin resistance, impaired glucose tolerance progresses to type-2 diabetes[3]. Diabetic peripheral neuropathy (DPN) is one of the most known chronic complications of type two diabetes and is defined as a neurological damage in patients with diabetes mellitus and it is a condition where blood supply to nerves in the feet or hands is impaired causing the nerve to malfunction and eventually die out and its cause burning pain, aching or numbness, pins and needles sensation. It affects 30% - 50% of diabetic patients .Taurine (2-aminoethanesulfonic acid) is essential naturally occurring beta-sulphonated amino acid which is not utilized in protein synthesis and never incorporated into muscle proteins, so it's found as a free molecule in the body or as simple peptides along with methionine and cysteine [4]. Increasing evidence suggested that taurine might play a role in improving DPN because of its ability to prevent SCs apoptosis. The main pathological feature of diabetic peripheral neuropathy is myelin sheath damage of peripheral nerve that was accelerated by schwann cells (SCs) apoptosis[5]. A recent study confirmed that taurine significantly lowers the blood glucose levels, reduces insulin resistance and dysfunctional nerve condition in diabetic mice[6]. Lipid abnormalities are commonly found in patients with diabetes mellitus, this abnormality called dyslipidemia which is one of the major risk factors that's leads to cardiovascular disease in diabetes mellitus. The characteristic features in lipid of diabetic patients with type two are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles[7], [8]. Cholesterol is one of the lipids and major sterol in animals, is both precursor to a wide variety of steroids and structural component of membranes. It is an essential component of the cellular membranes determining the fluidity and biophysical properties by increasing the compactness and lowering the permeability. It is also a source of bioactive molecules such as vitamin D, bile acids and steroid hormones [9]. Taurine changes the abnormal blood lipid profile that is associated with the diabetic condition. It was proved that elevated plasma triglycerides and LDL cholesterol in diabetics were lowered through administration of taurine [10]. Taurine negatively affects lipid levels by reducing lecithin cholesterol acyltransferase activity [11]. Taurine significantly reduced both resting systolic and diastolic blood pressure and these finding indicate that taurine can reduce the incidence of CVD by ~ 6% in both hypertensive and normotensive individuals [12] , [13] .

## Materials and Methods

**Study groups include** 28 control subjects (G1) and 53 Iraqi patients with type 2 diabetes(age from 40-65) and the patients classified into two subgroup: group (G2) (with a duration 2-8 years) including patients with type 2 diabetes without complication (T2DM) and group (G3) (with a duration 2-10 years) including patients with type 2 diabetes with peripheral neuropathy complication (DPN) .The patients enrolled in the study were attending to National Diabetes Center/ Al-Mustansiriya University during the period from December 2019 to march 2020 under the supervision of Dr. Firas younus Muhsin. Diagnostic criteria for the patients based on the American

Diabetes Association (ADA) (FBG≥ 126 mg/dL, plasma glucose≥200 and HbA1C≥6.5%) [14]. In this study we excluded patients with type one diabetic patients, type two diabetic patients who are taking insulin as a hypoglycemic therapy, nephropathy and retinopathy, patients with cardiovascular disease and those having liver diseases and other known diseases which are associated with glucose metabolism disordered. The control group was matched with the patients' groups in age, BMI and gender.

## Methods

Blood samples were obtained by venipuncture

from each individual about five ml after (10-12) hours of fasting. The blood sample was divided into two portions; 2 and 3 ml. The first portion was dispensed in tube containing ethylene diamine tetra acetic acid (EDTA) which used for the estimation of HbA1C. While the second portion was dispensed in a gel tube and left to clot at room temperature. The gel tube was centrifuged at (3000 r.p.m) for 10 minutes to collect serum which is used for estimation taurine, lipid profile (Cholesterol, TG, and HDL). Taurine was measured by using enzyme linked immunosorbent assay (ELISA) using the commercially available ELISA kit (Mybiosource, U.S.A). All procedures were carried out according to the manufacturer's instructions, Cholesterol (HUMAN, Germany), Triglycerides (TG) (HUMAN, Germany), and HDL (HUMAN, Germany). LDL was calculated from this equation: LDL-C= [Cholesterol - (T.G. /5) - HDL-C]. VLDL was calculated from this equation: VLDL-C (mg/dl) = Triglyceride/5 [15].

## Statistical Analysis

The data was statistically analyzed by SPSS software program version 22. The variables were reported as means ± standard deviation. The groups were compared by using one-way ANOVA and post hoc Tukey test, with a P value of <0.05 indicating the statistically significant difference.

## Results

The results of our study presented in Table (1) revealed that fasting blood glucose (FBG) showed a high significant increase (p=0.000) in G2 (154.38±50.61 mg/dl ) and G3 (220.88±91.58mg/dl) as compared to G1(86.61±5.12mg/dl), while G2 (p=0.000) showed a highly significant decrease as compared to G3(154.38±50.61mg/dl vs. 220.88±91.58mg/dl ).For HbA1c the results showed a high significant increase (p=0.000) in G2(8.09±1.29 % ) and G3 (8.72±1.86 % ) as compared to G1 (4.82±0.56 %) ,while there was no significant different (p=0.210) between G2 and G3

The results found in Table (1) revealed that the mean values of Taurine levels showed highly significant decrease (p=0.000) in G2 (1.78 ± 1.26ng/ml) and G3(1.55±1.99ng/ml) as compared to G1(3.13±0.64ng/ml). While, there was no significant between (G2) (p=0.818) and (G3) (1.78±1.26ng/ml vs. 1.55±1.99ng/ml).

The mean values of serum cholesterol showed no significant differences between G2 (182.77±38.66 mg/dl, p=0.059) and G3(178.22±40.17 mg/dl, p=0.160) as compared to G1 (161.10±21.48mg/dl), also there were no significant differences (p=0.880) between G2 and G3. For serum triglycerides (TGs) the results showed a high significant increase in G2 as compared to G1 (246.46±150.33 mg/dl, p=0.000) and in G3 (217.44±79.65 mg/dl, p=0.003) as compared to G1(128.68± 12.05 mg/dl) and there was no significant difference (p=0.524) between G3 and G2. HDL levels showed a high significant decrease in G2 (41.29±7.34 mg/dl, p=0.000) and in G3 (40.38±6.93 mg/dl, p=0.000) as compared to G1(57.57± 11.92 mg/dl) and there was no significant difference (p=0.929) between G3 and G2.

**Table 1: The characteristics of participants FBG, HbA1c, Taurine, Cholesterol, TG, HDL, LDL, VLDL among different groups (n=81).**

| Variables | G1 (Mean±SD) Control N=28 | G2 (Mean±SD) T2DM N=26 | G2 (Mean±SD) DPN N=27 | p-value | |
|---|---|---|---|---|---|
| FBG (mg/dl) | 86.61±5.12 | 154.38±50.61 | 220.88±91.58 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.000** |
| HbA1c (%) | 4.82±0.56 | 8.09±1.29 | 8.72±1.86 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.210 |
| Taurine(ng/ml) | 3.13±0.64 | 1.78±1.26 | 1.55±1.99 | G1*G2 | 0.002* |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.818 |
| Cholesterol(mg/dl | 161.10±21.48 | 182.77±38.66 | 178.22±40.17 | G1*G2 | 0.059 |
| | | | | G1*G3 | 0.160 |
| | | | | G2*G3 | 0.880 |
| TG (mg/dl) | 128.68± 12.05 | 246.46±150.33 | 217.44±79.65 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.003** |
| | | | | G2*G3 | 0.524 |
| HDL(mg/dl) | 57.57± 11.92 | 40.38±6.93 | 41.29±7.34 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.929 |
| LDL(mg/dl) | 78.64±21.94 | 100.62±35.94 | 100.15±36.84 | G1*G2 | 0.037* |
| | | | | G1*G3 | 0.040* |
| | | | | G2*G3 | 0.998 |
| VLDL(mg/dl) | 25.76±2.51 | 46.95±29.89 | 43.43±15.68 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.003** |
| | | | | G2*G3 | 0.784 |

A high significant increase was found in LDL levels of G2 (100.62±35.94 mg/dl, p=0.000) and of G3(100.15±36.84 mg/dl, p=0.000) as compared to G1(78.64±21.94 mg/dl) but there was no significant difference (p=0.998) between G2 and G3. Serum VLDL showed a high significant increase in G2 (46.95±29.89, p=0.000) and G3 (43.43±15.68 p=0.003) as compared to G1 (25.76±2.51 mg/dl), but there was no significant difference (p=0.784) between G3 and G2.

Table (2) showed the results of anthropometric measurements and indicated that BMI had no significant difference between G2 (30.62±6.33 kg/$m^2$, p=0.490), and G3 (32.26±6.47 kg/m2, p=0.078) as compared to G1(28.79±4.63 kg/m2), either there were no significant differences

(p=0.564) between G2 and G3. For the waist circumference (WC) the results showed a high significant increase in G2 (109.54±9.51 cm, p=0.000) and G3 (111.11±11.05 cm, p=0.000) as compared to G1 (96.21±14.75 cm), but there were no significant differences between G2 and G3 (p=0.883). The hip circumference (HC) results showed that there were no significant differences between G1 (p=0.173) and G2 (110.39±12.67cm vs. 116.15±9.84 cm), but a significant increase in G3 (p= 0.034) was found as compared to G1 (118.41±12.25 cm vs. 110.39±12.67 cm), also there were no significant differences (p=0.763) between G2 and G3. The obtained results of WHR in G2 (0.94±0.03) and G3 (0.94±.074) showed a highly significant increase (p=0.003) as compared to G1 (0.87±0.09) but there was no significant difference (p=0.997) between G2 and G3. The results of WtHR showed a highly significant increase in G2 (0.69±0.095, p=0.000) and G3 (0.67±0.083, p=0.001) as compared to G1 (0.58±0.076) but there was no significant difference (p=0.545) between G2 and G3.

**Table 2: The characteristics of anthropometric measurements among different groups (n=81).**

| Variables | G1 (Mean±SD) Control N=28 | G2 (Mean±SD) T2DM N=26 | G2 (Mean±SD) DPN N=27 | p-value | |
|---|---|---|---|---|---|
| BMI(kg/m2) | 28.79±4.63 | 30.62±6.33 | 32.26±6.47 | G1*G2 | 0.490 |
| | | | | G1*G3 | 0.078 |
| | | | | G2*G3 | 0.564 |
| WC(cm) | 96.21±14.75 | 109.54±9.51 | 111.11±11.05 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.883 |
| HC(cm) | 110.39±12.67 | 116.15±9.84 | 118.41±12.25 | G1*G2 | 0.173 |
| | | | | G1*G3 | 0.034* |
| | | | | G2*G3 | 0.763 |

***Cont...*** **Table 2: The characteristics of anthropometric measurements among different groups (n=81).**

| | | | | | |
|---|---|---|---|---|---|
| WHR | 0.87±0.09 | 0.94±0.03 | 0.94±.074 | G1*G2 | 0.003* |
| | | | | G1*G3 | 0.003* |
| | | | | G2*G3 | 0.997 |
| WtHR | 0.58±0.076 | 0.69±0.095 | 0.67±0.083 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.001** |
| | | | | G2*G3 | 0.545 |
| SBP (mmHg) | 120±1.90 | 144.07±21.98 | 147.59±28.75 | G1*G2 | 0.000** |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.812 |
| DBP (mmHg) | 79.92 ± 2.01 | 85.23± 13.37 | 94.74±16.21 | G1*G2 | 0.248 |
| | | | | G1*G3 | 0.000** |
| | | | | G2*G3 | 0.015* |

The SBP results showed that there was a highly significant increase in G2 (144.07±21.98 mmHg, p=0.000) also in G3 (147.59±28.75 mmHg, p=0.000) as compared to G1 (120±1.90 mmHg) but there was no significant difference (p=0.812) between G2 and G3. The DBP results showed no significant difference (p=0.248**)** between G1 and G2 (79.92 ± 2.01 mmHg vs. 85.23± 13.37mmHg).

While there was a highly significant increase in G3 (p=0.000) as compared to G1 (94.74±16.21 mmHg vs. 79.92 ± 2.01 mmHg). In addition a highly significant decrease (p=0.013) was found in G2 as compared to G3 (85.23± 13.37 mmHg vs. 94.74±16.21 mmHg).

**Table (3): Pearson correlation of Taurine in (G2) and (G3) groups.**

| **parameters** | **Taurine(ng/ml)** | | | |
|---|---|---|---|---|
| | **G2 (T2DM group)** | | **G3 (DPN group)** | |
| | **r** | **p** | **r** | **p** |
| FBG (mg/dl) | -0.059 | 0.773 | -0.063 | 0.754 |
| Hba1c (%) | 0.163 | 0.426 | -0.106 | 0.599 |

***Cont...*** **Table (3): Pearson correlation of Taurine in (G2) and (G3) groups.**

| | | | | |
|---|---|---|---|---|
| Cholesterol(mg/dl) | 0.083 | 0.688 | -0.394 | 0.042* |
| TG (mg/dl) | -0.235 | 0.247 | 0.012 | 0.955 |
| HDL (mg/dl) | -0.042 | 0.839 | -0.034 | 0.867 |
| LDL (mg/dl) | 0.148 | 0.469 | -0.379 | 0.051 |
| VLDL | -0.341 | 0.088 | 0.018 | 0.928 |
| BMI (kg/m2) | 0.045 | 0.827 | -0.029 | 0.884 |
| WC (cm) | -0.491 | 0.011* | -0.031 | 0.878 |
| HC (cm) | -0.509 | 0.008** | -0.034 | 0.868 |
| WHR | 0.069 | 0.738 | -0.021 | 0.917 |
| WtHR | 0.212 | 0.298 | 0.020 | 0.921 |
| SBP (mm/Hg) | -0.234 | 0.250 | 0.063 | 0.755 |
| DBP (mm/Hg) | -0.305 | 0.130 | 0.039 | 0.846 |

**R, Pearson coefficient**

***Statistically significant at p ⩽ 0.05**

Table (3) showed the correlations of serum Taurine with other biochemical parameters in Type 2 diabetes mellitus (G2) and diabetic peripheral neuropathy (G3). The results showed that Taurine was correlated negatively in G2 with WC (r= -0.491, p=0.011) and also, a highly negative correlation was found with HC (r= -0.509, p=0.008). Taurine had a negative correlation with cholesterol in G3 (r= -0.394, p=0.042). While there was no correlation relationship between taurine and other parameters in G2 and G3 respectively.

## Conclusion

Serum taurine levels were decreased in patients with T2DM and this was not correlated with FBG, HbA1c, and dyslipidemia. Also, we found an inverse correlation between taurine with waist (WC) and hip (HC) circumference associated with increasing systolic and diastolic blood pressure, we think taurine can be used to predict the progression of diabetes in obese subjects considering the elevated cardiometabolic risk.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# Immunohistochemistry Detection Apoptosis Related with ORF Virus Infection in Sheep Based on Caspase 3 Detection from Selected Farms in Basrah

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## Abstract

This study was designed for the molecular detect of apoptosis related with ORFV infection in sheep based on detected caspase (3) by immunohistochemistry. The samples were taken from the skin of the lips of animals infected with contiguous ecthema. The ability of the virus to induce apoptosis was also verified using cellular immunohistochemistry and the use of a polyclonal anti-caspase (3) antibody to detect apoptotic activity. The results of the study revealed positive expression of caspase (3), where a high percentage of caspase (3) was observed in the affected cells in the epidermis of the lip, in addition to the presence of overexpression in all layers of the epidermis, with the presence of small areas of apoptotic cells compared with the control group.

***Keywords:*** *immunohistochemistry, detection apoptosis, caspase (3).*

## Introduction

Orf is an infectious disease Orf will affect many sheep farmers so that the production rate will fall. Orf is caused by Orf Virus belonging to the family Poxviridae is in the genus Parapoxvirus[1]. The virus can be transmitted Through direct association with an infected animal, or by contact with it infected environments or materials, like during grazing[2]. The virus makes entry into the host through broken and damaged skin and replicates in the skin cells leading to formation of lesions[3]. Orf virus is a major occupational health problem for farmers, butchers, veterinary staff and goat skinners because they are exposed to Orf virus by infected cattle or sheep, or contaminated objects[4].

Medical indicators of the Sheep ORF virus infection include elevated temperatures, decrease appetite , depression, eye and nose mucosal inflammation, respiratory distress, various stages of skin lesions (from erythema to scabs on lips ), and lymph node enlargement[5].

Clinically, the skin is slowly progressive development of crusts, development of redness and flares, development of vesicles, and finally forming pustules[6]. Orf is notable for having lesions, inflammations, ulcers, and papules in the lips and nostrils[7]. On histopathological observation of skin tissues, there is epidermal hyperplasia with hyperkeratosis, parakeratosis and acanthosis of the epidermis, showing multilocular vesicles with degenerating cells with phyllodes[8]. Lesions occur as a sequence of erythema, follicular eruption, papules, vesicles, pustules, or crusts located around the muzzle, cheek, tongue, oral mucosa, and on ears or nose, etc. In the same way, ankles, eyelids, and breasts[9]. The ORF virus or ORFV is the causal agent that encodes the 119

protein that induces apoptosis. ORFV is the causative agent that encodes an ORFV119 protein that promotes apoptosis. Orf virus interacts with antigen presenting cells (APC) and epidermal cell and induces apoptosis which is the killing of the cells[10,11]. Orf virus also utilizes ubiquitin proteasome signal pathway This is to protect the virus particles from maturation and then released to the outside[12].

Stimulate Overexpression of ORF-3a, ORF-3b and ORF-7a induce cell death. Prove that overexpression of ORF-6 also causes cell apoptosis. Also, demonstrate that apoptosis can be blocked by inhibitors of the Caspase-3 and NF-κB[13].

The Caspase 3 is part of caspase-like family of proteins which are involved in proteolysis of proteins by macromolecular machines called caspases. Caspase activation is of immense importance in triggering and checking the mechanism of apoptosis programmed cell death[14].

The role of the caspase 3 enzyme in apoptosis is mediated by both external (death ligand) and internal (mitochondrial) signaling pathways[15,16].

Therefore, the zymogen cleavage site of caspase-3 enzyme is important to ensure its regulation. Cleavage of caspase-3 occurs to initiate a caspase-3-dependent signaling cascade that causes cellular events following mitochondrial permeabilization[17]. One signaling event is the introduction of granzyme B, which activated one of the effector for caspases, in cells that target apoptosis by killer T cells. These events trigger the caspase cascade, which culminates in the death of the cellular organelle , Caspase-3 plays a significant role in caspase-3 catalyzed procaspase[18]. In intrinsic activation mechanism, cytochrome c combines with caspase-9, Apaf-1, and ATP, with which the process of procaspase-3 is initiated[19]. It was noticed in vitro study that the molecules have sufficient power to activate caspase-3. However, other regulatory proteins are very essential in vivo.

Apoptosis is a major form of cell death during development and viral infection, and has important roles in the disease response to pathogens. ORFV is the causative agent that induces programmed cell death in the late stages, it contributes to release virus.

Orf virus also exploits the ubiquitin-proteasome system (UPS) signal transduction pathways and then circumvents intracellular signal transduction, thereby activating CD8+ T, in order to protect virus particles toward maturation and release outward[12]. Overexpression of ORF-3a, ORF-3b and ORF-7a leads to apoptosis. This is indicative of demonstrating that overexpression of ORF-6 further induces apoptosis and that Caspase-3 and JNK inhibitor inhibit ORF-6-induced apoptosis[13].

## Material and Method

The immunohistochemistry analysis was performed using an immunohistochemistry kit, 2-step plus Poly-HRP Anti Rabbit/Mouse IgG Detection System with DAB Solution (Elabscience, E-IR-R213, China) and as per manufacturer's instruction. The kit contained a 3% $H_2O_2$ and Normal goat serum as blocking agents, Polymer Helper as linker, Poly-peroxidase-anti-Mouse/Rabbit IgG secondary antibody and diaminobenzidine (DAB). Anti-Caspase 3 primary antibodies were used in this study to detect the apoptosis activity.

Section tissue was washed in distilled water and submerged in a saline bath (pH 9) of Tris (TBS) for five minutes. Tissue Sections The tissue sections are then placed in a glass jar filled with Citrate Buffer Antigen Retrieval pH 6, which has been preheated to 60°C and incubated for 25 minutes in a water bath at 97°C.. Tissue sections were left in a glass container for 20 minutes at room temperature, then rinsed with distilled water and immersed in a tampon bath for 5 minutes. Excessive buffer was tapped into

the tissue section and then gently wiped with tissue paper around the sections. The napkin sections on the glass slides were surrounded by a circle of wax and this was done using a special wax pen (Gene Tech Pen, Elabscience, E-BC-R531, China) Ensure that only the tissue section of the slide was confined to the reactant.. The tissue sections were then immersed in a 3% $H_2O_2$ block solution (Elabscience, E-IR-R213A, China) As a reagent to block, Cover and incubate the sections in a humidity chamber for a period of 10 minutes, before rinsing the sections with distilled water and immersing them in a TBS bath (pH 9) for 5 minutes. Excess buffer on the tissue sections was then removed by tapping gently on the slides and also gently wiping them with paper towels around the sections. The tissue sections were the flooded with the normal goat serum (Elabscience, E-IR-R213D, China) As a blocking reagent, cover the sections and incubate them in a humidity chamber for 30 min, before rinsing the sections with distilled water and immersing them in a TBS bath (pH 9) for 5 min.

The tissue sections were then applied with 0.1 mL of Poly-peroxidase-anti-Mouse/Rabbit IgG secondary antibody (Elabscience, E-IR-R213C, China), it was incubated in a room with a humidity similar to room temperature for a period of 30 minutes, then rinsed with distilled water and then immersed in a TBS bath (pH 9) for 5 minutes. Excess buffer on the sections was then removed by tapping and wiping gently with a tissue around the sections. Tissue sections were worked and applied with 0.1 ml of DAB. chromogen (Elabscience, E-IR-R213E, China) diluted 20 folds with DAB substrate solution (Elabscience, E-IR-R213F, China), then it is incubated in a humidity room for a period of 5 minutes. Then, we worked on rinsing tissue sections with distilled water before immersing them in a temporary TBS bath for 5 min.

Tissue sections were then stained with Mayer's hematoxylin stain for three minutes, followed by rinsing using tap water. Then tissue sections were dehydrated in five changes of three minutes each of 50%, 70%, 80%, 90% and 100% ethanol, respectively. Then tissue sections were immersed in two changes (ten minutes each) of xylene and then mounted with fixing media (DPX) and covered with cover clips. Finally, tissue sections were examined under a light microscope at 100x, 200x, 400x and 1000x magnifications.

## Result and Discussion

Figure (1) represented the immunohistochemical section of non-infected sheep lips skin, showing the negative expression of Caspase3 in epidermis and dermis layer. Figures (2,3,4,5A) represented the immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip, the overexpression of Caspase-3 was observed in dermis layers, also, a spaces of apoptotic cells was observed in positive expressed areas.

As well as figures (5B,6A,6B) showing the positive expression of Caspase-3 (brown color) was observed in epidermis of lip and the overexpression of Caspase-3 (brown color) was observed in all epidermis layers, the overexpression of Caspase-3 was observed in apoptosis affected area of epidermis, also, a spaces of apoptotic cells was observed in positive expressed areas. All this figures above were treated with A&B: X400, anti-Caspase-3 antibody and hematoxylin.

Apoptosis is a multistep process by which the evolutionarily maintained and genetically controlled cell death in response to a variety of different origins, sources and stimuli that can act to send signals from the outside of the cell or from inside the cell. The mechanism of programmed cell death proceeds by a series of morphological changes and transformations that are mediated by the activation and stimulation of a specific cysteine protease called caspases[20].

Apoptosis is a physiological mechanism that controls cell numbers during development and the response to external infection, including the response to viral and bacterial infections[21]. Viruses have developed strategies either by inhibiting or by inducing apoptosis of host cells, depending on specific interactions between the virus and the host [22].

Apoptosis may also be beneficial at later stages of infection by reducing the host's inflammatory response and facilitating virus spread. Caspases are critical mediators of programmed cell death (programmed cell death). Among them, caspase-3 is a frequently activated death protease that stimulates the specific cleavage of several key cellular proteins[19]. caspase-3 plays a central role in mediating nuclear apoptosis[23].

In this paper, we report that ORFV expresses apoptotic activity and identify the caspase-3 in lips skin lesion of sheep. IHC tested for epidermis of infected animals reveal expression of Caspase-3 antibody , these result indicated that orf virus have able to induce Apoptosis in epidermis cells .and orf virus has capacity to activation Caspase-3 by up regulation of pro apoptosis proteins and down regulation of anti-apoptotic proteins compared with control animals that no expression apoptosis this agreed with that detect orf virus (ORFV), the causative agent of orf, began codes for a protein ORFV119 that induces programmed cell death. ORFV119 then targets mitochondria (energy houses) in host cells, thereby inhibiting cell proliferation, and inducing programmed cell death.

Viruses developed various strategies and methods to counter host defenses. Early in the infection, our result dis agreed with[24] Which reveals that viruses prevent programmed cell death in order to facilitate the reproduction that occurs within the cell environment. However, in the late stages of infection, viruses induce apoptosis in order to spread the virus to neighboring cells or tissues.

Caspases-3 was play critical roles in various apoptotic pathways. Apoptosis (programmed cell death), an important form of cell death, is considered to have a major function Defense against virus infection in the host cell.. Both pathways activate caspases, thereby releasing several apoptosis-related proteins that induce cell death[25]. Apoptosis isn't just close associated with the development and occurrence of many cancers or immune disorder but also serves to restrict viral multyplication in host cells[26].

The result of current study reveal positive expression of Caspase-3 was observed in epidermis of lip (dark brown stain). Also to The overexpression of Caspase-3 was observed in all epidermis layers with presence of small spaces of apoptotic cells compared to control groups. These data showed that ORFV could inhibit cell prolife ration and the host needs to remove infected cells to maintain this agreement in a prime condition[27,28].

They reveal occurrence of cell death in late stage of infection, these indicated to promote dissemination into other cells or adjacent tissues by viral particles in late stages of infection by breaking down infected cells.

The study confirmed the presence of cells in the process of programmed cell death and clarified their presence and location in the subcutaneous and dermal layers as well as follicles and sebaceous glands in contagious Ecthyma lesions, this result agreed with [10], who reported the findings of apoptosis in ORF virus cutaneous effected cell by draw attention to the importance of the process of programmed cell death. By at least one viral protein or several proteins that exhibit the modulation, and act as signals and triggers for the purpose of initiating the process of apoptosis.

The downstream caspases (Caspase-3) stimulate the cleavage of protein kinases, DNA repair proteins, cytoskeletal proteins, and finally the "management"

which is the destruction of cellular functions. Caspases also influence and regulate cell cycle, signaling pathways, cytoskeleton architecture, and this ultimately leads to the morphological manifestations of apoptosis, eg membrane hypertrophy, DNA condensation and fragmentation[29]

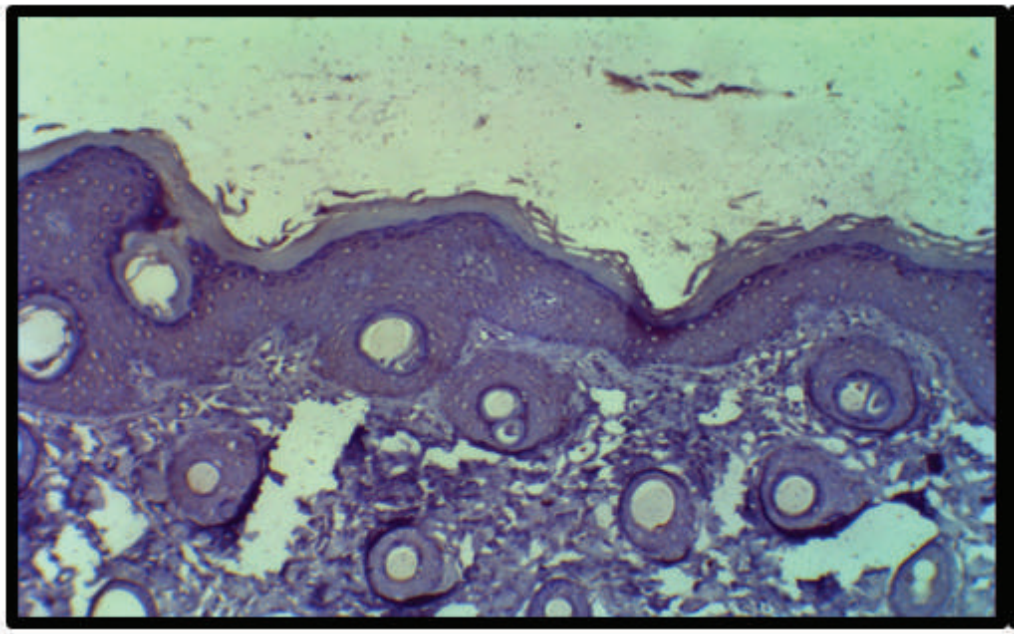

**Figure 1. Immunohistochemical section of non-infected sheep lips skin, showing the negative expression of Caspase3 in epidermis and dermis layer. (X100, anti-Caspase-3 antibody & hematoxylin).**

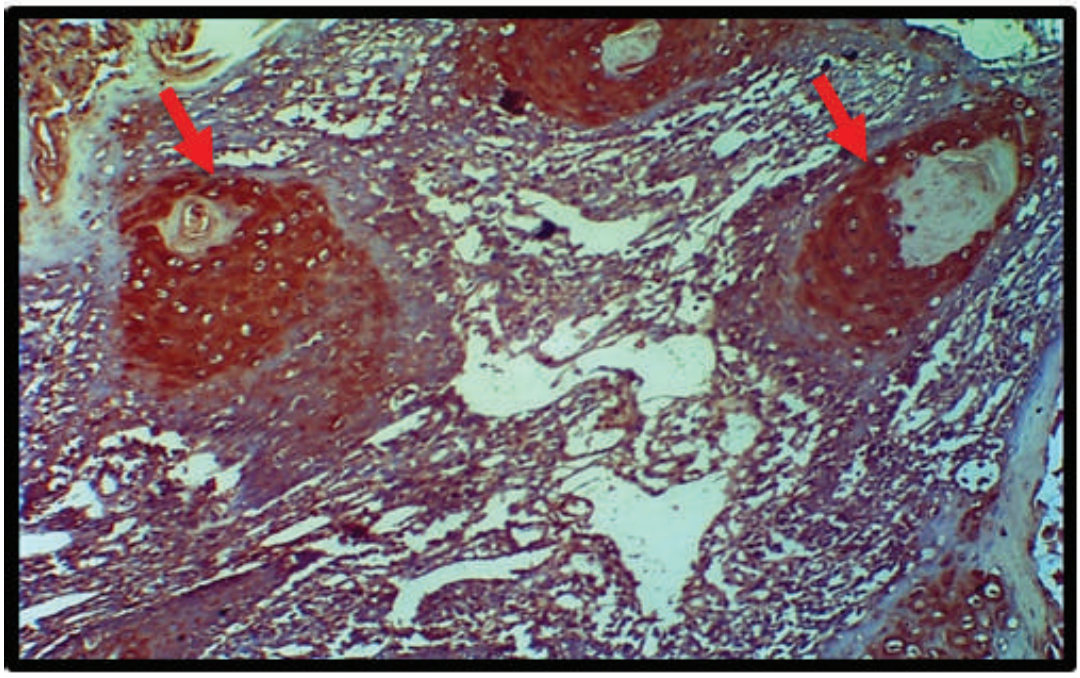

**Figure 2. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. (X100, anti-Caspase-3 antibody and hematoxylin).**

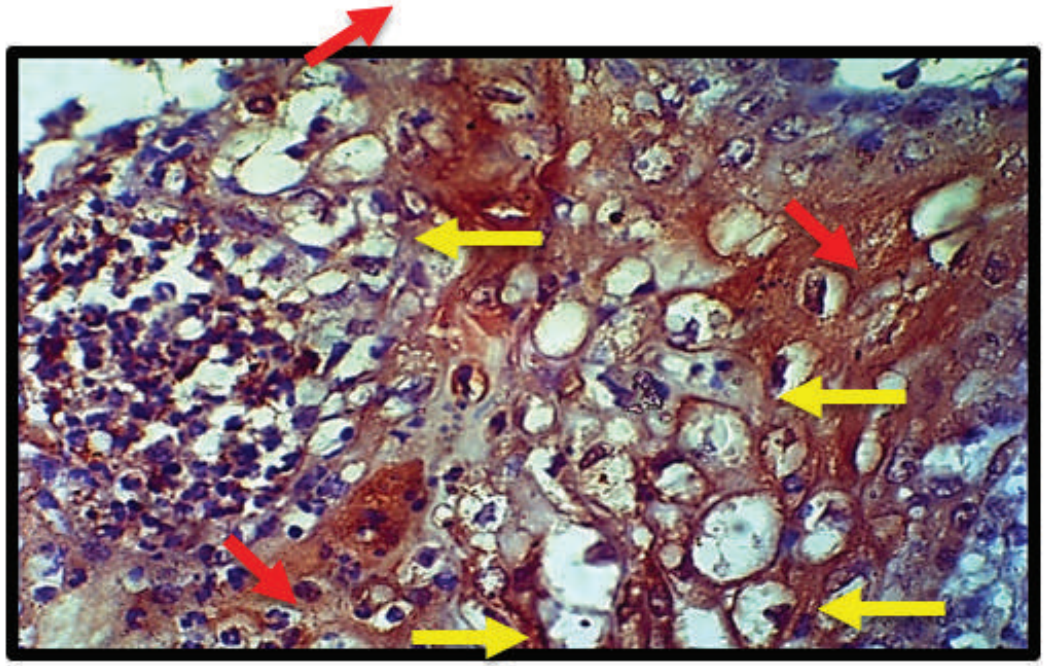

**Figure 3. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. Also, a spaces of apoptotic cells was observed in positive expressed areas ( ). (A&B: X100, anti-Caspase-3 antibody and hematoxylin).**

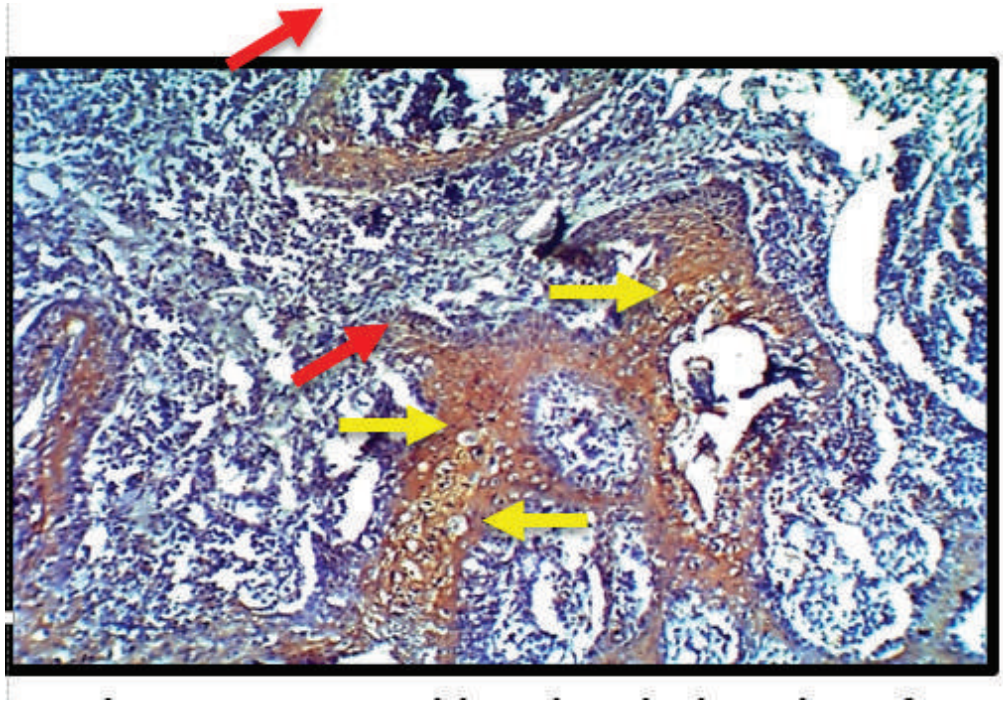

**Figure 4. Immunohistochemical section of sheep lips skin infected with ORF virus, showing the positive expression of Caspase-3 (brown color) was observed in dermis of lip. The overexpression of Caspase-3 ( ) was observed in dermis layers. Also, a spaces of apoptotic cells was observed in positive expressed areas ( ). (A&B: X100, anti-Caspase-3 antibody and hematoxylin). Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq**

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# Pregnancy Rate in Synchronized Iraqi Awassi Ewes Inseminated Artificially and Naturally

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## Abstract

This study was carried out to evaluate the efficacy of imported Australian frozen semen straws in achieving successful artificial insemination program in estrus synchronized ewes. This study conducted on twenty-one Awassi ewes, aged 2 - 4 years, and four fertile rams (two rams for natural mating and two for detecting of estrous by using apron) in Baghdad province (Rumintia Research station, department of animal resource research, state Board for Agriculture Research, Ministry of Agriculture/ Abu Ghraib district), for the period from November 2020 to March 2021. Pregnancy diagnosis was done by ultrasound to detect reproductive status of the ewes, then all non-pregnant ewes were synchronized by using vaginal sponges (60 mg of Medroxy acetate Progesterone / MAP) for 10days plus 500IU of Pregnant Mare Serum Gonadotropin (PMSG) in day 10, all animals were divided randomly into three equal groups each of seven ewes according to the type of insemination, G1 (1straw) and G2 (2 straws/ 8 hrs. interval) and G3 serviced naturally by fertile rams then the females were isolated from the males. The response of animals to hormonal treatment was 21, with a response rate of 100% in all groups with no significant difference with mean durations of response recorded 63.6± 11.7, 66.7± 12.2, and 62.8± 10.7 for the three groups, respectively. The pregnancy rate with single, double AI and natural mating was 41.86 and 71.41 and 71.42 percent, respectively with a highly significant difference ($P<0.01$) between G1 and G2. In conclusion, under field conditions, double AI with $20 \times 10^6$ sperm proved to be suitable for generating high pregnancy rates in Iraqi Awassi ewes.

***Keywords:*** *Ewe, Artificial insemination, Natural mating, Frozen semen.*

## Introduction

Ewes are considered one of the economically important farm animals in Iraq, as their meat consumption comes first, and their numbers were estimated for the year 2006 to be approximately 8,893,712. Therefore, improving their reproductive performance is an important input to increase their production efficiency, one of the encouraging things to do this improvement is that local ewes are characterized by their reproductive capacity throughout the year[1]. Reproductive technologies such as controlling estrus, superovulation, and the appropriate timing of artificial insemination that were used in the field of animal improvement were developed[2] Therefore, the correct use of these technologies in the field of reproductive management, work to increase the reproductive efficiency of animals[3] Among these important technologies that have been referred to is the artificial insemination technology. Ewes can be inseminating at any time of the year[3] , There are several methods of performing artificial insemination in small ruminants, vaginal AI, cervical AI, trans-cervical AI, and laparoscopic intrauterine AI. . The percentage of artificial insemination pregnancy varies

depending on the artificial insemination method and the type of semen used (fresh or frozen)[4].

## Materials and Methods

This study was carried out to evaluate the efficacy of imported Australian frozen semen straws in achieving successful artificial insemination program in estrus synchronized ewes. This study conducted on twenty one Awassi ewes, Aged 2 - 4 years, and four fertile rams (two rams for natural mating and two for detecting of estrous by using apron) in Baghdad province ( Rumintia Researches station ,department of animal resource researches, state Board for Agriculture Researches ,Ministry of Agriculture/ Abu Ghraib district) , for the period from November 2020 to March 2021. Pregnancy diagnosis was done by ultrasound to detect reproductive status of the ewes, then all non-pregnant ewes were synchronized by using vaginal sponges (60 mg of Medroxy acetate Progesterone / MAP) for 10days plus 500IU of Pregnant Mare Serum Gonadotropin (PMSG) in day 10, all animals were divided randomly into three equal groups each of seven ewes according to the type of insemination, G1 (1straw) and G2 (2 straws/ 8 hrs. interval) and G3 serviced naturally by fertile rams then the females were isolated from the males. All ewes were subjected to ultra-sonographical trans rectal and trans abdominal examination by using probe (8 MHz trans-rectal probe and 4 MHz trans-abdominal probe) starting from day40 post mating and AI.

## Results and Discussion

The estrus response of ewes (table 1) to hormonal treatment protocol for synchronization which included 60 mg MAP sponges for 10 days and 500 I.U (I.M) PMSG at the time of sponge's withdrawal, was 100% (7/7) in all groups with no significant differences ($P < 0.05$). These results showed that the treatment was very effective to induce synchronized estrus in ewes, it was also agreed with[5] who obtained estrus response of 96% after using the same synchronization protocol. These findings also consistent with those of [5] , who got a 55.5 % estrus response. The differences in response rate between various experiments may be attributed to the period of conducting experiment and differences in genetics between breeds of ewes. The pregnancy rate was 42.85% in G1 who were artificially inseminated with a single straw of Australian frozen semen, 71.42 % in G2 that were artificially inseminated with a double straw of Australian frozen semen, and 71.42 % in G3 which were mated naturally with fertile rams, as referred in the table (1). These findings were concurring with[6] , who discovered that a double artificial insemination with $200\times10^6$ sperm resulted in high pregnancy rates in Bangladeshi ewes and nearly like that of [7] When recording a pregnancy rate near to 70% in an experiment included several groups. However, the percentage we obtained for double artificial insemination was higher than that obtained by[6] , which were 20.8 % and 26.1 %, respectively. The low percentage of pregnancy rate that recorded by some researchers may be due to the origin of frozen semen which might be of low activity and may also be due to differences in the period of conducting the experiments[8]. discovered that fresh semen had higher fertility than frozen-thawed semen in cervical insemination. Natural mating and double artificial insemination had no significant differences, which could indicate that the double insemination is very close to natural mating in terms of the high quantity of spermatozoa put into the female genital tract. And it was higher than the findings of[6] , which were 16.7%, while it was like the findings of[9] , who found a percentage of 46.7 % in Turkish Awassi ewes, and less than the findings of[10] , which were 60 % and 61 % respectively.

**Table (1) Type of insemination, animals response, and pregnancy rate**

| Groups | No. of animal | Type of treatment | Type of insemination | Animals' response | | pregnancy rate | |
|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % |
| G1 | 7 | MAP60mg /10d with 500 IU of PMSG | A.I (single dose) | 7 | 100 | 3 | 42.85 b |
| G2 | 7 | MAP60mg /10d with 500 IU of PMSG | A.I(double dose) | 7 | 100 | 5 | 71.42 a |
| G3 | 7 | MAP60mg /10d with 500 IU of PMSG | Natural mating | 7 | 100 | 5 | 71.42 a |
| Total | 21 | | | 21 | 100% | 13/21 | |

Different small letter means sig. diff. between groups ($P < 0.01$)

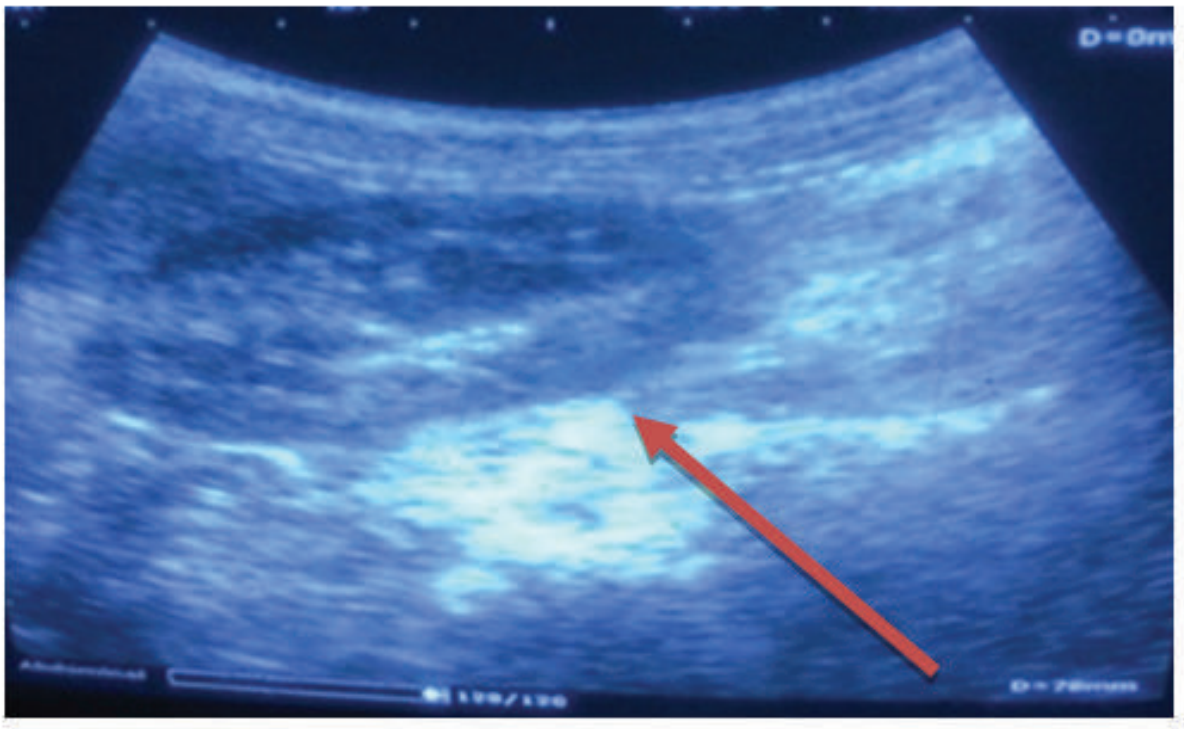

**Fig (1) fetus at 74 days of gestation in Iraqi Awassi ewe / Trans-abdominal probe (4 MHz)**

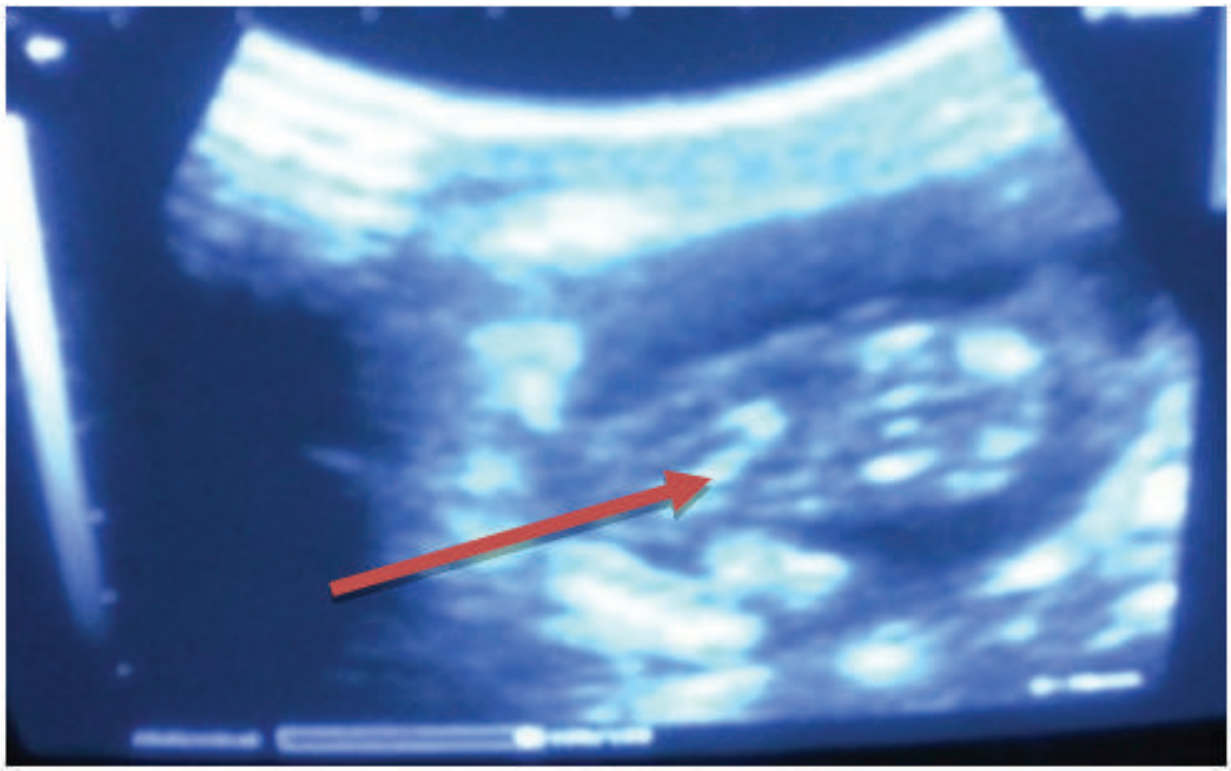

**Fig (2) fetus at 74 days of gestation in Iraqi Awassi ewe / Trans-abdominal probe ( 4MHz)**

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

**Funding:** Self-funding

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# Molecular Study of Eimeria species in Quail birds (Coturnix coturnix japonica) in Thi-Qar Province, Southern Iraq

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## Abstract

Fecal samples were collected from 330 quail birds from different areas of Thi-Qar province, Iraq. All samples were examined by traditional laboratory methods. Four species of *Eimeria* were diagnosed in quail (*Eimeria bateri*, *Eimeria uzura*, *Eimeria tsunodai* and *Eimeria fluminensis*), with a total infection was 64.54% (213 out of 330). DNA was extracted from 100 positive samples confirm the small subunit rRNA (18s) gene by Polymerase chain reaction using a general primer for *Eimeria* species, Seven genotypes were according to the identify of DNA sequences of *Eimeria* species in Japanese quail, the accession codes to NCBI-Genbank. using A comprehensive phylogenetic tree was generated in the present study, this details were being analyzed. This was the first molecular study in Iraq for *Eimeria* in common quail.

***Key Words:*** *Quail, Eimeria, Molecular,*

## Introduction

Avian coccidiosis is an enteric parasitic disease caused by multiple species of protozoan parasite of genus *Eimeria*, which are single celled, obligate intestinal epithelium parasites and is one of the commonest and economically most important diseases of poultry world-wide causing production losses causing high morbidity and mortality rate, (due to acute, bloody enteritis)[1,2].

Quail producted is consider as a branching of the modern poultry industry, Japan was the original home of quail birds that were domesticated as long as the twelfth century[3].

Coccidiosis associate with quails was first reported by Tyzzer (1929), When he described *Eimeria* dispersa in the bobwhite quail (Colinus virginianus)[4]. Several *Eimeria spp* were described from the different species of quails in different countries such as *Eimeria*. coturnicis from Coturnix coturnix; *E.uzura* and *E. bateri* from Coturnix Coturnix japonica in India[5], and *E. uzura* and *E. tsunodai* from *C. Coturnix japonica* in Japan[6]. The four more commons *Eimeria spp* from Japanese quails are: *E. bateri*[7], *E. uzura*, *E.tsunodai*[8] , and *E. fluminensis*[6]. Quail coccidiosis and its planned control have become a major target in different countries[9].

The development of molecular biology was one of the greatest achievements in biological science in the Twenty century, The discovery of Polymerase Chain Reaction (PCR) brought enormous benefits and scientific developments such as genome sequencing, gene expressions in recombinant systems, the study of molecular genetic, Molecular techniques are increasingly being used to detect parasites, these techniques have helped to identify the different genotypes of the Eimeria species in animals.

The DNA sequence of the first and second internal transcribed spacers (ITS-1 and ITS-2) of the nuclear DNA, which separate the ribosomal genes, is used most frequently, besides its heterogeneity in both sequence length and base composition of the ITS sequence, the rDNA is a member of a multiple copy gene family and provides large numbers of potential PCR targets[10,11].

Because of the lack of studies on the prevalence of *Eimeria Spp* in quail birds by using of genetic methods (PCR) and recorded the genotypes of the parasite this study designed.

## Materials and Methods

### Sample collection

Fecal samples were collected from 330 Quails birds (Coturnix Coturnix Japanica), from markets birds selling and farms, and from both sexes were (201 males and 129 females) and from different age groups from five regions in Thi Qar povince . This study started from December 2019 to September 2020. The samples were stored in clean plastic containers after taking (1 g) in clean eppendorf tube and preserved by freezing for the purpose of DNA extracting.

### Microscpically examination:

Samples were prepared for microscopic examination by direct smear method, positive samples were treated with (K2Cr2O7) at 2.5% for sporulation of oocysts and placed into Petri dishes at room temperature to study the morphological characteristics of *Eimeria spp*[12].

### Extraction of DNA:

Genomic DNA was extracted from one hundred positive samples of quail faeces using DNA Extraction Kit (Geneaid/ Korea) and following the manufacturers protocol.

### Primer:

The primers were provided as lyophilized form and were dissolved in a distilled water to give a final concentration 100 Pico mole/µl as primer stocks. These were kept at – 20C until further use in a concentration (0.5 Pico mole/ 20 µl in total PCR reaction). All these primers were supplied from Macrogen / Korea, (F- CGCGCAAATTACCCAATGAA, R-ATGCCCCCAACTGTCCCTAT) Amplicon size (450bp), The primers were taken from a reference[13].

### PCR Master Mix:

Multiplex PCR master mix was prepared with (AccuPower PCR PreMix Kit) and This master mix was manufactured according to the company's (AddBio/ Korea), Table (1).

### T1: Reaction of PCR master mix

### Thermocycler Conditions of PCR:

PCR thermocycler Conditions for *Eimeria sp*. gene were carried out as following in table (2)

**T2: Thermocycler Conditions of PCR**

| PCR steps | Temperature | Time | Repeated |
|---|---|---|---|
| Intial denaturation | 94°C | 5 minutes | 1 |
| Denaturation | 94°C | 30 Seco. | 35 cyles |
| Annealing | 55°C | 30 Seco. | |
| Extension | 72°C | 60 Seco. | |
| Final extension | 72°C | 5minutes | 1 |
| Hold | 12°C | ------ | ------ |

**Product PCR Analysis**:

Gel tray was placed in the electrophoresis tank, then 5 µl of PCR products were added into each comb's well, then the electric current was set at 100 volt and 80 AM for 30 min. Finally, PCR products were visualized by the gel documentation system.

Amplicon sequencing data and phylogentic tree:

Taken were 30 positive samples of different types according to the traditional diagnosis. The positive PCR products targeting the gene 18S rDNA.

The PCR products were sent to Macrogen Company in Korea in ice bag by DHL to carry out the DNA sequencing by sanger sequencing system, once the sequences obtained that were then submitted into NCBI-GenBank to get Genbank accession numbers.

The DNA sequencing analysis (Phylogenetic tree analysis) was conducted using Molecular Evolutionary Genetics Analysis of the (Mega X version 10) software, multiple sequence alignment analysis based on (Clustal W) alignment analysis[14]. The identified species typing analysis was done by phylogenetic tree analysis in comparison with in NCBI-Blast known sequences.

**Results:**

**Microscopic examination:**

The study revealed that 213 cases out of 330 were infected with *Eimeria* species. With a total infection rate of 64.54%. Four species of *Eimeria* were identified (*Eimeria bateri*, *Eimeria uzura*, *Eimeria tsunodai* and *Eimeria fluminensis*).

**Results of a conventional PCR:**

One hundred positive samples were taken to confirm the small subunit rRNA (18s) gene by Polymerase chain reaction using a general primer for *Eimeria* species. The results were evident on the agarose gel by electrophoresis at amplicon (450bp) Shown in the figure (1).

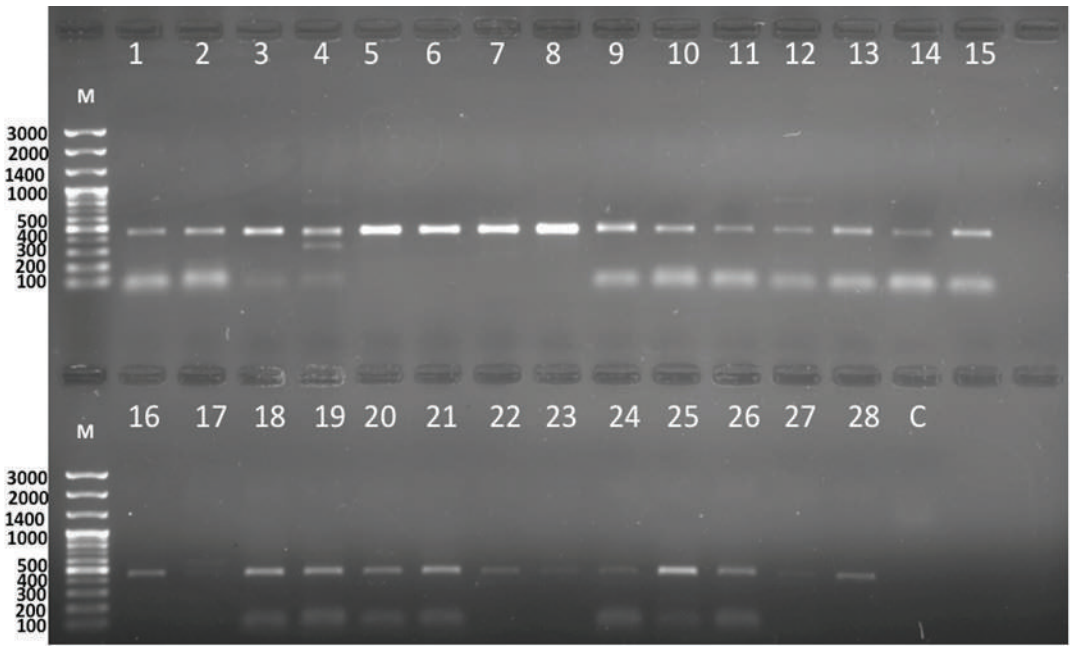


**Fig (1): gel electrophoresis image (1% agarose) shows the positive amplicons (size= 450 bp) of *Eimeria sp* by targeting the (18SrRNA) gene. (C) is control negative in which H2O was added instead of template DNA. (M) is molecular marker (100-3000bp) from AddBio, Korea.**

**Sequence analysis:**

DNA sequencing method were performed for phylogenetic confirmed detection and submitted in (NCBI-Genbank) database to get accession number codes for of local Eimeria spp in for the first time in the Iraq.

The results of the study were presented for the recorded genetic sequence analysis for the first time. Seven genotypes were investigated according to the identifing of DNA sequences of Eimeria species in Japanese quail. The accession codes for NCBI-Genbank were obtained and compared with the global strains deposited in NCBI-BLAST (USA, Canada, UK, Japan). There was high difference nucleic acid substitution and no obtained was pure identity.

**Phylogenetic analysis:**

It was provided more details about the creation of a tree concerning our identified *Eimeria spp* of neighbor joining- based These details were being analyzed using A comprehensive phylogenetic tree that was generated in the present study, which was based on the small ribosomal subunit of Eimeria spp. of the aligned nucleic acid sequences in this comprehensive tree.

Where was recorded seven strain (MW217223, MW217211), (MW217217, MW217212), (MW217218, MW217215, MW217226), (MW217230, MW217229, MW217228, MW217220, MW217216, MW217221, MW217213, MW217219), (MW217224), (MW217227), (MW217234, MW217235, MW217233, MW217232, MW217231, MW217209, MW217236, MW217237, MW217208, MW217225, MW217210, MW217222, MW217214). It was compared it with the strain NCBI-BLAST *Eimeria* species, figure (2).

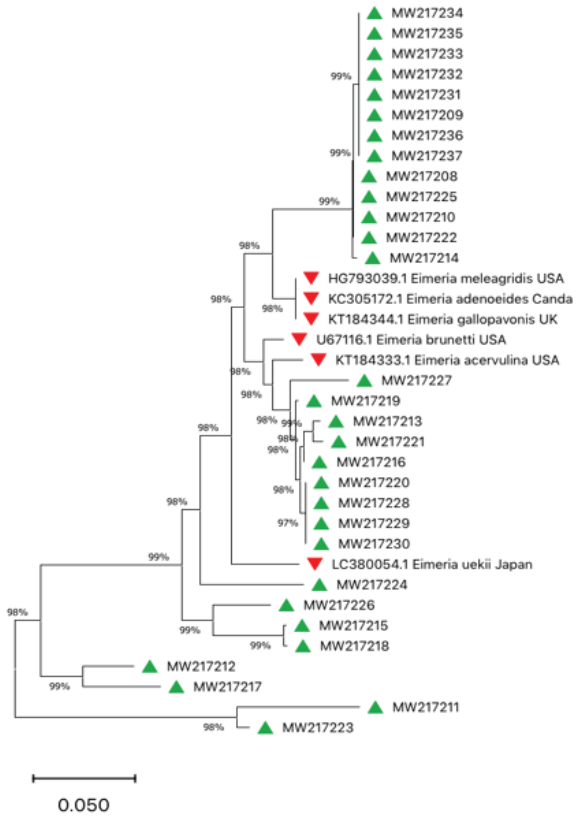


**Fig (2): Multiple sequence alignment of Eimeria sp. gene. The currently identified sequences were deposited in gene bank under the following accession numbers (MW217223, MW217211); (MW217217, MW217212); (MW217218, MW217215, MW217226); (MW217230, MW217229, MW217228, MW217220, MW217216, MW217221, MW217213, MW217219); (MW217224); (MW217227); (MW217234, MW217235, MW217233, MW217232, MW217231, MW217209, MW217236, MW217237, MW217208, MW217225, MW217210, MW217222, MW217214). These were analysed in comparison with the isolates from USA, Canada, UK, Japan).**

## Discussion

A total infection rate was 64.54%, from grand total of examineted quail (213 The of 330), our the total infected rate is agree with Abedel-Aal and El-Sayed[15] who recorded a percentage of 62.5% in Sharkia, Egypt, So was the approach to the result of Gebeyeh and Yizengaw[16] they recorded 65.10% in Ethiopia. These result were incompatible with Fadl *et al*.[17] recorded infection rate of 49% in Baghdad, Abdul-Latif *et al*. (2016) in Pakistan, who recorded a rate of 21%, moreover, Arafat and Abbas [18] documented infection rate lower than the results of our study it was 31.78% in Mansoura, Egypt. The difference in the *Eimeria* infection rates could be attributed to the number of samples, the density of the birds, type of rearing of the birds such as (Over crowding) may increase the *Eimeria* infection rate, its environmental condition (temperature and humidity), the program used for coccidiosis controland resistance against certain anticoccidial drugs and Mismanagement As well as adult birds are resistant to the parasite[19].

This study recording four species of *Eimeria sp* in quail (*E. bateri, E. tsunodai*, *E. uzura* and *E. fluminensis*), our results were Compatible with Teixeira and Lopes [6], Teixeira *et al*. [20], Berto *et al*. [21], Khudhair [22] and Arafat and Abbas [18].

**Molecular characterization**: *Eimeria* parasite in quials using PCR technique and 18s rDNA sequences were used in this study to confirm the identification of Eimeria species. The results confirmed the existence of *Eimeria* species in fecal samples for the quail.

PCR products showed amplification the presence of more than one species of *Eimeria* in quail. Generally, no much information available in molecular characterizations *Eimeria* of quails in the world. This is probably due to the fact that 18S rDNA of some genotype of *Eimeria* is semi-similar in nucleotides sequences and molecular length between *Eimeria sp*, Perhaps due to the lack of molecular studies *Eimeria* in quail to a few species and the fact that *Eimeria* less impact on the economic loss.

Sequence analysis of the 18srDNA gene has been used to provide evidence of quails infection with different *Eimeria* species. All the 30 PCR-positive specimens 30, were successfully sequenced and analysed. The nucleotide sequences of the 18s rDNA genes revealed the presence of seven genotypes Eimeria spp. and no reference strains of this species were available from other and sequencing depots in NCBI Genbank data base and It was obtained accession number codes for local Eimeria species for the first time in Iraq. In the present study, the sequence of Eimeria spp (genotype second) had considerable high homology more than 97% with Eimeria acervulina, While it was the first genetic sequence had homology more than 94.54% with Eimeria meleagridis and the most common type in was the results of 18S rDNA sequence analysis, probably because of the large number of Eimeria bateri in the microscopic examination.

The evolutionary history was inferred using the Neighbor-Joining method . The optimal tree with the sum of branch length is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches[23].

The analysis involved 30 nucleotide sequences. Codon positions included were Its-1,2 Noncoding. All positions containing gaps and missing data were eliminated. There was a total positions in the final dataset. Evolutionary analyses were version (Mega x 10) software, multiple sequence alignment analysis based on (Clustal W) alignment analysis[14].

phylogenetic analyses with GenBank-acquired ITS-1 rRNA sequences from seven chicken Eimeria spp. revealed that 95% was the lowest bootstrap

value separating any 2 Eimeria species. The ITS-1 rRNA region was chosen for the molecular analysis for this study due to previously documented use in distinguishing Eimeria spp. in chickens (Schnitzler et al., 1997). Addition to the fact that the resultant sequences can be used for meaningful intraspecies phylogenetic analyses.To our knowledge, this is the first survey of coccidia in quails.

The species composition in other bird hosts varies, A survey of 109 pen-raised wild turkeys from 12 locations disclosed that 74 (68%) of the birds were positive for coccidia[24]. Of the positive birds, *Eimeria meleagrimitis* and *Eimeria gallopavonis*, which are 2 of the most pathogenic species, were the most frequently detected species. In contrast, McDougald *et al*.[25] found *Eimeria acervulina* in 93% (n540) and Eimeria tenella in 14%(n56) of Argentina poultry farms. *Eimeria tenella* is the most pathogenic coccidia species in chickens, but was the least frequently detected species, whereas *E. acervulina* is mildly pathogenic, but was the most frequently detected species. Other studies of *Eimeria spp*. in wild northern bobwhite disclosed varying prevalences. Duszynski and Gutierrez[12] did not observe any oocysts from the intestinal contents of 10 wild bobwhites from Roosevelt County, New Mexico. Williams *et al*.[26] and Kocan *et al*.[27] reported *Eimeria spp*. oocysts from 36% (n 5 9) of bobwhites from eastern Kansas and 28% (n 5 30) of bobwhites from oklahoma, respectively. The Eimeria spp. in these surveys were not identified, and no attempts were made to infect other hosts or to determine their pathogenicity by experimental infection.

It is unknown if released quail can serve as a disease threat for wild quail; however, it would seem that coccidia would not be an issue in adult wild birds given the dispersal of wild animals and the self-limiting nature of coccidia infection[28].

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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

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# Study the Factors affecting the Production of Coagulase Enzyme from Clinical Bacteria Isolated

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## Abstract

This study included isolation and diagnosis of Coagulase Positive Staphylococci bacteria from clinical samples of (21) samples from the Teaching Hospital for Women and Children in Ramadi and included nasal swabs, Ear swabs, Normel skin swabs, Wound swabs, Urine Swabs and Abscesses.

These isolates were diagnosed according to phenotypic and biochemical tests and vatic. After diagnosing the developing colonies, it was found the Gram-positive bacteria formed a percentage (71.42%) of the clinical samples out of the total isolates that gave growth on the culture media, and the Staphylococci positive for the plasma coagulase enzyme (Coagulase Positive Staphylococci) was found. (71.42%) of the total Staphylococci bacteria obtained during isolation and diagnosis of clinical samples and a type of Staphylococcus positive for coagulase (CPS), *Staphylococcus. aureus*, was diagnosed based on the Slide and Tube Coagulase Test.

The sensitivity of CPS-positive staphylococcus to the antibiotic against the bacteria was tested and it was found that the most effective antibiotic against *S. aureus* bacteria was Azithromycin as well as the antibiotic Gentamycin, as indicated by *S. aureus* has demonstrated resistance to Erythromycin as well as Ciprofloxacin.

The different conditions for the production of the plasma coagulase enzyme (Coagulase) for the bacterial isolate of local number (5) were studied, and it was noticed that there is a great similarity in these conditions except for some differences. The study and it was noticed that the appropriate temperature in the production of the enzyme for the local bacterial isolate number (5) is the temperature (35) C, and it was noticed that the pH that led to the increase in the production of the enzyme is (PH10).After (24hr) incubator , and the results were consistent in terms of good production of the enzyme in the mentioned isolation when using a incubator shaker at a speed (100) .

***Key words:*** *coagulase enzyme production , staphylococcus aureus.*

## Introduction

Coagulase is an enzyme that is produced by some types of bacteria . The enzyme clots the plasma component of the blood. The only significant disease-causing bacteria of humans that produces coagulase is *Staphylococcus aureus*. The blood clotting enzyme (Coagulase) produced by *S.aureus* bacteria plays a major role in the blood clotting process [1].

Coagulase is an extracellular protein polypeptide consisting of 690 amino acids of molecular weight KDa (70 - 60) containing heterotrophic amino acids [2] . This enzyme consists of three regions first. It is the N-terminous region that contains the Prothrombin binding site and the second is the Central region and

is highly preserved.

coagulase is a prototype of a group of proteins called ZAAPs Adhesion Protein & Zymogen Activator. It has also been observed that some do not consider the plasma coagulase enzyme as an enzyme. Or it may be called ECMBPs) Extracellular matrix binding protein, which gives the surface proteins of bacteria the ability to adhere to some components (ECM) Extracellular matrix For the host[3].

Staphylococci bacteria are the only ones in their production of this enzyme, so we find that this enzyme has received a lot of attention. Staphylococcus is called Staphylococagulase. In 1932, researchers, Beren and Peters Champan, suggested that the plasma coagulant test is important in diagnosing pathogenic bacteria[4].

## Materials and Methods

Clinical samples were obtained for all ages and both sexes for the period from (October 5, 2020 to January 25, 2021), which amounted to (21) samples, from the Teaching Hospital for Women and Children in Ramadi, where they were transferred to the hospital laboratory, Bacteriology Department . The clinical samples included nasal swabs, Ear swabs, Normal skin swabs, Wound swabs, Urine Swabs and Abscesses swabs.

*S. aureus* bacteria were isolated from clinical samples, and grown on mannitol saline agar medium, which is the selection medium for that bacterium. Colonies of bacteria producing clotting enzymes developing on that medium were observed.

The isolates were re-purified by sub-culturing them with several grafts on the medium of the saline mannitol agar, as the developing colonies were taken on the medium of the mannitol agar and were implanted by a planning method with a sterile flame loop vector and the dishes were incubated at 37 ° C for a period of (24- 48 hours) to obtain Pure single colonies.

**Microscopic Examination**

Microscopy was performed to find out the response of the bacterial isolation to the Gram stain. Part of a growing colony was taken in the middle of Mannitol Salt Agar medium by means of a loop, then a bacterial smear was made from it on a clean glass slide and stained with Gram stain. Then they were examined with a light microscope using the oil lens, and the shape and color of the bacterial cells were observed.

**Biochemical Test**

**Coagulase Test**

**Tube Coagulase Test**:

Free Coagulase was investigated using a tube test, where (0.8) ml of blood plasma was added to (0.2) ml of Brain heart infusion broth medium and inoculated with bacterial isolates growing at an age of (18-24) hour. In small tubes and incubated at a temperature of (37) C for a period of (4) hour , during which the occurrence of coagulation, which indicates the positivity of the test, was monitored, while the tubes in which clotting did not appear at room temperature were left until the next day[7].

**Slide Coagulase Test**

This method was carried out to investigate the clumping factor enzyme-linked to plasma by using a glass slide and placing a drop of blood plasma on it and then adding to it frish colonies of staphylococcus bacteria at an age of (18-24 hr.) developing in the medium of a Brain heart infusion agar and mixed well, where the appearance of clumping within (5-10) seconds is an indication of the positivity of the test. Another glass slide was used and a drop of bacterial suspension was placed on it with the physiological solution, which represents the negative control[8] .

## Statistical Analysis

All experiments were designed according to the SAS - Statistical Analysis System[11] in analyzing the data to study the effect of different parameters on the studied traits according to Complete Randomize Desigen (CRD). The significant differences between the averages were compared with the Least Signification Difference (LSD) test. At a probability level (0.05).

## Results and Discussion

The results of the isolates shown in Table (1) showed obtaining 15 coagulase-producing bacterial isolates from 21 bacterial isolates. These isolates varied in their ability to produce the coagulase based on the zone of clotting around the colony developing on the medium of the intestine of gut chickens agar . With a pH of 7 at 37 ° C for a 24-hour incubation period.

The colonies with clotting zone diameters appeared on the medium of the chicken gut, which was used as a nitrogen source.

These samples formed a percentage (71.42%), while the number of samples that gave a negative result for laboratory culture was (6) samples formed a percentage (28.57%), and these results are approximately consistent with what he mentioned[12]. It was found that the percentage of clinical samples that gave a positive growth for laboratory culture was (70%).

**Table (1) Bacterial Isolates That Produce Plasma Coagulase Enzyme (Coagulase)**

| T | NO. Isolation | Location | diameter coagulation (cm) |
|---|---|---|---|
| 1 | 1 | Teaching Hospital for Women and Children | 4.5 |
| 2 | 5 | Teaching Hospital for Women and Children | 6 |
| 3 | 6 | Teaching Hospital for Women and Children | 2.5 |
| 4 | 7 | Teaching Hospital for Women and Children | 3.5 |
| 5 | 14 | Teaching Hospital for Women and Children | 3 |
| 6 | 20 | Teaching Hospital for Women and Children | 2.5 |
| 7 | 21 | Teaching Hospital for Women and Children | 4.5 |
| 8 | 23 | Teaching Hospital for Women and Children | 3 |
| 9 | 24 | Teaching Hospital for Women and Children | 5 |
| 10 | 25 | Teaching Hospital for Women and Children | 5.5 |
| 11 | 29 | Teaching Hospital for Women and Children | 3 |
| 12 | 44 | Teaching Hospital for Women and Children | 5 |
| 13 | 50 | Teaching Hospital for Women and Children | 5 |
| 14 | 51 | Teaching Hospital for Women and Children | 7 |
| 15 | 60 | Teaching Hospital for Women and Children | 4 |

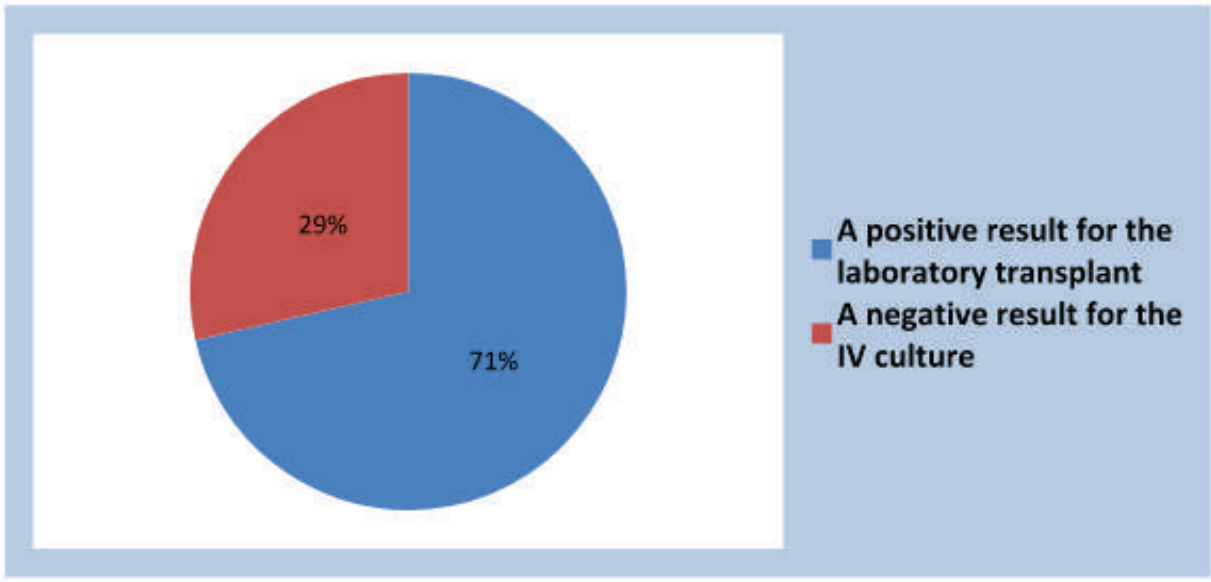


**Figure (1) The percentage of clinical samples that gave growth on the culture media.**

The bacterial isolates of the genus Staphylococcus were initially identified based on their phenotypic characteristics when grown on the nutrient agar. Opaque, then it turned pale yellow when growing for a longer period, and this corresponds to what was mentioned .

**Staphylococci Sensitivity for Antibiotic**

In this study, the sensitivity of selected isolates to. (10) Antibiotic By using the standard antibiotic diffusion method, the bacterium *S. aureus* was shown *S. aureus* under study is resistant to some antibiotics, including the antibiotic Erythromycin, reaching (86.66%). This is close to what was mentioned by Devapriya *et. al*. [17], who obtained a percentage of (88%) of the isolates resistant to this antibiotic.

Wozniak [18] indicated that his isolates of *Staphylococcus aureus* showed resistance to the anti-Ciprofloxacin by (80%) and this is close to what we found, as the isolates resistant to this antibody reached about (73.33%).

Also, the highest sensitivity of *S. aureus* bacteria was obtained with the Azithromycin, where the proportion (86.66%) of *S. aureus* isolates was sensitive to this antagonist, which was mentioned by Laub, Krisztina, *et al*.[19]. The bacterial isolates showed high sensitivity to Gentamycin, as the percentage of sensitive isolates reached (66.66%) , This is close to the findings Swarooprani *et. al*.[20], Where about (72.4%) of the isolates were sensitive to this antagonist.

The results of this study showed that *S. aureus* bacteria isolates under study possessed multiple resistance to antibiotics, which made them one of the pathogens occurring in hospitals as well as their possession of many virulence factors represented by the production of toxins, enzymes and other virulence factors[21].

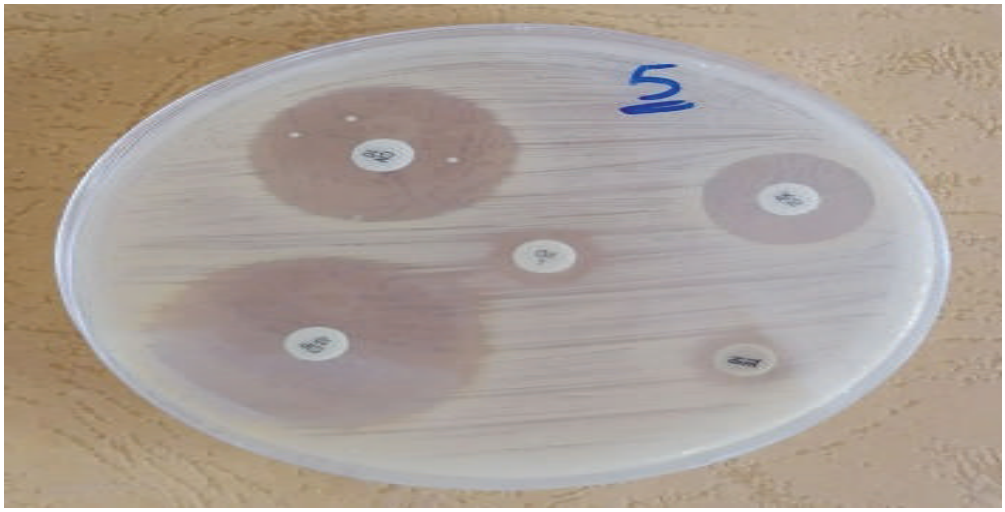


**Figure (4) *S.aureus* susceptibility test**

**Factors affecting of production coagulase :**

**Effect of pH.**

The results of the statistical analysis showed that the pH (10) had a significant difference affecting the productivity of the plasma coagulant enzyme on the rest of the pH numbers of the other isolates (5) of *S. aureus* bacteria.

The pH affects the production of the enzyme through its effect on the properties of the nutrient medium, the solubility of nutrients and their readiness for the organism, and this in turn affects the growth of microorganisms as well as the influence of the enzymes produced by these organisms by the pH of the growing agricultural media.

This result differs with the findings of Wilcox *et al*.[22] , Which explained the use of the pH (7.5) in the production of the enzyme plasma coagulase (Coagulase), and I suggest the reason for this difference to the type, nature and components of the food medium (chicken gut) locally prepared It is considered the first of its kind in such a study.

**Table (2) The effect of pH on the production of Coagulase enzyme from Staphylococci isolates.**

| No. | PH | enzymatic activity. Units / ml |
|---|---|---|
| 1 | 5 | 0.460 ± 0.031 b |
| 2 | 6 | 0.425 ± 0.025 b |
| 3 | 7 | 0.421 ± 0.022 b |
| 4 | 8 | 0.483 ± 0.037 b |
| 5 | 9 | 0.643 ± 0.062 b |
| 6 | 10 | 1.010 ± 0.077 a |
| | LSD value | 0.328 * |
| The averages carrying different letters within the same column differ significantly between them. * (P <0.05). | | |

**The effect of temperature.**

The effect of temperature on the production of the (Coagulase) from the bacterial isolates under study using different temperatures ranged between (25, 30, 35, 40,45, 50 and 55) C, and it was found that the highest productivity of bacterial isolate (5) for bacteria *S. aureus* at a temperature of (35) C.

The reason for the increase in the productivity of the enzyme at the high temperature may be attributed to the fact that the high temperature affects the speed of enzymatic reactions inside the cell or on some factors that aid the growth of the isolate, such as the decrease in the percentage of dissolved oxygen[23].

The results of the present study agree somewhat with the results of Sturm *et al*.[24], Which demonstrated the use of a temperature of 37 ° C in the production of the enzyme plasma coagulant of *S. aureus*.

**Table (3) The effect of temperature on the production of Coagulase enzyme from Staphylococci isolates .**

| No. | Temperature | enzymatic activity. Units / ml |
|---|---|---|
| 1 | 25 | 0.435 ± 0.025 |
| 2 | 30 | 0.434 ± 0.022 |
| 3 | 35 | 0.518 ± 0.037 |
| 4 | 40 | 0.452 ± 0.032 |
| 5 | 45 | 0.355 ± 0.026 |
| 6 | 50 | 0.490 ± 0.029 |
| | LSD value | 0.217 NS |
| NS: Not significant. | | |

**Effect of Inoculum size.**

Inoculation of production medium with different sizes of inoculum (0.5, 1, 1.5, 2, 2.5, 3 ml / ml medium) with uniform density 8 x $10^{-5}$ colonies / ml of *S. aureus* bacteria for isolation (5). To find out its effect on the production of (Coagulase), the results show that the highest production of the enzyme from *S. aureus* bacteria when adding the inoculum volume is 3 ml / 100 ml.

These results are in agreement with what was obtained[25] , that the best inoculum size for producing coagulase from *S. aureus* was when using a vaccine volume of 3 ml / 100 ml.

**Table (4) The effect of the inoculum size on the production of Coagulase enzyme from Staphylococci isolates.**

| No. | The size of the inoculum | enzymatic activity. Units / ml |
|---|---|---|
| 1 | 0.5 | 0.835 ± 0.082 |
| 2 | 1 | 0.853 ± 0.071 |
| 3 | 1.5 | 0.851 ± 0.092 |
| 4 | 2 | 0.923 ± 0.156 |
| 5 | 2.5 | 0.887 ± 0.087 |
| 6 | 3 | 0.978 ± 0.079 |
| | LSD value | 0.187 NS |
| NS: Not significant. | | |

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# MYC Gene Mutations as Causative Pathways for Development and Treatment of Hematological Malignancies

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## Abstract

MYC is a proto-oncogene with deregulation of >50% of human cancers. The dysregulation of MYC cause tumorigenesis, cell growth and proliferation, cell growth, and apoptosis. Novel therapy approaches lead to the direct inhibition of the MYC gene by disruption of MYC/Max complex, MYC destabilization, inhibition of MYC translation and/or transcription. Cetuximab and panitumumab act on the epidermal growth factor receptor (EGFR). Cetuximab is an immunoglobin G1 isotype of a monoclonal antibody that produces antibody-dependent cell-mediated. Panitumumab is an immunoglobulin G2 isotype monoclonal antibody. This antibody acts on different site of EGFR with different degree of affinity. Genome-wide association studies e.g., TWAS determine the aggregate genotypes. Enhancer is cell-specific gene regulation that cluster for binding sites of a transcription factor with spatially coordinated to control the expression of one or more specific target genes. In this review, we will summarize novel drugs in targeting cancerous MYC in haematological malignancy.

***Keywords:*** *MYC gene, enhancer, Genome-wide association studies (GWAS), Cetuximab, Panitumumab, monoclonal antibody.*

## Introduction

Considerable literature demonstrated the involvement in the expansion of autoimmune disorder and specific cancer, particularly by proto-oncogene. MYC's future function in disease progression control as well as its integration into disease management has been documented. The three L-MYC, N-MYC and c-MYC are the member of the MYC proto-oncogene family with significant roles in numerous physiological methods i.e., cell cycle regulation and cell division, protein synthesis, cell adhesion, proliferation, cell differentiation, angiogenesis, immune activation, and apoptosis(Beltran, 2014; B.-J. Chen, Wu, Tanaka, & Zhang, 2014; Nie et al., 2012; Posternak & Cole, 2016). Also. MYC is a multifactorial TFs that has a significant function in tumorigenesis. The rearrangement of MYC leads to developments of diffusion large B cell lymphoma (DLBCL) and high-grade B cell lymphoma (HGBL) with the rearrangements of BCL6 and BCL2 by MYC translocation, referred to as double-hit lymphoma (DHL) and triple hit lymphoma (THL) (Landsburg et al., 2016). The World Health Organization (WHO) 2017 update about the DHL and THL prognostic implication that defines the cytogenetically as the

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category of "High-grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements" (Grimm & O'Malley, 2019).

The gene regulation is monitored by MYC family member with the basic helix loop helix zipper (bHLHZ) of a transcription factor (TFs). MYC is highly expressed during cell proliferation from foetal development to cell division of adult. The highest level of MYC expressed in the brain tissue during embryogenesis. However, MYC expression is downregulated after embryonic development (Beltran, 2014; Blagosklonny & Pardee, 2002; Fernandez et al., 2003).

There is an interaction between MYC and several proteins to form the multicomponent for the gene expression and transcription regulation and chromatin structure in the normal cell. The dimerization of max protein and MYC protein regulates transcription by binding MYC-box sequence with transcriptional coactivators and also in the regulation of MYC target gene. The interaction of WDR5 with MYC-max leads to the recognition of the target gene. Also, the interaction of MYC-max and coactivators i.e., TF II triggers transcriptional elongation (Beltran, 2014; Blagosklonny & Pardee, 2002; Gandarillas, 2012; Levens, 2013).

The MYC gene stability is influenced by coding mutations that enhance its level over altering basic action (Liu et al., 2006). The sequence of the protein coding is left intact by the chromosomal abnormalities of the MYC gene. MYC acts as oncogenic with its abnormal gene expression. MYC gene moves in Burkitt lymphoma and plasmacytoma with immunoglobins gene (Boxer & Dang, 2001; Levens, 2013). The MYC overexpression is promoted by gene amplification, translocation of chromosomal and mutation in the signalling pathway without any dependence on growth factors stimulation that promotes unstrained proliferation and finally tumorigenesis. Thus, MYC cause extensive transcriptional reprogramming with angiogenesis activation and suppression of host immune response. Therefore, MYC is considered to involve in more than 50% of all human cancer(Dang, 2012; Y. Li, Casey, & Felsher, 2014; Lin et al., 2012).

**Haematological Malignancy**

Acute Myeloid Leukemia (AML) and Chronic Myelogenous Leukemia (CML)

AML is a malignant disease with significant genetic heterogenicity and failure of bone marrow that leads to immature myeloid cell proliferation. The hematopoietic precursors are arrested in an early stage of development. The occurrence of more than 20% of blasts in the bone marrow distinguishes most AML subtypes from other associated blood disorders (Mughal et al., 2017; Yun et al., 2019). The c-MYC is a downstream target that occurred by molecular abnormalities for the AML patient's prognosis influenced by genetic mutations as shown in figure 1. The MYC expression is highest identified in AML patients that demonstrated poorer overall survival in cytogenetic risk groups(Mughal et al., 2017). The decreased cell growth increased apoptosis with lower MYC expression is a result of the lack of adenylate cyclase that supports AML in human (S. L. Chen et al., 2020; M. Li et al., 2019).

CML is described as a clonal myeloproliferative disorder that is identified by the occurrence of balanced genetic translocation of the Philadelphia chromosome. This increases the proliferation of granulocytes and their immature precursors without the loss of their capacity to differentiate. Around 20% of adults with leukaemia have specifically CML. The CML blood and bone are suggested by the unexplained leucocytosis including immature myeloid cells (Granatowicz et al., 2015). BCR/ABL1 fusion gene is also associated with CML. MYC-Max heterodimer regulates BCR promoter. The BCR and BCR/ABL1

downregulation is significantly caused by MYC expression silencing BCR/ABL1 positive CML cell lines that cause reduced proliferation and induction of cell death. However, MYC overexpression is observed in the CML blast crisis. The beta-catenin MYC target gene activates in blast crisis patients. Thus, MYC overexpression has a significant role in BCR/ABL1 up-regulation leading to blast aggressiveness acquired during CML evolution (Sharma et al., 2015; Wang, Ikura, Eto, & Ota, 2004).

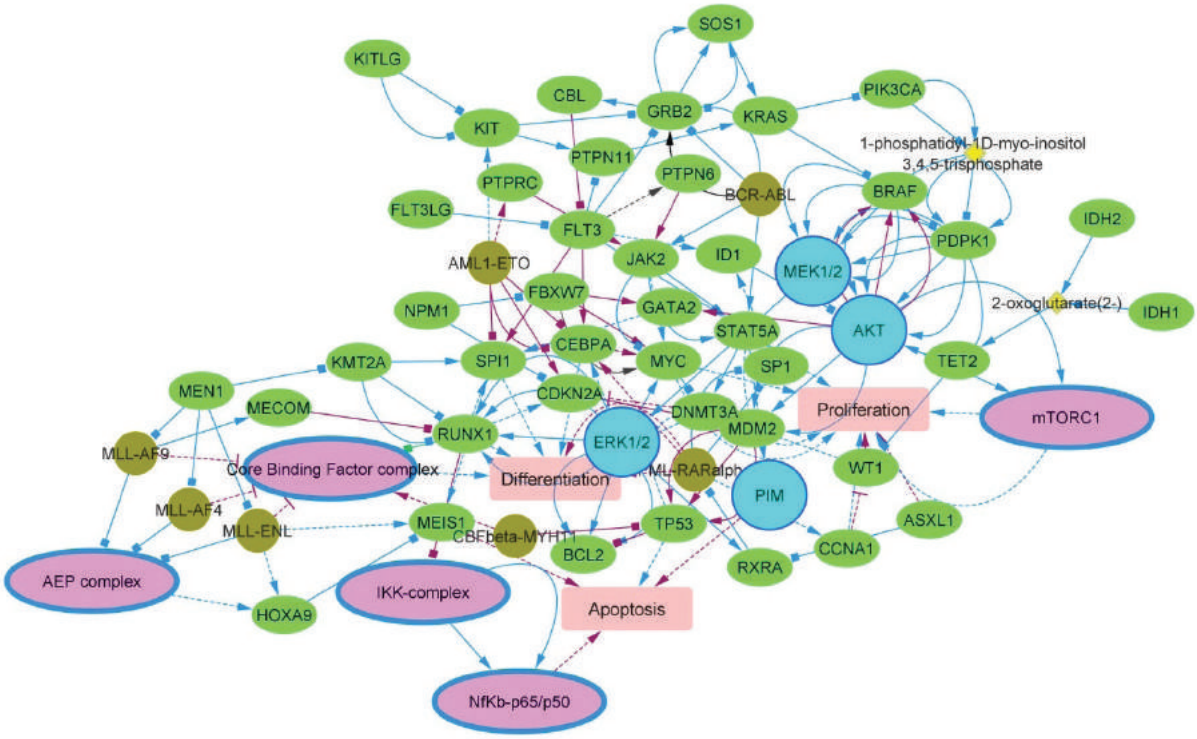


**Figure 1: Acute myeloid Leukemia network:**

Nodes for all proteins including MYC is shown in green colour circles, fusion proteins are shown in Gray colour, all complexes are shown in large pink circles enclosed with light blue line, protein family proteins are shown in cyan colour circles, small molecules receptors are shown in yellow colour and all the phenotypes are shown in light pink rectangular boxes. Different edges represent the interaction with different molecules. Cyan colour edges show the up regulation of different molecule with the effect of other molecules and vice-versa. Magenta colour edges represent the down regulation of different molecules with respect to each other.

Diffuse Large B-Cell Lymphoma (DLBCL) and Hodgkin's lymphoma

Nodular lymphocyte-predominant Hodgkin's lymphoma and classic Hodgkin's lymphoma are two distinct disease entities of Hodgkin's lymphoma, a rare B-cell malignant neoplasm. DLBCL is the most prevalent lymphoid neoplasm in adults, responsible for 32.5 % of all non-lymphoma Hodgkin's cases diagnosed per year. DLBCL is a heterogeneous disease with variable clinical outcomes to molecular pathogenesis and its biology. DLBCL is divided into three molecular subtypes: germinal core activated B-cell lymphoma, B-cell lymphoma, and primary mediastinal massive B-cell lymphoma. MYC

overexpression is observed in both DLBCL and Hodgkin's lymphoma in which DLBCL patients demonstrated the rearrangements and expression of the MYC and/or BCL2 gene. Burkitt lymphomas and DLBCL have a larger level of MYC staining over other lymphomas (Chisholm et al., 2015; Zelenetz et al., 2016).

## Materials and Methods

The GRCh38.p13 long promoter sequence of Human gene chr8:127,735,434-127,742,951(GRCh38/hg38); chr8:128,747,680-128,755,197(GRCh37/hg19 by Entrez Gene); and chr8:128,747,680-128,753,674(GRCh37/hg19 by Ensembl) information were gathered using the gene card human database. The gene databases COSMIC, and Ensembl were used to evaluate the comprehensive and updated information on transcription factors (TFs) leading haematological malignancy due to myc genome changes.

Our study explains the effect of Gefitinib, Erlotinib, Panitumumab, Cetuximab and other compounds that target the MYC gene. Also, we discuss the gene enhancer, genome-wide association study PhenoPred prediction of MYC gene associated diseases and Sorting Intolerant from Tolerant (SIFT).

## Results and Discussion

Therapeutic approaches targeting MYC gene

MYC remains the several significant biological pathways involved in cancerous cell proliferation and growth. The MYC-mediated tumorigenesis experimental model demonstrated that MYC cause addiction to established tumours whereas MYC expression deregulation led to failure of addiction. New therapeutic approaches use these MYC induced changes by targeting interruption of MYC-Max dimerization and MYC target genes, inhibition of MYC expression, MYC-Max DNA binding (Palaskas et al., 2011). Other therapeutic approaches focused on targeting MYC target gene i.e., glutaminase (GLS), lactate dehydrogenase A (LDHA), and ornithine decarboxylase (ODC) and also aurora or cyclin-dependent kinases inhibitors (D. Yang et al., 2010). Transgenic murine lymphomas are sensitive to Chk1 inhibitors linked to MYC-induced replicative stress (Murga et al., 2011). The post-translational modifications cause TFs to disorganised segments in controlling the TFs function and stability. The therapeutic approaches depend on protein-protein interaction, DNA binding and disrupting expressions. Here, we summarize the new therapeutic approaches and compounds (Tables 1) that describes the therapeutic targeting on the MYC gene i.e., activation of post-translational modifications, Dimerization of partner protein and MYC transcription.

**Table 1: Drugs & Compounds for MYC Gene**

| Name | Status | Role | Mechanism of Action |
|---|---|---|---|
| Cisplatin 23 102 | Approved 25 | | Inhibits DNA synthesis, a chemotherapy drug, Potent pro-apoptotic anticancer agent; activates caspase-3, Platinum |
| Gemcitabine 23 102 | Approved 25 | | Ribonucleotide reductase and DNA synthesis inhibitor, Nucleoside Analogs |
| Aspirin 25 | Approved, Vet approved 25 | Channel blocker, Target, downregulation | |

***Cont...*** **Table 1: Drugs & Compounds for MYC Gene**

| | | | |
|---|---|---|---|
| Nadroparin 25 | Approved, Investigational 25 | Target, inhibitor | |
| rituximab 102 | Approved 25 | | Therapeutic Antibodies |
| Trametinib 102 | Approved 25 | | MEK 1/2 inhibitor, MEK Inhibitors, Kinase Inhibitors, Mitogen-activated protein/extracellular signal-regulated kinase (MEK) inhibitors |
| Venetoclax 102 | Approved, Investigational 25 | | Bcl-2 inhibitor, potent and selective |
| Alisertib 101 | Investigational 25 | inhibitor, Biomarker | Aurora-A kinase inhibitor, AURKA Inhibitors, Kinase Inhibitors |
| Dinaciclib 101 | Investigational 25 | inhibitor, Biomarker | CDK inhibitor, CDK2 Inhibitors, Kinase Inhibitors |
| 10058-F4 88 | | | C-MYC-Max dimerization inhibitor |
| AMG900 101 | | Biomarker, inhibitor | AURKA Inhibitors, Kinase Inhibitors, Inhibitors of mitosis Aurora kinase inhibitors |
| BAY1000394 101 | | Inhibition, Biomarker | CDK4 Inhibitors, CDK2 Inhibitors |

The MYC expression is reduced by epigenetic silencing of the MYC gene. The bromodomain and extra-terminal motif (BET) bromodomains are effective against MYC by the inhibitor of histone methyltransferases, DNA methyltransferases, histone demethylases, and histone deacetylases (Poole & van Riggelen, 2017). The MYC expression is decreased by BET inhibitor by inhibiting the binding of BRD4 at acetylated histones inside the MYC promoter (Kato et al., 2016). G-quadruplexes inhibit the MYC transcription and silences gene expression within the nucleus hypersensitive element (NHE) III region of MYC promoter result in MYC mRNA suppressions and induction of cytotoxicity (Hu et al., 2018).

Some compounds bind directly and inactive downstream MYC function reducing the acquired resistance and better efficacy, bHLHZ is required for MYC dimerization to Max and DNA binding at e-box sequences. MYC/MAX dimer disruptors i.e., 10058-F4 and 10074-G5 binds within the bHLHZ domain and interrupt MYC/MAX dimerization. Struntz et al studied the MYC: MAX stabilization in vitro and in vivo with the use of molecule KI-MS2-008 results in degradation and attenuation of the MYC transcriptional gene (Struntz et al., 2019).

The MYC expression and activity are controlled by several proteins modification, albeit indirect, post-translational therapeutic modifications and method of decreasing MYC function. The mechanism of post-translation modification for the MYC gene include "1) kinases that phosphorylate S62-MYC; (2) phosphatases that dephosphorylate S62-MYC; (3) the PIN1 proline isomerase; and (4) enzymes that affect MYC ubiquitin-dependent proteolysis".

**Gefitinib and Erlotinib effect on Apoptosis and c-MYC Expression.**

A new therapeutic approach is required for the management of haematological malignancy by targeting one of the biological processes i) apoptosis leading to cell death ii) irreversible cell-cycle blockade iii) terminal differentiation. Target therapy

interrupts the vital cell process and destroys tumour cell. Selective tyrosine kinase inhibitors i.e., imatinib is a targeted therapy for hematologic malignancies. Gefitinib and erlotinib inhibit epidermal growth factor receptor of haematological malignancy (Bell et al., 2005; Herbst et al., 2002; Hidalgo et al., 2001). Stegmaier et al. discovered gefitinib to initiate the cell differentiation in 3 AML e.g., HL60, U37 and Kasumi-1 that inhibits the EGFR expressing cells. Erlotinib is an antineoplastic drug that induces apoptosis on MDS and AML cells (Stegmaier et al., 2005).

Despite A549 non -small cell lung cancer, off-target occurred in the haematological cell lines KG-1, HL60 and P39 due to inhibitory activity of the erlotinib on EGFR causing deficient of EGFR expression. The detection of phosphatidylserine exposure by annexin V-FITC conjugates due to erlotinib induce apoptosis to lead to viability loss in KG-1 cells but P39 and HL60 fail to do so. Phosphoproteome analysis evaluates most KG-1 but fewer P39 and HL60 absolute numbers are reduced by erlotinib. Early phosphatidylserine exposure demonstrated apoptosis, loss of nuclear DNA and nuclear karyorrhexis and pyknosis in incubated KG-1 cell. The autophosphorylation of the cancerous JAK2 kinase on tyrosine is reduced with erlotinib administration (Simone Boehrer et al., 2008). The cytofluorometric analysis proved that erlotinib therapy causes hyperphosphorylation of TF stat-5 by JAK2 on tyrosine. Thus, Phosphoproteome and cytofluorometric analysis validate that erlotinib therapy alone can reduce the constitutive STAT-5 activation in KG-1 cells (S. Boehrer et al., 2008). The RNA interferes with downregulation with JAK2 expression leading to erlotinib-induced apoptosis. The STAT-5 activation was abolished with JAk2 expression alone and simultaneously induce apoptosis in KG-1 cell lines. Similar, the level of apoptosis has induced either erlotinib alone or a combination of erlotinib and JAK2 knockdown. Thus, erlotinib causes apoptosis by inhibiting JAK2, PDGFR knockdown (Abou Dalle et al., 2018). Golub et al. stated proliferation and differentiation arrest as antineoplastic approaches on EGFR inhabitation of gefitinib on haematological malignancy (Stegmaier et al., 2005). Erlotinib and gefitinib destroyed the tumour cell-based on chromatin condensation and fragmentation of nuclear and phosphatidylserine exposure leading to apoptosis. Moreover, these drugs either following ligation of death receptors activating the apical caspase in the death-inducing signal complex (intrinsic pathway) or liberation of the caspase activator cytochrome c in mitochondria outer membrane permeabilization (MOMP), (extrinsic pathway) leading to apoptosis. it is clear that erlotinib activates the intrinsic pathway, based on the finding that mitochondria released cytochrome c (and other death effectors such as endonuclease G) before caspase-3 was stimulated, and that caspase inhibition was unable to avoid mitochondrial outer membrane permeabilization and cell death (Simone Boehrer et al., 2008; Doan et al., 2013; Lainey et al., 2013). Figure 2 describes the gefitinib and erlotinib effect on Apoptosis and c-MYC Expression.

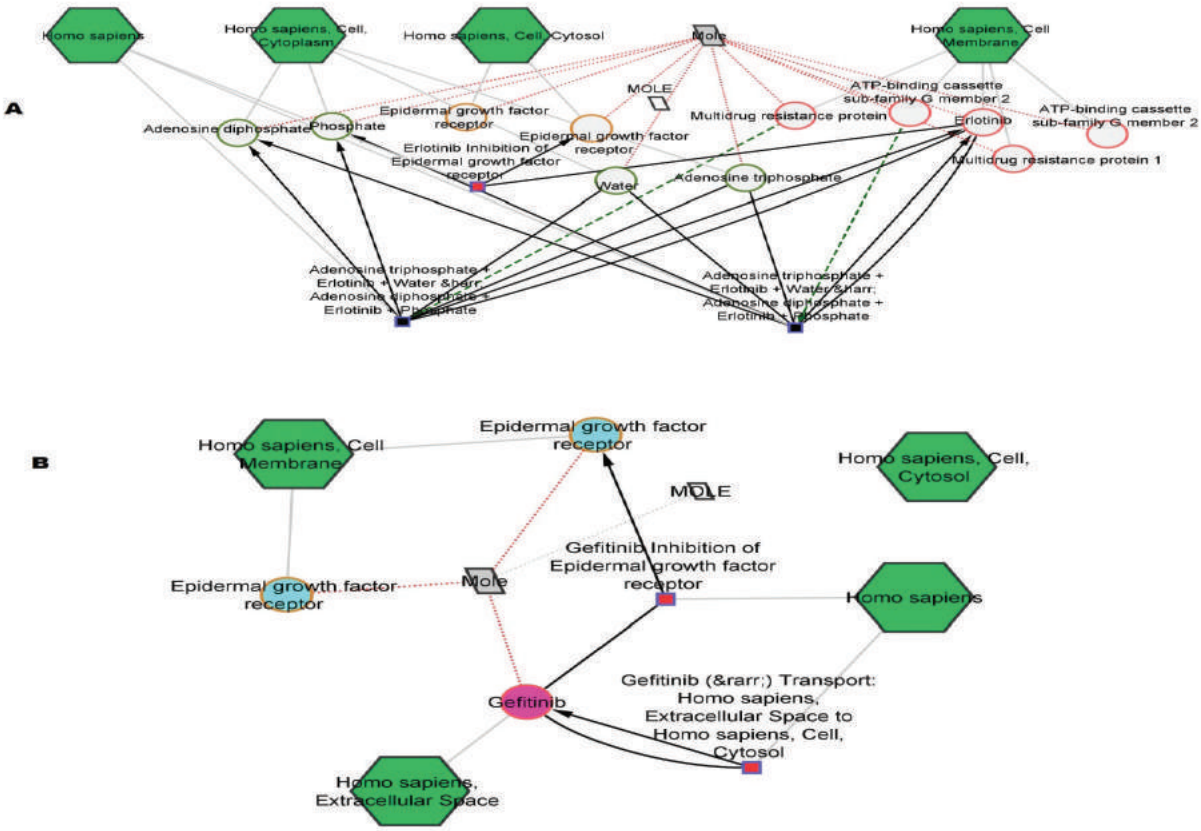


**Figure 2: Gefitinib and Erlotinib effect on Apoptosis and c-MYC Expression.**

The prooncogenic activities reduced apoptosis, enhanced cell proliferation enhanced angiogenesis and metastatic malignancy are associated with EGFR signalling pathway dysregulation. The receptor binding activates downstream signalling pathway include PI3K/Akt (phosphoinositide 3-kinase/protein kinase B), JAK/STAT (Janus kinase/signal transducers and activators of transcription), and MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase. The frequent activation of these pathway cause survival and proliferation of tumorous cells (García-Foncillas et al., 2019; Kim & Grothey, 2008; Shim, 2011).

The function of monoclonal antibodies cetuximab and panitumumab act by binding to EGFR extracellular domain III that prevents EGFR ligand binding and locking in autoinhibitory monomeric conformation(Shim, 2011; Zhou et al., 2012). Panitumumab and cetuximab are human and mouse/human chimeric monoclonal antibodies respectively. The binding affinity of Panitumumab for the EGFR is 8 times more than Cetuximab. The dimerization and ligand binding activates tyrosine kinase receptors that are internalised by clathrin-dependent endocytosis. The tyrosine kinase activity of activated EGFR is terminated. This process regulates the cell surface receptors number. The deubiquitinating enzymes deubiquitinate ubiquitinated receptors that are again recycled back to the cell membrane. Anti-EGFR therapy resistance has been linked to receptor ubiquitination (Lu et al., 2007). However, cetuximab and panitumumab proved their prognostic effects for the EGFR overexpress that is responsible for haematological malignancy (Kim & Grothey, 2008). Panitumumab and cetuximab compete for the binding

site of EGF. Panitumumab's binds with mutational epitope with ≥50% binding affinity for EGFR residue P349, I438, F412, D355 and P362. Similarly, cetuximab binding affinity for EGFR residue I467, K465, K443, H409, Q408, Q384, S468, P387, D355 and F352 (Voigt et al., 2012). Since D355 is present inside the binding site of all three molecules that cause competition between EGF and monoclonal antibodies. cetuximab binding overlaps with EGF binding site at 5 location i.e., S468, K443, Q409, Q408H, D355 whereas panitumumab binding overlap with 2 locations at D355 and K443 (Voigt et al., 2012). Also, the binding affinity for EGFR is different for panitumumab and cetuximab with dissociation constants of 0.05 nM and0.39 nM respectively (Kim & Grothey, 2008). Thus, figure 3 summarises the pathway of panitumumab and cetuximab.

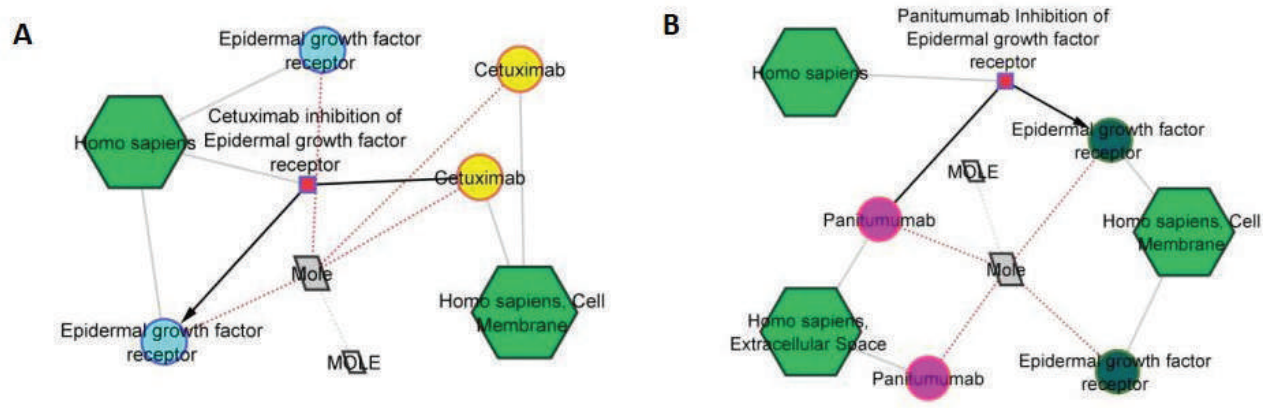


**Figure 3: Panitumumab and Cetuximab Drugs action pathway.**

**Enhancer**

Enhancer is usually distributed all over the genome with cis-regulatory DNA sequence. Enhancer is TFs binding elements that regulate the gene expression in a specific spatiotemporal specific manner (Levo & Segal, 2014). Thus, cell and tissue development fate are greatly influenced by enhancer based transcriptional regulation (Taminato et al., 2016). The transcriptional machinery model supports the conformation of chromosome capture that identify direct interaction with the adjacent chromatin regions. Human consists of hundreds of thousand enhancers. Each enhancer is linked with several TFs. Recent development leads to enhancer detection by enhancer reporter assays at the non-coding DNA sequence. The massive parallel reports assays identify variable histone modification marks at different organs (Inoue & Ahituv, 2015). Several genome-wide programmes for enhancer recognition and annotation are based on massive parallel report assays. Active enhancer ensures bidirectional transcriptions that lead to the formation of enhancer RNA (eRNA) products. The most challenging process is linking the enhancer to their target gene (Pennacchio, Bickmore, Dean, Nobrega, & Bejerano, 2013; Shlyueva, Stampfel, & Stark, 2014; Yao, Berman, & Farnham, 2015).

Early discovery used molecular genetics methods to assess enhancer effects on a single gene. Recently, various predictive investigation identified gene enhancer is linked to various TFs binding site as in table 2. Chromosome conformation capture identifies the enhancer and their target gene by

restricting the physical DNA loop. The target gene expression and enhancer variation are identified by expression quantitative trait locus (eQTL) analyses. Enhanceropathies are enhancer related disease occurred either due to TFs mutation intermingling with enhancer or enhancer mutation themselves (Mifsud et al., 2015; Whalen, Truty, & Pollard, 2016). E.g., the Sonic hedgehog gene (SHH) is enhancer mutation diseases that cause developmental abnormalities preaxial polydactyly (Lettice et al., 2003).

**Table 2: GeneHancer based predicted genomic regulatory elements related to the MYC gene.**

| GeneHancer (GH) Identifier | GH Type | GH Score | Gene Association Score | Total Score | Transcription Factor Binding Sites |
|---|---|---|---|---|---|
| GH08J127732 | Promoter/Enhancer | 2.3 | 528.9 | 1200.29 | 394 TFs, HNRNPK, ZNF217 |
| GH08J128181 | Enhancer | 1.6 | 23.4 | 36.88 | 196 TFs, EP300, FOXK2 |
| GH08J127806 | Promoter/Enhancer | 1.8 | 13.9 | 25.49 | 140 TFs, ZNF217, TCF12 |
| GH08J127895 | Enhancer | 1.4 | 13.2 | 19.2 | 297 TFs, ZBTB40, ZNF217 |
| GH08J127793 | Promoter/Enhancer | 1.8 | 10.6 | 19.09 | 170 TFs, SIN3A, MYC |
| GH08J128166 | Enhancer | 1.7 | 10.5 | 17.7 | 354 TFs, LARP7, EP300 |
| GH08J126824 | Promoter/Enhancer | 1.8 | 9.6 | 17.58 | 49 TFs, CTCF, TCF12 |
| GH08J127972 | Promoter/Enhancer | 1.6 | 9.9 | 16 | 83 TFs, ZBTB5, RELA |
| GH08J127924 | Promoter/Enhancer | 1.5 | 10.2 | 15.71 | 69 TFs, TCF12, FOS |
| GH08J127758 | Enhancer | 1.3 | 11.8 | 15.34 | 101 TFs, TCF12, TOE1 |
| GH08J127863 | Enhancer | 1.3 | 11 | 14.58 | 161 TFs, ZNF217, TCF12 |
| GH08J127888 | Promoter/Enhancer | 1.4 | 10.2 | 14.42 | 115 TFs, CTCF, SIN3A |
| GH08J127799 | Enhancer | 1.2 | 11.5 | 13.31 | 196 TFs, ZNF217, TEAD4 |
| GH08J126876 | Promoter/Enhancer | 1.2 | 10.2 | 12.02 | 75 TFs, ZNF217, CTCF |
| GH08J127966 | Enhancer | 1.1 | 9.9 | 10.99 | 137 TFs, CTCF, TCF12 |
| GH08J128313 | Enhancer | 1 | 9.9 | 10 | 75 TFs, TCF12, SP1 |

***Cont...*** **Table 2: GeneHancer based predicted genomic regulatory elements related to the MYC gene.**

| | | | | | |
|---|---|---|---|---|---|
| GH08J128203 | Enhancer | 1 | 9.8 | 9.55 | 20 TFs, GATAD2B, ZNF592 |
| GH08J127923 | Enhancer | 0.8 | 10.2 | 7.98 | 17 TFs, FOXA2,NR3C1 |
| GH08J128162 | Enhancer | 0.7 | 9.5 | 6.72 | 10 TFs, EP300, SMC3 |
| GH08J128385 | Enhancer | 0.6 | 10.1 | 6.19 | 15 TFs, GATAD2B, HES1 |
| GH08J128647 | Enhancer | 0.6 | 10.1 | 6.11 | 13 TFs, ZBTB5, GATAD2B |
| GH08J127816 | Enhancer | 1 | 5.8 | 6 | 92 TFs, TCF12, TEAD4 |
| GH08J128041 | Enhancer | 0.6 | 10 | 5.85 | 4 TFs, FOS, CEBPB |
| GH08J127886 | Enhancer | 0.6 | 10.2 | 5.68 | 11 TFs, IRF1, ZNF592 |
| GH08J128649 | Enhancer | 0.5 | 10.7 | 5.62 | 9 TFs, FOS, FOSL2 |
| GH08J127666 | Enhancer | 1.4 | 3.6 | 5.18 | 98 TFs, ZNF217, TCF12 |
| GH08J127922 | Enhancer | 0.5 | 10.2 | 5 | 7 TFs, ZNF316, MAFF |
| GH08J127763 | Enhancer | 0.4 | 11.8 | 4.96 | 4 TFs, POLR2A, FOXP2 |
| GH08J127821 | Enhancer | 0.8 | 5.7 | 4.76 | 24 TFs, USF1, TEAD4 |
| GH08J126874 | Enhancer | 0.4 | 10.2 | 4.27 | 4 TFs, MEF2B,GATA3 |
| GH08J127813 | Enhancer | 0.7 | 5.6 | 3.89 | 9 TFs, CTBP1, RBM25 |
| GH08J127245 | Enhancer | 1.2 | 2.9 | 3.45 | 17 TFs, ZNF426, NFE2 |
| GH08J127826 | Enhancer | 0.6 | 5.7 | 3.13 | 3 TFs, TCF7L2, ZBTB40 |
| GH08J127292 | Enhancer | 1.5 | 1.7 | 2.64 | 136 TFs, EP300, ZBTB40 |
| GH08J127396 | Enhancer | 1.4 | 1.8 | 2.57 | 74 TFs, EP300, CTCF |
| GH08J127959 | Enhancer | 1.4 | 1.4 | 1.9 | 191 TFs, PHB2, ZBTB5 |
| GH08J127389 | Enhancer | 1.1 | 1.6 | 1.78 | 16 TFs, POLR2A, EP300 |

***Cont...*** **Table 2: GeneHancer based predicted genomic regulatory elements related to the MYC gene.**

| | | | | | |
|---|---|---|---|---|---|
| GH08J127339 | Enhancer | 1.1 | 1.5 | 1.62 | 28 TFs, FOS, EP300 |
| GH08J127645 | Enhancer | 0.9 | 1.8 | 1.54 | 7 TFs, EP300, ZNF263 |
| GH08J127265 | Enhancer | 0.9 | 1.5 | 1.35 | 4 TFs, POLR2A, JUND |

**Genome-wide association study**

Genome-wide association study (GWAS) is a tool that identifies thousands of single nucleotide polymorphism (SNPs) of the human genome to detect the loci of the gene causing diseases. Linkage disequilibrium (LD) is associated with GWAS as summarised in Table 3. Strong LD demonstrated among SNP and true mutation. GWAS use a hypothesis with the case-control strategy that is based on "common disease–common variation" (Ponder, 2001). The disease-associated SNP marker with allelic frequency is different among the case and control group that aids to determine the SNP locus. The genetic polymorphism present in the human genome is tested by the International HapMap Project. GWAS in advance in the detection of gene loci by coupling with fast-developing high-throughput genotypic technology (Green, Watson, & Collins, 2015). Yang et al studied for the first in Chinese population. The study could not provide significant results of genome-wide analysis and could not be included in the GWAS catalogue (T. L. Yang et al., 2008).

Sud et al. identified 6 potential mutation of chromatin loci i.e., 6q22.33 (rs9482849, 6q23.3 (rs6928977), 3q28 (rs445989), 13q34 (rs112998813), 10p14 (rs3781093), 6q22.33 (rs9482849), for development of Hodgkin's lymphoma due to genetic mutation. These mutations are associated with Hodgkin's lymphoma and diffuse large B-cell lymphoma(Cerhan et al., 2014; Sud et al., 2017). Mitchell et al. identified 8 gene mutation on chromosome 7p15.3 responsible for multiple myeloma (Mitchell et al., 2016).

**Table 3: Phenotypes from GWAS CatLog for MYC Gene**

| Phenotype | Gene Relation | Best Score | Mean Score | # of Snps | # of Studies |
|---|---|---|---|---|---|
| glioma | GWAS | 64.7 | 31.8 | 1 | 1 |
| colorectal cancer, colorectal adenoma | GWAS | 63.5 | 42.7 | 3 | 1 |
| childhood onset asthma | GWAS | 23.0 | 16.3 | 1 | 2 |
| prostate specific antigen measurement | GWAS | 20.2 | 14.9 | 2 | 1 |
| bladder carcinoma | GWAS | 17.7 | 11.6 | 2 | 4 |
| prostate carcinoma | GWAS | 17.2 | 12.6 | 2 | 2 |
| cleft palate, cleft lip | GWAS | 15.1 | 15.1 | 1 | 1 |
| Eczema | GWAS | 14.5 | 14.5 | 1 | 1 |
| colorectal cancer | GWAS | 14.2 | 12.7 | 3 | 4 |

***Cont...*Table 3: Phenotypes from GWAS CatLog for MYC Gene**

| | | | | | |
|---|---|---|---|---|---|
| hemoglobin measurement | GeneHancer | 13.7 | 13.7 | 1 | 1 |
| allergy | GWAS | 13.2 | 13.2 | 1 | 1 |
| endometrial carcinoma | GWAS | 13.1 | 9.9 | 3 | 2 |
| breast carcinoma | GWAS | 13.0 | 11.8 | 1 | 2 |
| Hodgkins lymphoma | GeneHancer | 13.0 | 9.8 | 1 | 3 |
| diffuse large B-cell lymphoma | GWAS | 12.0 | 11.2 | 2 | 1 |
| ovarian carcinoma | GWAS | 11.5 | 10.0 | 1 | 2 |
| asthma | GWAS | 10.7 | 9.2 | 1 | 2 |
| renal cell carcinoma | GWAS | 10.3 | 10.3 | 3 | 1 |
| body height | GWAS | 9.5 | 8.5 | 2 | 2 |
| allergic sensitization measurement | GWAS | 9.3 | 9.3 | 1 | 1 |
| pancreatic carcinoma | GWAS | 9.0 | 8.2 | 2 | 3 |
| Inhalant adrenergic use measurement | GeneHancer | 8.2 | 8.2 | 1 | 1 |
| multiple sclerosis | GWAS | 8.1 | 8.1 | 1 | 1 |
| endometrial endometrioid carcinoma | GWAS | 8.1 | 8.1 | 1 | 1 |
| platelet crit | GeneHancer | 8.1 | 8.1 | 1 | 1 |
| chronic lymphocytic leukemia | GWAS | 7.7 | 7.7 | 1 | 1 |
| allergic rhinitis | GWAS | 7.4 | 7.4 | 1 | 1 |
| erythrocyte count | GeneHancer | 7.4 | 7.4 | 1 | 1 |
| alcohol use disorder measurement, alcohol dependence | GWAS | 7.3 | 7.3 | 1 | 1 |
| systemic lupus erythematosus | GeneHancer | 6.7 | 6.6 | 2 | 1 |
| tonsillectomy risk measurement | GeneHancer | 6.0 | 6.0 | 1 | 1 |
| waist-hip ratio | GeneHancer | 5.7 | 5.7 | 1 | 1 |

**Sorting Intolerant from Tolerant (SIFT)**

Sorting Intolerant from Tolerant (SIFT) is a protein prediction algorithm that predicts amino acid substitution as deleterious, prioritise nonsynonymous and missense variants as summarized in table 4 (Kumar, Henikoff, & Ng, 2009; Ng & Henikoff, 2002; Sim et al., 2012). A protein can be able to handle an amino acid transition while still functioning normally. It could be able to act naturally, or it could be intolerant to the amino acid. SIFT determines whether an amino acid transition is tolerable or harmful to protein function. SIFT considers protein conservation in homologous sequences as well as the magnitude of the amino acid substitution. It's been seen in a variety of cancer, mutation, and genetic research (Mitsui et al., 2012; Tennessen et al., 2012).

**Table 4: List of deleterious variants of MYC predicted by SIFT (Sorting Intolerant From Tolerant) program.**

| SNP | REF ALLELE | ALT ALLELE | AMINO ACID CHANGE | GENE ID | SIFT SCORE | SIFT MEDIAN | NO OF SEQS AT POSITION | SIFT PREDICTION |
|---|---|---|---|---|---|---|---|---|
| rs28933407 | C | T | P71S | ENSG00000136997 | 0.018 | 2.69 | 93 | DELETERIOUS |
| rs28933407 | C | T | P57S | ENSG00000136997 | 0.021 | 2.39 | 65 | DELETERIOUS |
| rs28933407 | C | T | P72S | ENSG00000136997 | 0.043 | 2.45 | 61 | DELETERIOUS |
| rs28933407 | C | T | P71S | ENSG00000136997 | 0.044 | 2.45 | 61 | DELETERIOUS |
| rs61752959 | G | A | Q33Q | ENSG00000136997 | 0.609 | 2.46 | 50 | TOLERATED |
| rs61752959 | G | A | Q48Q | ENSG00000136997 | 0.614 | 2.52 | 54 | TOLERATED |
| rs61752959 | G | A | Q47Q | ENSG00000136997 | 0.615 | 2.52 | 54 | TOLERATED |
| rs61752959 | G | A | Q47Q | ENSG00000136997 | 0.68 | 2.77 | 74 | TOLERATED |
| rs121918683 | A | C | N86T | ENSG00000136997 | 0.394 | 2.92 | 26 | TOLERATED |
| rs121918683 | A | C | N101T | ENSG00000136997 | 0.402 | 2.9 | 31 | TOLERATED |
| rs121918683 | A | C | N100T | ENSG00000136997 | 0.403 | 2.89 | 31 | TOLERATED |
| rs121918683 | A | C | N100T | ENSG00000136997 | 0.489 | 3.03 | 42 | TOLERATED |
| rs121918684 | G | C | E53D | ENSG00000136997 | 0.479 | 3.09 | 49 | TOLERATED |
| rs121918684 | G | C | E53D | ENSG00000136997 | 0.491 | 2.73 | 43 | TOLERATED |
| rs121918684 | G | C | E54D | ENSG00000136997 | 0.505 | 2.73 | 43 | TOLERATED |
| rs121918684 | G | C | E39D | ENSG00000136997 | 0.591 | 2.63 | 39 | TOLERATED |
| rs121918685 | C | G | P59A | ENSG00000136997 | 0 | 2.4 | 66 | DELETERIOUS |
| rs121918685 | C | G | P74A | ENSG00000136997 | 0 | 2.46 | 62 | DELETERIOUS |
| rs121918685 | C | G | P73A | ENSG00000136997 | 0 | 2.46 | 62 | DELETERIOUS |
| rs121918685 | C | G | P73A | ENSG00000136997 | 0.003 | 2.69 | 94 | DELETERIOUS |
| rs146505192 | T | C | F7L | ENSG00000136997 | 1 | 3.54 | 26 | TOLERATED |
| rs146505192 | T | C | F22L | ENSG00000136997 | 1 | 3.34 | 25 | TOLERATED |
| rs146505192 | T | C | F21L | ENSG00000136997 | 1 | 3.79 | 29 | TOLERATED |
| rs146505192 | T | C | F21L | ENSG00000136997 | 1 | 3.34 | 25 | TOLERATED |
| rs200431478 | C | T | S362F | ENSG00000136997 | 0.003 | 2.46 | 71 | DELETERIOUS |
| rs200431478 | C | T | S361F | ENSG00000136997 | 0.003 | 2.46 | 71 | DELETERIOUS |

Computational biology aims to predict correctly new gene-disease interactions as summarized in table 5. The so-called guilt-by-association (GBA) technique, in which new candidate genes are discovered by their association with genes already known to be active in the disorder being tested, has proven to be a very effective method. Several different kinds of data can be used to draw this relation. Goh et al. use a network that connects gene with the related disease (Goh et al., 2007). Tian et al. unite genetic interactions and protein interactions for the expression of gene correlation (Tian et al., 2008). Ulitsky et al. merge interactions from yeast two-hybrid experiments and published networks (Ulitsky & Shamir, 2007). Human Reference Protein Database is the most frequently database to determine directly protein-protein interactions (Goel, Harsha, Pandey, & Prasad, 2012).

**Table 5: PhenoPred predicted MYC gene associated diseases**

| Known Disease Gene | |
|---|---|
| DOID:63 - temp holding | |
| DOID:162 - cancer | |
| DOID:462 - Malignant Neoplasms | |
| DOID:2227 - Malignant neoplasm of lymphatic and hemopoietic tissue | |
| DOID:2319 - Neoplasm by Special Category | |
| DOID:8584 - Burkitt's tumor or lymphoma | |
| DOID:8716 - Lymphosarcoma and reticulosarcoma | |
| Predicted Disease | Score |
| DOID:3135 - Malignant neoplasm of bone, connective tissue, skin and breast | 5.87 |
| DOID:3094 - Neoplasms, Neuroepithelial | 5.42 |
| DOID:171 - Neuroectodermal Tumors | 5.17 |
| DOID:3620 - Central Nervous System Neoplasms | 5.00 |
| DOID:3195 - Neural Neoplasm | 4.98 |
| DOID:1193 - Nervous System Neoplasms | 4.92 |
| DOID:3041 - Familial Cancer | 4.83 |
| DOID:3093 - Neoplasms, Nerve Tissue | 4.70 |
| DOID:3165 - Skin Neoplasms | 4.70 |

***Cont...*** **Table 5: PhenoPred predicted MYC gene associated diseases**

| | |
|---|---|
| DOID:2994 - Germ cell tumor | 4.66 |
| DOID:688 - Neoplasms, Embryonal | 4.65 |
| DOID:3095 - Neoplasms, Germ Cell and Embryonal | 4.59 |
| DOID:1115 - Sarcoma | 4.42 |
| DOID:2627 - Glioma | 4.37 |
| DOID:122 - Abdominal Neoplasms | 4.36 |
| DOID:208 - Neoplastic Syndromes, Hereditary | 4.33 |
| DOID:3023 - Common Tumor | 4.27 |
| DOID:4159 - Cancer of Skin | 4.25 |
| DOID:685 - Common Carcinoma | 4.20 |
| DOID:170 - Endocrine Gland Neoplasms | 4.10 |

## Conclusion

The proto-oncogene, MYC dysregulation promotes tumorigenesis, apoptosis, cell proliferation. MYC gene alteration has been detected in haematological malignancy particularly in Hodgkin disease and B-cell neoplasm with aggressive behaviour. The Discovery of novel therapeutics approaches accelerates our understanding of tumorigenesis mechanism in MYC associated haematological malignancy. We have emphasized the transcriptional regulation network of MYC during leukaemia that enlighting the molecular pathway for MYC associated apoptosis, cell growth and cell proliferation. Here we have summarised various pharmacological therapy directly or indirectly inhibiting the MYC (Table 1). Enhancers are specialised regions of the genome that regulate the levels of expression of target genes. They will exist at a great distance from their target gene and loop in complex systems to do so. The field has been attempting to answer the question of which enhancers interfere with target genes for many years, and several experimental approaches to do so have substantial statistical, viability, or reproducibility disadvantages.

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**Data Availability Statement**

Research data are not shared. The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflicts of Interest**: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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