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Editorial

Health Benefits of Honey

Prof. Dr. Azhar Masud Bhatti
Editor-in-Chief

Honey is beneficial for health. It is an antioxidant, anti-inflammatory, antimicrobial, help in wound healing especially in burn. It also helps in improving digestive, heart health and supporting gut bacteria. Furthermore, it helps in relieving cough, sore throat and better for many other skin conditions. Honey has also beneficial properties in improving immunity and neuroprotective effect.

It also lower blood pressure and benefit for management of type 2 diabetes. It improves cholesterol and triglyceride level. It contains a variety of nutrients, several vitamins and minerals.

Characteristics and Composition of Honey: A highly perishable, thin sweet liquid is transformed into a stable high density viscous, acidic, high energy food. Because of its low PH, high osmalatity and low moisture micro-organisms responsible for spoilage do not grow in it. Moisture in honey ranges between 15 to 25% depending upon the origin of nectar and weather conditions. At 17.4% moisture, it is in equilibrium with 58% relative humidity of atmosphere, neither gaining nor losing moisture from the environment.

Major components of honey are sugars, of which mono-saccharides, glucose and fructose make up around 70% of the total. Disaccharides including sucrose add 10% and water in which sugars are dissolved, 17 to 20%. So far 181 different substances have been identified in honey and some of these are not known to exist any where else. The exact composition of honey depends mainly on the plant source it is derived from, but also on the weather soil and other factors and two honeys are never the same.

Sugar and Sweetness of Honey: Eighty percent (80%) of honey consists of sugars. Some honey taste sweeter than others because sugars differ in their sweetness. Two main sugars are fructose and glucose. Fructose is slightly sweeter than sucrose. Glucose is less sweeter than sucrose. Glucose is less sweeter and Maltose is even lesser. Relative sweetness of different sugars is not the same at all concentrations. Dilute solutions of sucrose (upto 10%) taste sweeter than corresponding solutions of invert sugars (glucose and fructose). But as the concentration increases the sweetness of sucrose lags more and more behind. If sucrose is taken as 100 then equivalent glucose and fructose is 66 and maltose is 30. Many mixtures of sugars taste sweeter than would be expected by adding their separate effects together. Bees like to collect sucrose, glucose, maltose and fructose in that order, but choices also vary with concentrations. Reducing disaccharides (maltose) present in honey increase during storage. The shelf life of honey is 2.5 years, but is wholesome after decades.

Proteins and Amino Acids in Honey: Honey contains traces of proteins derived from honey bees. Amino acids are also present, the most abundant being praline, followed by lysine, glutamic acid and aspartate. Others present are alanine, araginine, cystine, glycine, histidine, isoleucine, methionine, phenyle alanine, serine threonine, tryptophan, tyrosine and valine. These amino acids are also useful in distinguishing one honey from other and can tell if the honey is natural at all. Amino acids are break down products of proteins which are of been origin, but proteins of plant origin are also present. The ash contents range from 0.02 to 1 percent. Dark honey has higher mineral contents Potassium is the highest (100 times as much as IRON), twenty-eight minerals have been found in honey. Hydroxyl methyl furfural is another very important constituent present in honey and the amount of this chemical in honey is an indirect indicator of quality.

While the list of the vitamins is impressive, the quantities are nutritionally insignificant to humans. Vitamin BI, Vitamin B 12, B complex, Riboflavin, Nicotinic acid, B6, Pantothenic acid and vitamin C (ascorbic) are present in honey.

Enzymes: There are three important enzymes in honey. Invertase, diastase, (amylase), and glucose oxidase. Catalase and acid phosphatase are also present. Invertase is produced hypo-pharyngeal glands of bees, and changes sucrose into glucose and fructose. So if the enzyme is still present in unfinished honey, the process could c continue, even if the honey has been extracted from the comb. Invertase is destroyed by heating.

Diastase (amylase) is produced in similar fashion and is also found in plants. It breaks down starch and is involved in digestion of pollens in bees. It is destroyed by heat. Glucose oxidase is produced in the hypopharyngeal glands of bees, and oxidizes glucose in uripened honey. It is virtually inactive in full density honey, but becomes active in diluted honey. Changing glucose into Gluconic acid, and hydrogen per oxide. It is sensitive to sunlight as well as to heat. Substances called INHIBINES thought to be present causing inhibition of growth of bacteria, is actually glucose oxidase. Catalase and Acid phosphatase are also found in honey but are in-significant.

Honey contains acids (average PH 3.9) which contributes to its resistance to damage by microorganism. The tarness they produce e also enhances its flavor. Main acid is Gluconic acid, and is produced by the action of glucose oxidase on glucose. Formic acid has been found consistently, other acids found are benzoic acid, butyric, citric, isovaleric, lactic, malic, oxalic phenyl acetie, propoionic, pyroglutamic, succinic and valeric acids.

Physical Properties of Honey: All honeys are liquid when produced by bees. They are super-saturated solutions. On storage at room temperatures which is 10-20 C lower than the hive temperatures, the first sugar to crystallize out is glucose. The granulation is also affected by the presence of suspended minute particles in the honey that act as nucleus. Flash heating is utilized to dissolve any crystals present in the honey before sale on commercial basis.

Each capped cell of comb provides an environment for honey storage that is protected from moisture, dust and the contaminants. This inhibits granulations of honey in the comb.

Medical Uses of Honey: Honey has been used successfully in the treatment of diarrhea. It has proven capability to kill the causative organisms, provide nutrition, helps absorption of sodium and other electrolytes and water. Substitution of honey on ORS instead of glucose has definitely reduced hospital stay of children with diarrhea.

Honey has been found effective in wound healing. It increases granulation, protects from infections and reduces healing time. It has been used with great success in infected wounds.[1]

Honey when used in breakfast for the types II diabetic patients did not cause deterioration effect on the blood glucose level.[2]

It has also been used in prevention of cornea; and other eye problems, articles are mostly in Russian language and not available for translation.[3]

Summaries of the original articles are presented.

Honey in the Treatment of Infantile Gastro Enteritis: IE Haffe Jee, A. Moosa. Dept. of Pediatrics, child health. Faculty of medicine, university of Natal, Durban. South Africa.[4]

Honey has been effectively used in infantile diarrhea, because of bacteriostatic activity against entero pathogenic organisms. This study was designed to document this effectiveness.

169 infants and children were given trial. Control group was selected randomly, and treated with the standard regimen, while trial group was treated with the ORS containing honey (50ml/1). Examination of stool was carried out for parasites and cultures were done to isolate the invading organisms, Elisa was used for Rota virus and clinitest for reducing substances.[4]

Recovery time in viral diarrhea was same both in honey treated and controlled. But in bacterial diarrhea the honey treated patients had a shorter recovery time, 58 hours as compared to 93 hours in control. Rehydration time was shorter in honey treated, 18 hours as compared to 23 hours in controls. Reducing substances and plasma sodium concentration showed no difference.

This study has shown that honey shortens the duration of diarrhea in patients with bacterial gastro enterits caused by organisms such as slamonella, Shigella and E.Coli. In non bacterial gastro enteritis honey had the same effects as glucose on the duration of diarrhea. Also oral honey may safely be used as substitute for pure glucose in oral rehyderation solutions, provided these contain electrolytes in the currently recommended concentration and honey providing III mols of each glucose and fructose per liter, (2Gm/100ml).

Honey could also be used to promote sodium and water absorption from howel as oral rice water or sucrose does because of high osmotic contents. Fructose is absorbed by facilitated diffusion rather by active transport and is not coupled with sodium ions, helping prevention of hyper-natremia. Furthermore honey does not result in osmotic diarrhea, when used in proper dilution in oral rehyderation solution (50ml/1).

Oral rehyderation solution used by the researchers has a total glucose fructose concentration of 220 mmol/L (4gm/100ml) and even this did not appear to aggravate diarrhea.

Anti Bacterial Action of Honey: Jedda A, Ramsroop KVG, et al. KE Hosp. Durban. South Africa[5]

This study was carried out at King Edward Hospital Durban, to evaluate the anti-bacterial effects of honey in varying dilution on common gram-positive and gram-negative organisms.

Pure honey was found to be a very potent inhibitor of growth of bacteria as Salmonella, Shigella, entero pathogenic E. Coli, and V. Cholera, all of which are known to cause considerable morality from diarrhoeal diseases in developing countries. Honey also inhibits growth of Pseudomonas aerogenosa, Strepto coccal group B and Strep. Pyognenese, Strep. Fecalis and listeria monocytogens (Major bacteria causing infections in newborn age group. Explains the reason for using honey as the first food in most newborns in our country).

Growth of H. influenza is also inhibited by honey. A common organism in the younger age group causing pneumonia, ear infection and meningitis.

The possible mechanisms of action of honey are following:

1. Shrinkage and disruption of bacterial cell wall because of osmotic effect of hygroscoric sugars in honey.
2. Low pH.
3. Substance called “inhibin” with bacterial properties has been postulated to be present in honey.

Acceleration of Wound Healing by Topical Application of Honey: Ariesh Bergman, Joseph Yanal, berry Weiss, David Bell, Menachem P. David Tele Aviv (Israel).[6]

Honey helps wound healing was documented by this study. Mice were used, incision were given, treated with local application of honey and definite improved healing was noted.

These researchers used male mouse, and incisions of 10x10mm were made at nape of neck. A thin layer of boiled pure honey was applied before dressing in

experimental groups, whereas saline solution was used in control group: following results were noted:

1. The epithelialization was larger in honey treated animals than in control.
2. The increase from control level that was 58% on day 3 rose to 114% on day 6.
3. Thickness of granulation tissue was greater among honey treated animals the in controls. The increase was 69% by day 6 and remained to that level till day 9.
4. The area of the wound approximately doubled by day 3 from original 100 mm2 in both groups. However, the area of the wound remained than in the controls. The reduction in size was 37% on day 6, and 48% on day 9.
5. No clinical infection was noted in both of granulation tissue and accelerated epithelization of healing tissues, by honey.

Additional anti-microbial properties may be due to hypertonicity and low pH, which inhibits bacterial growth. Contribution to the wound healing may also be due to in part to its being an excellent energy source in a catabolic situation. Similarly the mechanism for its hyproscopic effect in reducing edema and its action as viscous barrier to wound invasion and fluid loss are known. Honey contains enzymes such as Catalase which may also affect the healing process.

Honey in the Treatment of Infected Wound: Professor Chirife et al lancet 24, 1982. Professor Chirife and his colleagues documented the inhibition of growth of micro-organisms due to honey.[7]

In developing countries honey has been used successfully for the treatment of infected wounds. In clinical and in vitro studies honey has been found to prevent growth of the common pathogens, including streptococci and coagulase positive staphylo-cocci. However, growth of certain candida species is not inhibited. The low pH (3.7) of honey creates very unfavourable environment for bacterial growth which can not be achieved by granulated sugar. The tissue dehydration is rapid and can cause excessive desication and thus interferes with healing. This however can be avoided by application of saline soaks.

Honey for Necrotic Breast Ulcers: D A Mossel lancet Nov, 15, 1980. University of Utrecht Netherlands.[8]

There were reports of isolation of clostridia Botulism from some samples of honey. The author opinions that by using honey on open wound we will be exposing a patient to an unnecessary risk,

This is a review of literature, and Dr. Mossel writing this review agrees, that honey soothers the wound protects it from infection and dose not allow proliferation of organisms. He thinks that honey is not itself sterile. Midura et al isolated Clostreidia botulism type B and A from some samples from Hungary. Although botulism contracted from infected wounds is not a common disease. It is unwise to expose a patient to can extra risk which is medically unacceptable.

Surgery and Honey

Surgery in Western Kenya: Honey has been used as sterile coating of surgical wounds. It helps healing of wound, and protects it from infections. It helps healing of wounds and protects it from infections. This authors spent 2 years in Kenya and used honey post operatively with good results.

Honey was used as a thin layer on open wounds 2 to 3 times daily with excellent results. Honey rendered these wounds not only bacteriologically sterile in 3 to 6 days but also encouraged the formation of healthy granulation.

Honey at Breakfast Have no Additional Acute Hyperglycemic Effect Over Bread in Type II Diabetic Patients: BRONT F, et al, Diabetolgia, 1985. 28; 213-217.[2]

Blood glucose level is highest after breakfast even more than 2 hours post meal or after mid day level. In this study glucose levels were checked after honey meal and compared with post bread levels and found no significant difference.

The researcher concluded after the study that in type II diabetics, in acute condition substitution of part of a meal by a reasonable amount of honey has no deleterious on blood glucose regulation and insulin secretion.

Al–Quran: God ordered honey bee," She should make house in mountains, trees and high places. She ought to eat the juices of fruits, trees and stay clean. There will be drinkable liquid extracted from the abdomen. The liquid multiformed will have healing power."[9]

Table No.1: (Derived from Food and Nutrition Encyclopedia Vol. 1)

Nutrient	Unit Per 100 Gms.	Per Table Spoon (20 gm)
Energy	61 K cal.	310
Proteins	0.1 gm	0.30
CHO	165 gm	823
Calcium	1 mgm	5
Phosphorus	1.2 mgm	6
Sodium	1 mgm	5
Magnesium	0.6 mgm	3
Potassium	10.2 mgm	51
Iron	0.1 mgm	0.50
Zinc	0.02 mgm	0.1
Copper	0.04 mgm	0.20
Vit. C	0.2 mgm	1.0
Riboflavin	0.008 mgm	0.04
Niacin	0.30 mgm	0.30
Pantothenic acid	0.004 mgm	0.02
Folic acid	0.6 mcgm	3

Table No.2: Minerals in Honey:

Minerals	Dark Honey	Light honey
Potassium	1676	205
Chloride	113	52

Sulphur	100	58
Calcium	51	49
Sodium	76	18
Phosphorus	47	35
Magnesium	35	19
Silicon	14	9
Iron	9.4	2.4
Maganese	4.1	0.3
Copper	0.6	0.3

Table No.3: Average (%) of Major Constituents of Honey:

Fructose	21-53.9	38.2
Glucose	22.4-44.4	31.3
Sucrose	0.0-2.4	8.3
F+G+S		70.8
Water	13.4-26.6	17.2
Reduced di saccharides calculated as maltose 7.3%		
Higher sugars	1.5%	
Total acid	0.57%	
Ash	0.17%	
Nitrogen	0.94%	

List of Sugars Present in Honey:

Monosaccharides (70% of honey): Glucose, Fructose, Disaccharides, Sucrose 1-3%

Reducing disaccharides calculated as maltose (7%): Maltose, Iso maltose, Niogerose, Turanose, Kojibiose, Netrenhalose, Centibiose, Laminaribiose, Ceucrose

Trisaccharides and higher sugars (1.5%):

Melezitose, Raffinose, Iso panose, Iso mato tetrose, 64 alpha gluco syle sucrose,

Are galacto mannose

Elrose, Dextrinose, Matotribose, Iso Maltose pentose, Centose, 1-Kestose, Panose, Panose, Iso maltortriose, 3-Alpha isomaltose, Sylglucose

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Original Article

Diagnostic Accuracy of Cold and Electric Pulp Test in Determining Pulpal Status in Saudi Sub-Population

Omar Aljasir[1], Muhammad Atif Saleem Agwan[2], and Syed Fareed Mohsin[3]

ABSTRACT

Objective: This study evaluated the accuracy of cold and electric pulp tests in defining pulpal status in the Saudi sub-population.

Study Design: Cross-sectional analytical study

Place and Duration of Study: This study was conducted at the College of Dentistry, Qassim University, Saudi Arabia, from September 2023- February 2024.

Methods: This cross-sectional study included 118 participants aged 18–60 years with no prior clinical diagnosis of pulpal disease. Pulpal status was determined by the presence (vital) or absence (necrotic) of bleeding from the pulp chamber following access opening. Both cold and electric pulp tests were conducted with at least a 5-minute interval between them. The sensitivity, specificity, and accuracy of each test were evaluated, and reproducibility was measured using the intraclass correlation coefficient.

Results: The study found that, based on the cold test, 62 (52.5%) teeth were non-vital and 56 (47.5%) were vital. In comparison, the EPT identified 77 (65.3%) teeth as vital and 41 (34.7%) as non-vital. The cold test demonstrated greater diagnostic accuracy (0.808) than the EPT (0.639) in determining pulpal status.

Conclusion: The findings indicate that the cold test exhibited higher overall accuracy (80.8%) and specificity (85.5%) than the electric pulp test (63.9% accuracy and 49.1% specificity) in a Saudi sub-population. Although EPT showed slightly higher sensitivity (77.8% vs. 76.2%), the cold test provided superior predictive values, making it a more dependable method for assessing tooth vitality.

Key Words: Accuracy, electric pulp test, cold test, pulp vitality

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INTRODUCTION

In endodontics, pulp testing serves as a vital diagnostic aid that guides appropriate treatment planning.[1] Though histological analysis is the most reliable method for evaluating pulp vitality, it is impractical before treatment because the pulp is encased within hard tissues.[2]

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Therefore, assessing pulpal status remains challenging, however it is considered acceptable while both sensibility and pulp vitality are maintained. Sensibility is the ability to react to an external stimulus. Sensibility pulp tests offer qualitative sensory information to deduce the pulp's "vitality" and general condition.[1] Thermal and electric pulp sensibility tests are applied to indirectly evaluate pulpal condition by assessing the nerves inside the dental pulp.[2, 3] Interpreting sensibility test outcomes needs attention, as they are subjective and depend on both the patient and the operator.[4]

Thermal pulp testing includes coating chemical agents to the tooth surface to either elevate (heat pulp testing HPT) or lower (cold pulp testing CPT) the temperature, thereby provoking sensory reactions from the pulp through thermal transmission.[1] A lot of pulp tests are commonly applied in combination to develop more accurate results.[5]

The cold pulp test is frequently used vitality test by dental professionals, providing perceptions into the pulp's position ("non-vital/vital") or inflammatory status (irreversible/reversible).[6] Examples of CPT agents comprise ethyl chloride, ice, CO2 snow, and refrigerant sprays (such as dichlorodifluoromethane, tetrafluoroethane, or a propane/butane/isobutane gas mixture saved in pressurized cans). Refrigerant sprays

have the advantage of not requiring specific storage conditions and enabling accurate application with a cotton pellet.[7] Currently, frequently used CPT agents in clinical situations are refrigerant sprays due to their ease of storage, comparative affordability, and convenience of application.[1]

Electric pulp testing (EPT) includes applying an electrical stimulus to the tooth to initiate the pulpal nerve filaments connected with pain and produce a reaction from the patient. Though extensively used, the electric pulp test (EPT) is highly technique-sensitive. Reliable results depend on several factors, such as delivering an adequate stimulus, positioning the electrode correctly, properly isolating the tooth, applying a suitable conducting medium, maintaining a consistent testing procedure, and carefully interpreting the findings.[8]

Vitality pulp tests assess numerous parameters that reflect the vascularity of the tooth pulp that accurately determines the vitality of pulp. Techniques for instance, laser Doppler flowmetry (LDF), pulse oximetry (PO), laser speckle imaging (LSI), transmitted laser light (TLL), dual wavelength spectrophotometry (DWS), and transmitted light plethysmography (TLP) employ optical technology.[9] These techniques are entirely noninvasive, painless, and objective; the patient is not required to provide a subjective response.[9]

Sensitivity defines to the percentage of correct cases recognized by a diagnostic test, while specificity indicates the percentage of non-cases correctly recognized. Positive predictive value measures percentage of positive test results, which are true cases, while negative predictive value measures percentage of negative test results, which are true non-cases.[10]

By estimating the specificity and sensitivity of electric pulp and cold tests, the study purposes to enhance clinical decision-making, lessen needless procedures, and enhance patient outcomes in dental clinics. Therefore, this study assessed the accuracy of cold and electric pulp tests in assessing pulp condition in a clinical setting in the Saudi sub-population.

METHODS

This cross-sectional study's approval was obtained by the Institutional Review Board of Qassim University (Reference # 21-14-14). The duration of the study was about six months from Sept 2023- Feb 2024. Every patient gave their signed, informed consent, before participation. The study included 118 subjects aged between 18 and 60 years, who had no prior clinical diagnosis of pulp status. Only teeth without prior endodontic treatment, with intact crowns, and in patients with healthy periodontium were included. Exclusion criteria encompassed full coverage crowns, extensive restorations, current trauma, reverted pulp chambers, or calcification within root canal system, as well as patients undergoing orthodontic treatment or with systemic diseases.

Pulp Testing Methods: An independent researcher, not informed about the clinical indicators, symptoms, past dental records, and radiographs, performed endodontic diagnostic tests. Participants signaled sensation by raising their hand. Each test was conducted with at least a 5-minute interval, by rubber dam isolation and polyester strips to isolate the tooth.

Cold pulp testing: A thin film of Vaseline was coated to the labial or buccal tooth surface as a separating medium. A No. 2 cotton pellet sprayed with refrigerant (Endo-Ice, Coltène/Whaledent, Cuyahoga Falls, OH, USA), was then positioned on the mid-buccal surface for up to 15 seconds or till the participant signaled feeling cold sensation.

Electric pulp testing: Teeth were isolated, dried, and covered with toothpaste before applying the EPT (Parkell Digitest II™). An increasing electrical stimulus was delivered to the mid-buccal surface until the patient responded, with a response reflecting vitality and no response reflecting non-vitality. A two-minute interval was kept between tests to allow pulpal recovery.

Ideal standard: The study determined pulp status using the existence (indicating vitality of pulp) or nonexistence (indicating necrotic pulp) of blood flow after access opening as the reference standard as reported by Janani K et al.[11]

Statistical analysis: The SPSS Version 23.0 was used to analyze the data. Demographic details such as age, gender, and tooth location were documented as frequencies and percentages. Sensitivity, specificity and accuracy were measured. True positive (TP), false positive (FP), true negative (TN), and false negative (FN) responses were identified to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and incidence for each test. Reproducibility was assessed using the Intraclass correlation coefficient.

RESULTS

A total of 118 patients with presenting complaints of toothache and to assess the pulp health, were involved. Most of the patients fall within the 31-40 age group 42(35.6%), followed by the 20-30 age group 40(33.9%). There were 58(49.2%) males, and 60(50.8%) females. The mandibular left quadrant is the most common location for toothache 40(33.9%), while the mandibular right quadrant is the least common 21(17.8%). Pain on biting is the most common presenting complaint 41(34.7%), followed by spontaneous pain 28(23.7%) and sensitivity 21(17.8%), as depicted in Table 1.

Fig. I presents the distribution of vitality and non-vitality of teeth based on different diagnostic tests: Cold test results showed a slight majority of the teeth classified as non-vital 62(52.5%), while 56(47.5%)

identified as vital. In contrast, the EPT results indicate that most teeth were vital 77(65.3%), with a smaller proportion indicated as non-vital 41(34.7%). For the bleeding after access opening, 63(53.4%) of the teeth exhibited bleeding, suggesting vitality, whereas 55(46.6%) did not show bleeding, indicating non-vital status.

Table No. 1: Demographic details of the patients (n=118).

Variables		n(%)
Age group	20 -30	40(33.9%)
	31- 40	42(35.6%)
	41- 50	22(18.6%)
	51-60	14(11.8%)
Gender	Male	58(49.2%)
	Female	60(50.8%)
Tooth location	Maxillary right quadrant	22(18.6%)
	Maxillary left quadrant	35(29.6%)
	Mandibular right quadrant	21(17.8%)
	Mandibular left quadrant	40(33.9%)
Presenting complaint	spontaneous pain	28 (23.7%)
	pain of biting	41(34.7%)
	sinus	6 (5.1%)
	sensitivity	21(17.8%)
	food packing	9 (7.6%)
	More than one signs and symptoms	13(11.0%)

Table 2 shows the specificity and sensitivity of the cold test and EPT in assessing tooth vitality, with bleeding on chamber opening used as the reference standard. The cold test classified 56 teeth as vital, of which 48(76.2%) bled upon chamber opening and 8(14.5%) did not. It also identified 55 teeth as non-vital, with 47(85.5%) showing no bleeding and 15(23.8%) bleeding. This relates to a sensitivity of 76.2% and specificity of 85.5%. EPT classified 77 teeth as vital, of which 49(77.8%) bled and 28(50.9%) did not, while 41 teeth were non-vital, with 14(22.2%) showing bleeding and 27(49.1%) not bleeding. This reflects a sensitivity of 77.8% but a lower specificity of 49.1%.

Table No. 2: The specificity and sensitivity of cold test and EPT.

Variables		Bleeding after access cavity		Total
		Yes	No	
cold test	**Vital**	48 76.2%	8 14.5%	56 47.5%
	Non-Vital	15 23.8%	47 85.5%	62 52.5%
Total		63 100.0%	55 100.0%	118 100.0%
Electric pulp testing	**Vital**	49 77.8%	28 50.9%	77 65.3%
	Non-Vital	14 22.2%	27 49.1%	41 34.7%
Total		63 100.0%	55 100.0%	118 100.0%

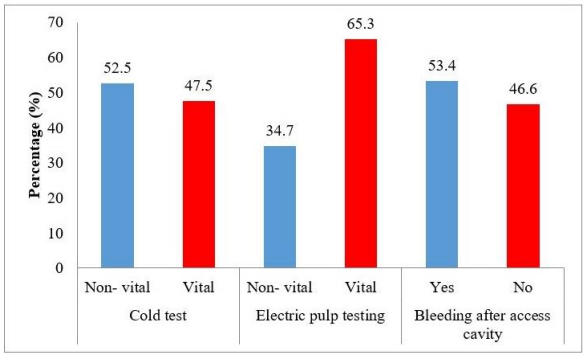

Figure No. 1: The graphical demonstration of the distribution of vital and non-vital teeth using pulp testing methods.

Table 3 shows that the cold test demonstrated higher overall diagnostic accuracy (0.808) than the EPT (0.639) for assessing pulpal status. Both tests showed comparable sensitivity (cold: 0.762, EPT: 0.778), indicating similar ability to identify vital teeth. However, the cold test showed much greater specificity (0.855 vs. 0.491), making it more dependable in detecting non-vital teeth.

Table No. 3: The diagnostic accuracy of cold test and EPT.

Variables	Accuracy	Sensitivity	Specificity	PPV	NPV	Reproducibility
Gold standard vs Cold test	0.808	0.762	0.855	0.857	0.758	0.760
Gold standard vs Electric pulp testing	0.639	0.778	0.491	0.636	0.659	0.431

DISCUSSION

This study assessed the diagnostic accuracy of pulp testing methods in evaluating the pulp health of permanent teeth in the Saudi sub-population.

Test accuracy is typically assessed by relating the findings of an index test with a reference standard, often referred to as a "gold" standard, which confirms presence or absence of the target condition.[12-14] The present study proved that direct vision in access cavity preparation served as the gold standard for

differentiating between non-vital and vital teeth. Similarly, Ghouth et al.'s study employed a reference standard that involved either pulpal debridement or finalized root canal therapy.[15]

One of the cross-sectional studies determined the diagnostic accuracy of the cold test and EPT in single-rooted teeth with irreversible pulpitis. Clinically, 81 teeth (45%) were non-vital, while the remaining 99 teeth (55%) were found to be vital. EPT demonstrated high sensitivity (94.95%), specificity (92.59%), and diagnostic accuracy (93.89%), suggesting it is more reliable than the cold test.[16] However, the present study denies these findings, showing that the cold test reported higher sensitivity (76.2%) and specificity (85.5%) as compared to EPT (sensitivity: 77.8%, specificity: 49.1%). Moreover, the cold test also presented higher overall accuracy (80.8%) than the EPT (63.9%). The PPV and NPV were higher for the cold test (PPV: 85.7%, NPV: 75.8%) than for EPT (PPV: 63.6%, NPV: 65.9%).

In the present study, the cold test presented greater overall diagnostic accuracy (0.808) in comparison with the electric pulp test (0.639) for assessing pulpal status. Both tests indicated high sensitivity (cold: 0.762, EPT: 0.778), presenting effectiveness in detecting vital pulp. However, the cold test showed noticeably higher specificity (0.855 vs. 0.491), making it more reliable in detecting non-vital teeth. These results prove that the cold test was more precise and consistent diagnostic way in clinical scenario compared to the electric pulp test. Comparable results were stated in a previous study, where the cold test exhibited higher sensitivity (87%) and accuracy (87%) as compared to the bridging EPT (66% and 67%, respectively).[17]

A research by Peterson et al. who stated the electric pulp test (EPT) had an accuracy as high as 81%, whereas further studies presented accuracies of 75%[18] and 76%[19] for EPT. For the cold test, other studies have shown accuracies of 90%[18] and 86%.[17] As far as the present study is concerned, the cold test revealed an accuracy of 0.808 for assessing pulp status, while the electric pulp test indicated a lower accuracy of 0.639.

In the present study, the cold test demonstrated greater diagnostic accuracy (0.808) than the EPT (0.639) for assessing pulpal status. Both showed comparable sensitivity (cold: 0.762, EPT: 0.778), but the cold test had much higher specificity (0.855 vs. 0.491), making it more reliable in detecting non-vital teeth. The cold test also had superior predictive values (PPV: 0.857, NPV: 0.758) compared to the EPT (PPV: 0.636, NPV: 0.659). These findings showed partial similarity to a research by Weisleder et al., which stated PPVs of 0.93 for the cold test and 0.83 for the EPT, and NPVs of 0.74 for the cold test and 0.87 for the EPT.[20] Similarly, a research of Villa-Chávez et al. reported a NPV of 0.89 for the heat test, 0.90 for the cold test, and 0.83 for the EPT, with a PPV of 1.0 for all three tests with an incidence of 45%.[19] Likewise, another study reported that the accuracy of the cold, heat, and EPT was 78%, 74%, and 62%, respectively. The sensitivity tests identified irreversible pulpitis with greater probabilities: the NPV was 63% for cold, 67% for heat, and 54% for EPT, while the PPV was 83% for cold, 91% for heat, and 95% for EPT.[21]

This study had a few limitations. Both cold and electric pulp tests involve a degree of subjectivity, as the patient's response to stimuli can vary based on individual pain thresholds and psychological factors. Particularly in teeth with numerous roots or calcified canals, cold and electric pulp tests may not always accurately reflect the pulpal state. Larger and more varied samples should be considered in future studies to enhance the generalization of results across different populations.

CONCLUSION

This study concluded that the cold test showed overall greater accuracy (80.8%) and specificity (85.5%) as compared to electric pulp testing (63.9% accuracy and 49.1% specificity) in Saudi sub-population. Whereas EPT has a little bit higher sensitivity (77.8% vs. 76.2%), the cold test delivers superior predictive values, making it a more dependable diagnostic aid for assessing tooth vitality.

Author's Contribution:

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Drafting or Revising Critically:	Muhammad Atif Saleem Agwan, Syed Fareed Mohsin, Omar Aljasir
Final Approval of version:	All the above authors
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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Original Article

Outcomes of the Complications Between Early and Late Stent Removal in Hypospadias Repair

Imran Qadir[1], Ahmad Wahab Arshad[2], Sadaqat Ali[3], Tahir Shahzad Nawaz Babar[4], Hamza Sohail[4] and Nisar Ahmad[5]

ABSTRACT

Objective: To compare the postoperative complications between early (5th postoperative day) and late (10th postoperative day) stent removal following Snodgrass repair for distal penile hypospadias.

Study Design: Randomized controlled trial study

Place and Duration of Study: This study was conducted at the Department of Pediatric urology, Children Hospital Faisalabad from 01 November-2024 to July 2025.

Methods: This trial was done after the approval by the ethical committee of the institutional review Board (IRB). A total of 86 treatment-naïve patients of distal penile hypospadias were included and randomly segregated into two groups. Where group A early stent removal considered at 5th day While late stent removal counted at 10th day was Group B. Standard Snodgrass repair was performed in all patients by the same surgical team. Complications like i.e. urethrocutaneous fistula (UCF) and wound dehiscence were assessed at 2 weeks, 1 month, and 3 months postoperatively. Data analysis was done with the help of SPSS version 26, and p-values <0.05 were considered significant.

Results: Patients were segregated in two groups, each group consists of 43 patients. Group-A removed catheter at 5th day of surgery, while in group-B removed catheter at 10th day of surgery. Bleeding from wound site found in group-A (8.14%) 07 patients, while in group-B 05 (5.81%). Wound infection in group-A (6.98%) 06 patients, while in group-B 07 (8.14%). UTI in group-A (2.33%) 02 patients, while in group-B 04 (4.65%). Urinary retention in group-A (6.98%) 06 patients, while in group-B 05 (5.81%). Urinary Extravasation in group-A (10.47%) 09 patients, while in group-B 12 (13.95%).

To access the late compilations twenty seven patients (31.47%) were uneventful in group-A while twenty nine patients (33.72%) in group-B were remained uneventful. UC Fistula in group-A (10.47%) 09 patients, while in group-B 08 (9.30%). Wound Dehiscence in group-A (8.14%) 07 patients, while in group-B 06 (6.98%).

Conclusion: Early stent removal following Snodgrass repair may potentially reduce postoperative morbidity and hospital stay without increasing complication rates.

Key Words: Postoperative Complications, Early and Late Stent Removal, Hypospadias Repair

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INTRODUCTION

Hypospadias is considered as a commonly found congenital urethral anomaly of the genitalia in male population, distinctively by an abnormally placed urethral opening on the ventral side of the penis. The reason of this condition, is due to incomplete fusion of urethral folds during embryological development [1,2]. The prevalence varies globally, affecting approximately one in 250 live male population [3,4]. It is commonly classified into anterior, middle, and the posterior types based on the position of the meatus. The anterior and middle forms are more frequent and usually repaired in a single stage, whereas the posterior forms may require staged procedures [5,6].

Surgical correction of hypospadias aims to achieve both functional and cosmetic restoration of the penis. The primary goals include creating a straight penis (orthoplasty), positioning the meatus found at the tip of the glans, and ensuring a good urinary stream with minimal postoperative complications. Numerous surgical techniques evolved with the passage of time, but the Snodgrass technique (Tabularized Incised Plate

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urethroplasty), introduced in 1994, has become the most widely accepted due to its simplicity, reliability, and excellent cosmetic outcomes[7].

Despite advancements in surgical methods, complications i.e. urethrocutaneous fistula, dehiscence of wound, meatal stenosis remain major concerns[8,9]. The timing of stent removal after repair is a crucial factor influencing outcomes. Extended catheterization may enhance healing by preventing urinary leakage across the repair site, but prolonged use increases discomfort, urinary tract infections, and bladder spasms. Conversely, early removal of the stent may reduce hospital stay and improve patient comfort, but may pose a risk of suture line disruption. Therefore, determining the optimal timing for stent removal is essential for improving surgical outcomes[10].

Previous studies have reported mixed results. Fakhry et al. (2021) suggested no major difference between the early and the late removal of stent[11], while Kumar and Ram (2022) observed higher bladder spasms in the late removal group[12]. However, there remains a small amount of local data, especially in the Pakistani population. This study seeks to provide evidence-based insight to guide paediatric surgeons & paediatric urologist in optimizing postoperative management following Snodgrass repair.

METHODS

This randomized controlled trial (RCT) was conducted in the Pediatric urology Department, Children Hospital Faisalabad, over a duration of 12 months following approval. A total of 86 patients. Where the 43 participants in each group, selected through the non-probability convenient sampling technique of sampling. The study included treatment-naïve patients with distal penile hypospadias, aged between 6 months and 12 years. Patients having hypospadias repair in the past, syndromic conditions or major congenital malformations, bladder dysfunction, deranged renal function, or those presenting with mid or proximal hypospadias will be excluded from the study.

Methodology: After obtaining ethical approval and informed consent, 86 cases were enrolled. All underwent Snodgrass repair under aseptic conditions. Standard surgical steps included penile degloving, correction of chordee if present, incision made on the midline of urethral plate, and tabularization over the stent. The neourethra was reinforced using a dartos flap. Patients were assigned randomly into two different groups: Group-A (stent removal at 05th Postoperative day) and Group B (stent removal at 10th postoperative day). Follow-up evaluations were done at 2 weeks, 1 month, and 3 months to assess early and late complications.

Statistical Analysis: Data analysis was done with the version 26 of SPSS. Quantitative data were expressed as mean ± SD, and qualitative data were presented as the frequencies and the percentages. Chi-square test, was used to compare the complication rates between the groups. P-value <0.05 was considered as statistically significant.

RESULTS

In our study there were total N=86, Male patient Aged ranged from 02 years to 14 years, The mean age of the entire study of 86 male patients is 8.58 years. Patients were segregated in two equal groups where each group consisted on 43 patients. Group-A patients have been removed catheter at 5th post operative day, while in group-B the catheters was removed at 10th post operative day. Bleeding from wound site found in group-A (8.14%) seven patients, while in group-B five (5.81%). Wound infection in group-A (6.98%) six patients, while in group-B seven (8.14%). UTI in group-A (2.33%) two patients, while in group-B four (4.65%). Urinary retention in group-A (6.98%) six patients, while in group-B five (5.81%). Urinary Extravasation in group-A (10.47%) nine patients, while in group-B twelve (13.95%).

To access the late compilations 27 patients (31.47%) were uneventful in group-A while 29 patients (33.72%) in group-B were remained uneventful. UC Fistula in group-A (10.47%) nine patients, while in group-B eight (9.30%). Wound Dehiscence in group-A (8.14%) seven patients, while in group-B six (6.98%).

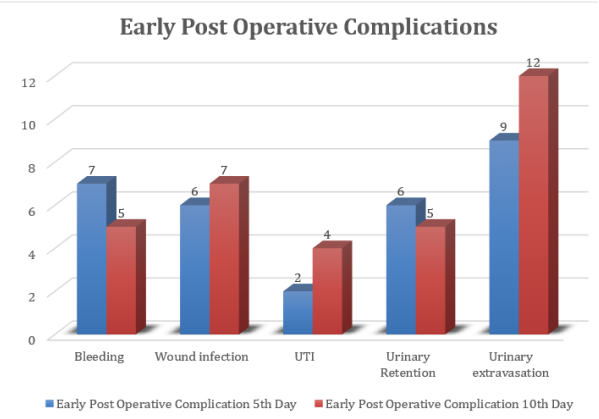

Figure No.1: Early Post-Operative Complications

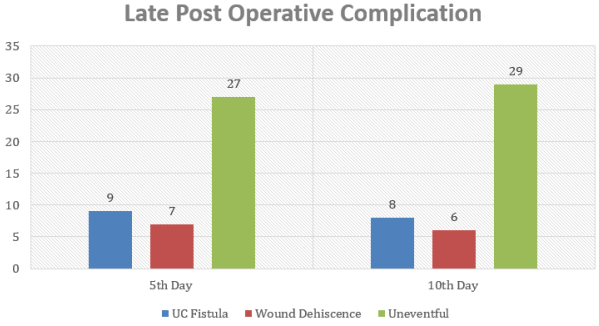

Figure No.2: Late Post Operative Complication

T-Test: Age vs. Early Complication: The T-test compares the mean age of male patients who developed an early post-operative complication against those who did not.

T-Test Results:

- **Calculated T-Statistic (T):** 0.354
- **P-value:** approx 0.725

Conclusion: We are unable to reject the null hypothesis because the P-value of 0.725 is significantly higher than the significance level (alpha=0.05). There is no statistically significant difference in mean age between male patients who experienced an early complication and those who did not.

2. Chi-Square Test: Early Complication vs. Late Complication

This test assesses if there is an association between experiencing an early post-operative complication and a late post-operative complication.

Table No.1: Contingency (Observed Frequencies)

	Late Comp. None	**Late Comp. Present**	**Row Total**
Early Comp. None	19	4	**23**
Early Comp. Present	37	26	**63**
Column Total	**56**	**30**	**N=86**

P-value [Fisher's Exact Test]: 0.045

Since the P-value of 0.045 is less than the significance level (alpha=0.05), we reject the null hypothesis. This indicates a statistically significant association between the occurrence of an Early Post-Operative Complication and a Late Post-Operative Complication among these 86 male patients. More specifically, it appears that having a complication earlier in life is linked to having a complication later on.

DISCUSSION

This study explored the relationship between timing of stent removal and postoperative complications in distal penile hypospadias repaired using Snodgrass technique. The findings correspond with international literature that early stent removal does not significantly increase the risk of urethrocutaneous fistula or wound dehiscence. Early removal improves patient comfort, reduces hospital stay, and minimizes catheter-associated infections.

Hypospadias repair is ideally performed at 6–18 months to reduce psychological and developmental impact, though studies show mixed results on whether older age increases complications. TIP remains the most widely used technique due to its simplicity and low complication rates, while alternatives like Mathieu, Duckett, and Koyanagi are used in specific cases. Across techniques, urethral fistula is the most common complication, followed by meatal stenosis, glans dehiscence, and chordee, with higher rates in proximal cases. Risk factors include poor urethral plate quality, constipation, and prolonged procedures, while preventive measures like showering protocols and antibiotics show limited benefit. In the present study, complication rates were higher than some reports, but frequent follow-up and recent surgical improvements suggest evolving outcomes.

Several studies, including those by Fakhry et al. (2021), have demonstrated that the success of Snodgrass repair remains high with low complication rates regardless of stent duration. However, some authors advocate for delayed stent removal to ensure optimal healing. This study contributes to local data, offering valuable evidence to guide postoperative care in pediatric surgical units across Pakistan[11].

Kumar and Ram conducted a study on 62 patients who underwent TIP hypospadias repair and concluded that early catheter removal did not influence the occurrence of long-term complications. However, the study's limitations included a small sample size and its descriptive design. Therefore, prospective randomized controlled trials are necessary to evaluate the safety of early catheter removal and its impact on quality of life during the early postoperative period[12].

According to Scarpa, no catheter-related complications, including blockage or malfunction, were observed in group A, and no urinary tract infections were recorded. Following catheter removal, three episodes of urinary retention occurred: one case in the stented group (1/18) and two cases in the unstented group (2/26). All episodes occurred in patients older than 24 months and were managed with temporary urinary catheterization under sedation. No long-term complications, such as fistula or stenosis requiring surgical intervention, resulted from these episodes. During the follow-up period, four cases of persistent fistula were identified, with two cases in group A and two in group B. Our protocol is to delay fistula repair until at least six months after the initial surgery. One patient underwent reoperation seven months after the primary procedure, while the remaining cases were diagnosed later and treated at ten, twelve, and fourteen months, respectively. Among the unstented patients, apart from one individual who developed meatal stenosis followed by a fistula, only one case of subclinical meatal stenosis was observed[13].

In his study, Tatanis et al, reported that a 20-hour post-op catheter placement after hypospadias repair is an effective alternative whose outcomes are comparable to conventional catheter duration methods, where it decreases the discomfort of patient while not raising the complication risk[14].

Hadidi studied on 63 patients having perineal hypospadias. Study included 59 patients who were observed regular follow-up then which were reviewed. Where suprapubic cystostomy was performed and

catheter was placed only for 04 days, which shorten the stay in the hospital and reduced discomfort of patient. It gives satisfactory results and considered the standard technique in hypospadias of perineal position[15].

Drake et al studied revealed that late removal of the catheter increased the stenosis meatal (12.7%) significantly as compared to early removal of catheter during the 3rd post-operative days[16].

In the study by Daher, catheter removal was performed after one week in group I and after three weeks in group II. No patients in either group experienced catheter-related bladder spasms. The overall complication rate was significantly higher in group I compared with group II (22.1%, n = 21 vs. 7.4%, n = 7; P = .005). Reported complications included meatal stenosis, which occurred in four patients in group I and two patients in group II, and urethrocutaneous fistulas, observed in 17 patients in group I and five patients in group II. No cases of postoperative wound infection, urinary tract infection, urethral diverticulum, glans dehiscence, or complete wound dehiscence were reported. Coronal fistulas were significantly more common in group I than in group II (13.7% vs. 3.2%, P = .01). However, there were no statistically significant differences between the two groups in the rates of mid-shaft fistulas (1.1% vs. 1.1%, P = .994), penoscrotal fistulas (3.2% vs. 1.1%, P = .317), or meatal stenosis (4.2% vs. 2.1%, P = .414). All complications occurred within the first six months postoperatively, with no late complications observed. Meatal stenosis was managed with urethral dilatation, whereas urethrocutaneous fistulas required surgical correction[17].

Gabra studied 119 patients and found the statistically significant association between the type of hypospadias and the duration of urethral stenting ($p < 0.001$). Patients with distal hypospadias were more likely to have catheter removal within five days, whereas those with proximal hypospadias tended to require longer stenting. In addition, patients who developed a urethral fistula were significantly more likely to have catheter removal after five days compared with those without fistula formation ($p = 0.037$). No significant associations were observed between prolonged urethral stent duration and other postoperative complications[18].

Tubularized incised plate urethroplasty (TIPU) with snodgrass modification remains the most common technique for distal hypospadias repair, offering good functional and cosmetic outcomes, though fistula remains a frequent complication with rates around 5.9% in the literature. In the presented series, the fistula rate was 9% (similar between stented and unstented groups), and urinary retention occurred mainly in toilet-trained children older than 24 months, consistent with prior reports. While stents are traditionally kept for 2–7 days, unstented repairs allowed earlier discharge (median 3 vs. 7 days) without significantly higher complication rates, though older age at surgery was linked to poorer compliance and increased risks. Overall, surgeon experience, patient age, and stent use influence outcomes, with early catheter removal potentially balancing hospital stay reduction against manageable complications[19].

Hypospadias is considered one of the most common conditions found in the boys are born with, and the goal of surgery is always the same: to give them a penis that looks and works normally so they can grow up without physical or emotional difficulties. The TIP-Urethroplasty technique has become the go-to method for distal cases because it's straightforward, reliable, and usually gives good results. Still, like any surgery, complications can happen—most often fistulas, meatal narrowing, or wound breakdown—usually within the first six months. Surgeons have learned that adding protective tissue layers, like spongioplasty, helps lower the risk of fistulas and supports healing[20].

One of the big debates is whether to use stents or catheters after surgery. Stents can be uncomfortable and may cause bladder spasms, while catheters are generally better tolerated and seem to reduce complications, especially when left in place for longer. In fact, some studies suggest keeping a bladder catheter for up to three weeks can cut the risk of fistulas significantly, though it does mean managing spasms with medication. Age also plays a role: operating earlier—ideally between 6 and 18 months—tends to lower complication rates, while waiting until after 18 months can make healing harder.

In short, the best outcomes come from balancing technique, timing, and aftercare. Using protective tissue, choosing the right diversion method, and operating at the right age all help minimize risks and give children the best chance at a normal, healthy future.

CONCLUSION

Early stent removal following Snodgrass repair of distal penile hypospadias is considered the safe and the effective alternative to delayed removal. It does not significantly increase complications and contributes to enhanced patient recovery and comfort.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Imran Qadir, Ahmad Wahab Arshad, Sadaqat Ali
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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Effect of Benson Relaxation Technique on Anxiety Among Women with High-Risk Pregnancies in a Tertiary Care Hospital, Lahore, Pakistan

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ABSTRACT

Objective: To evaluate the effectiveness of the Benson Relaxation Technique in reducing anxiety among Women with high-risk pregnancies.

Study Design: A pre-post quasi-experimental study

Place and Duration of Study: This study was conducted at the Obstetrics and Gynecology Department of Services Hospital, Lahore, in collaboration with the Department of Nursing and Behavioral Sciences, University of Health Sciences, Lahore during April 2024 to December 2024.

Methods: A total of 40 Women with high-risk pregnancies were recruited and underwent Benson Relaxation Technique sessions for three consecutive days. Anxiety levels were assessed before and after the intervention using the Hamilton Anxiety Scale (HAM-A). Data were analyzed using paired sample t-tests for normally distributed data and the Wilcoxon signed-rank test for non-normally distributed data. A p-value of <0.05 was considered statistically significant.

Results: Anxiety, pre-intervention results showed that 3 (7.5%) Women had no anxiety, 7 (17.5%) had mild anxiety, 12 (30%) had moderate anxiety, and 18 (45%) experienced severe anxiety. Post-intervention, 21 (52.5%) had no anxiety, 16 (40%) had mild anxiety, and only 3 (7.5%) had moderate anxiety, demonstrating a substantial improvement.

Conclusion: The findings indicate that the Benson Relaxation Technique is an effective non-pharmacological intervention for reducing anxiety among Women with high-risk pregnancies. Its integration into prenatal care programs could improve maternal mental health and overall pregnancy outcomes.

Key Words: High-Risk Pregnancy (HRP), Benson Relaxation Technique (BRT).

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INTRODUCTION

High-risk pregnancy denotes gestations complicated by maternal, fetal, or obstetric conditions that substantially increase the likelihood of maternal or perinatal morbidity and mortality; women with high-risk pregnancies experience elevated rates of psychiatric symptoms, particularly antenatal anxiety, compared with low-risk groups[1,2]. Systematic reviews and targeted studies indicate that anxiety among pregnant women is common and frequently higher in those with medical or obstetric complications, with several studies documenting heightened prevalence and symptom severity in high-risk cohorts and recommending routine screening in tertiary and specialized prenatal services[1,2,3.] In Pakistan and similar low- and middle-income settings, antenatal anxiety has been reported at substantial levels during recent years, including during the COVID-19 pandemic, highlighting the burden of perinatal anxiety in local clinical populations that attend tertiary hospitals [3].

Antenatal anxiety in high-risk pregnancies has clinically important consequences for both mother and fetus. Anxiety activates neuroendocrine and autonomic pathways notably the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system leading to hormonal (e.g., cortisol, catecholamines) and cardiovascular responses that can adversely influence maternal hemodynamics and placental perfusion and have been implicated mechanistically in hypertensive disorders of pregnancy, such as preeclampsia, and other perinatal complications[4,5,6]. Non-pharmacological psychological interventions are recommended and increasingly studied for antenatal anxiety because of the need for low-risk, scalable options during pregnancy; systematic reviews show a breadth of practical

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approaches (mindfulness, CBT, relaxation, psychoeducation, breathing techniques) across low- and high-risk obstetric samples, and meta-analytic syntheses emphasize that relaxation approaches form a consistent, evidence-based component of multi-modal care for prenatal anxiety and stress.[2,7,8] In clinical practice, relaxation techniques including diaphragmatic breathing, progressive muscle relaxation (PMR), autogenic training, and mind-body methods such as Benson relaxation have been applied in pregnancy and in related medical populations with reported benefits for subjective anxiety, physiological stress markers (e.g., cortisol, blood pressure), and patient-centered outcomes[7,6,5].

The Benson Relaxation Technique (BRT) is a standardized, brief mind-body method that combines a focus word or phrase with passive return of attention and a relaxed posture to elicit the relaxation response; it has been adapted across medical contexts because it is brief, inexpensive, and amenable to self-practice [9]. Clinical and quasi-experimental evaluations in recent years document that BRT and related autogenic approaches can reduce self-reported anxiety and lower blood pressure in patients with cardiovascular conditions and other medical[10]. Although robust randomized evidence of BRT specifically in pregnancy remains limited, contemporary trial protocols and small intervention studies propose and examine BRT as an adjunct to routine prenatal care for hypertensive disorders and preeclampsia, underscoring both its theoretical suitability and current research momentum toward evaluating BRT for obstetric risk groups[11].

There is a pressing need to assess the impact of the Benson Relaxation Technique (BRT) on maternal anxiety among women with high-risk pregnancies at a tertiary care hospital in Lahore, Pakistan. This need arises from (1) the high prevalence and significance of antenatal anxiety in these women; (2) the connection between anxiety and obstetric complications; (3) the positive effects of relaxation methods on anxiety; and (4) the practical benefits of BRT as a brief, low-cost, and culturally adaptable intervention.

METHODS

This quasi-experimental pre- and post-test study was conducted to evaluate the effectiveness of Benson's Relaxation Technique (BRT) in reducing anxiety among women with high-risk pregnancies admitted to the Obstetrics and Gynecology Department of Services Hospital, Lahore, in collaboration with the Department of Nursing and Behavioral Sciences, University of Health Sciences, Lahore during April 2024 to December 2024. A total of 40 women diagnosed with high-risk pregnancies were selected through a probability-based simple random sampling technique. The sample size was determined to achieve a 90% statistical power and a 5% level of significance based on previously published data.

Women aged between 18 and 35 years, able to communicate in Urdu, and willing to participate were included, while those with psychiatric illnesses, on anxiolytic medications, or unable to complete the intervention were excluded. The study adhered to the ethical principles outlined in the Declaration of Helsinki. The intervention involved administering Benson's Relaxation Technique (BRT), developed by Herbert Benson in 1970, which aims to reduce sympathetic nervous system activity and induce relaxation. Sessions were conducted individually in a calm, quiet environment under the investigator's supervision. Each session lasted for 15 to 20 minutes and was performed once daily for three consecutive days. Participants were instructed to sit or lie comfortably, close their eyes, relax all muscles, and breathe slowly while repeating a calming word, such as "peace" or "relax," on exhalation, and to ignore distracting thoughts.

Data were collected before and after the intervention using standardized, validated tools, including the Hamilton Anxiety Rating Scale (HAM-A) and the Perceived Stress Scale (PSS), both translated into Urdu and pilot-tested for cultural suitability. The HAM-A scale comprises 14 items assessing psychological and physical symptoms of anxiety, while the PSS consists of 10 items measuring perceived stress levels. Baseline demographic information, including age, education, parity, and socioeconomic status, was also recorded.

Data analysis was performed using SPSS version 25. Descriptive statistics, including mean, standard deviation, frequency, and percentage, were used for the demographic variables. The Shapiro–Wilk test was used to assess data normality; as anxiety and stress scores were non-normally distributed, the Wilcoxon signed-rank test was used to compare pre- and post-intervention scores. A p-value of less than 0.05 was considered statistically significant. The study was completed over nine months, including participant recruitment, intervention delivery, and data analysis, ensuring methodological rigor and reliability.

RESULTS

A total of 40 women with high-risk pregnancies participated in this study. The mean age was 26.62 ± 3.94 years (range 18–34 years). All participants were married (100%). Most belonged to the middle socioeconomic class (75%), and 30% had completed matriculation as their highest level of education. The majority were multigravida, with varied parity and number of children. Demographic characteristics are summarized in Table 1.

The Hamilton Anxiety Scale (HAM-A) was used to evaluate anxiety levels before and after Benson's Relaxation Technique (BRT). The mean pre-intervention HAM-A score was 23.05 ± 10.17, and the

mean post-intervention score was 8.02 ± 4.36, indicating a highly significant reduction ($p < 0.001$, Wilcoxon signed-rank test). The distribution of anxiety severity before and after intervention is shown in Table 2. A significant improvement in anxiety levels was observed after intervention ($p < 0.001$).

Table No. 1. Demographic Characteristics of Participants (n = 40)

Variable	Category	Frequency (n)	%age
Age (years)	Mean ± SD (Range)	26.62 ± 3.94 (18–34)	—
Marital Status	Married	40	100
	Unmarried	0	0
Number of Pregnancies Conceived	1	11	27.5
	2	9	22.5
	3	10	25
	4	8	20
	5	2	5
Number of Living Children	None	3	7.5
	1	12	30
	2	12	30
	3	9	22.5
	4	3	7.5
	5	1	2.5
Socioeconomic Class	Lower	4	10
	Middle	30	75
	Upper	6	15
Educational Status	Uneducated	5	12.5
	Middle	6	15
	Matric	12	30
	Intermediate	8	20
	Graduate	7	17.5
	Master's	2	5

Table No. 2: Severity of Anxiety Before and After Intervention (n = 40)

Anxiety Level	Before Intervention n (%)	After Intervention n (%)
No Anxiety	3 (7.5)	21 (52.5)
Mild Anxiety	7 (17.5)	16 (40.0)
Moderate Anxiety	12 (30.0)	3 (7.5)
Severe Anxiety	18 (45.0)	0 (0.0)
Total	**40 (100)**	**40 (100)**

A statistically significant reduction in mean HAM-A scores was observed following BRT ($p < 0.001$). (Table 3).

The mean PSS score declined from 20.88 ± 3.79 before intervention to 13.87 ± 8.89 after intervention ($p < 0.001$), demonstrating a significant decrease in stress levels (Table 4).

Table No. 3: Descriptive Statistics for Hamilton Anxiety Scale (HAM-A) Scores (n = 40)

Parameter	Before Intervention	After Intervention
Mean ± SD	23.05 ± 10.17	8.02 ± 4.36
Median (IQR)	22.50 (16.75)	7 (7)
Minimum – Maximum	3 – 43	2 – 18
p-value (Shapiro–Wilk)	0.736	0.018
p-value (Wilcoxon signed-rank test)	—	< 0.001

Table No. 4: Descriptive Statistics for Perceived Stress Scale (n = 40)

Parameter	Before Intervention	After Intervention
Mean ± SD	20.88 ± 3.79	13.87 ± 8.89
Median (IQR)	22 (3)	12 (12.75)
Minimum – Maximum	8 – 26	0 – 35
p-value (Shapiro–Wilk)	< 0.001	0.028
p-value (Wilcoxon signed-rank test)	—	< 0.001

Each of the 14 HAM-A items demonstrated considerable improvement following BRT. Before intervention, high proportions of women reported moderate to very severe symptoms such as tension, fear, insomnia, muscular discomfort, and cardiovascular complaints. After BRT, most participants shifted to the "not present" or "mild" categories across all items, indicating global anxiety relief. Details are provided in Table 6. Marked reductions were noted across all symptom domains following BRT ($p < 0.001$).

Table No. 5: Item-wise Analysis of Hamilton Anxiety Scale Components Before and After Intervention (n = 40)

HAM-A Item	Category	Before n (%)	After n (%)
1. Anxiety (Anguish/ Resentful)	Not Present	12 (30.0)	28 (70.0)
	Mild	11 (27.5)	12 (30.0)
	Moderate – Very Severe	17 (42.5)	0 (0.0)
2. Tension (Restlessness, Stress)	Not Present	7 (17.5)	18 (45.0)
	Mild	7 (17.5)	22 (55.0)
	Moderate – Very Severe	26 (65.0)	0 (0.0)
3. Fear (Phobia/Worry)	Not Present	8 (20.0)	22 (55.0)
	Mild	6 (15.0)	15 (37.5)
	Moderate – Very Severe	26 (65.0)	3 (7.5)

4. Insomnia (Fatigue/Nightmares)	Not Present	5 (12.5)	15 (37.5)
	Mild	7 (17.5)	19 (47.5)
	Moderate – Very Severe	28 (70.0)	6 (15.0)
5. Intellectual Symptoms (Poor Concentration/Memory)	Not Present	11 (27.5)	24 (60.0)
	Mild	7 (17.5)	13 (32.5)
	Moderate – Very Severe	22 (55.0)	3 (7.5)
6. Depressed Mood	Not Present	5 (12.5)	17 (42.5)
	Mild	10 (25.0)	19 (47.5)
	Moderate – Very Severe	25 (62.5)	4 (10.0)
7. Muscular Symptoms (Aches/Stiffness)	Not Present	3 (7.5)	19 (47.5)
	Mild	9 (22.5)	17 (42.5)
	Moderate – Very Severe	28 (70.0)	4 (10.0)
8. Sensory Symptoms (Hot/Cold Flushes, Weakness)	Not Present	11 (27.5)	22 (55.0)
	Mild	10 (25.0)	16 (40.0)
	Moderate – Very Severe	19 (47.5)	2 (5.0)
9. Cardiovascular Symptoms (Palpitations/Chest Pain)	Not Present	14 (35.0)	26 (65.0)
	Mild	6 (15.0)	12 (30.0)
	Moderate – Very Severe	20 (50.0)	2 (5.0)
10. Respiratory Symptoms (Dyspnea/Chest Tightness)	Not Present	10 (25.0)	29 (72.5)
	Mild	11 (27.5)	9 (22.5)
	Moderate – Very Severe	19 (47.5)	2 (5.0)
11. Gastrointestinal Symptoms (Nausea/Abdominal Pain)	Not Present	12 (30.0)	27 (67.5)
	Mild	7 (17.5)	10 (25.0)
	Moderate – Very Severe	21 (52.5)	3 (7.5)
12. Genitourinary Symptoms (Frequency/Impotence)	Not Present	15 (37.5)	19 (47.5)
	Mild	7 (17.5)	14 (35.0)
	Moderate – Very Severe	18 (45.0)	7 (17.5)
13. Autonomic Symptoms (Sweating/Dry Mouth)	Not Present	—	18 (45.0)
	Mild	—	19 (47.5)
	Moderate – Very Severe	—	3 (7.5)
14. Behavior at Interview (Restlessness/Fidgeting)	Not Present	17 (42.5)	26 (65.0)
	Mild	5 (12.5)	10 (25.0)
	Moderate – Very Severe	18 (45.0)	4 (10.0)

DISCUSSION

The findings from this study provide compelling evidence of the effectiveness of the Benson Relaxation Technique (BRT) in reducing anxiety and stress among women experiencing high-risk pregnancies. A total of 40 participants exhibited significant reductions in anxiety as measured by the Hamilton Anxiety Scale (HAM-A) and perceived stress levels measured by the Perceived Stress Scale (PSS). The mean pre-intervention HAM-A score of 23.05 ± 10.17 decreased to 8.02 ± 4.36 post-intervention, reflecting a highly significant reduction ($p < 0.001$). This noteworthy finding aligns with the existing literature, which suggests that relaxation techniques, including BRT, can reduce maternal anxiety and improve overall wellbeing during pregnancy[12].

As supported by Zenouzi et al[13], relaxation techniques, particularly BRT, have shown efficacy in alleviating stress and anxiety in pregnant women. Participants reported reduced maternal stress and improved mental health. This aligns with our observation that BRT not only alleviates anxiety but also modifies behavioral patterns associated with anxiety symptoms, such as tension and insomnia, as evidenced by the substantial shift towards lower anxiety severity levels post-intervention.

In Table 2, the distribution of anxiety severity reveals a striking change: the percentage of participants with no anxiety increased from 7.5% to 52.5%. In comparison, severe anxiety cases dropped from 45% to 0% following the BRT intervention. This improvement underscores the potential of BRT as an effective non-pharmacological intervention during high-risk pregnancies, consistent with findings by Elgwad et al. [14], who identified significant reductions in stress and anxiety through the application of similar relaxation therapies in their patient populations.

The results on perceived stress, shown in Table 4, also echo findings from previous studies, such as those by Abera et al. 15, who reported that structured relaxation approaches significantly reduced perceived stress among pregnant women. Our study reported a significant reduction in mean PSS score from 20.88 ± 3.79 to 13.87 ± 8.89 ($p < 0.001$). Each symptom showed significant declines post-intervention, aligning with findings from Hasanzadeh et al., who reported parallel improvements in physical health and emotional wellbeing following relaxation interventions in obstetric populations.[16]

Moreover, the demographic characteristics of our sample suggest greater exposure to the uncertainties associated with high-risk pregnancies, making participants particularly vulnerable to anxiety [17]. Reflecting on Abera et al.'s findings, which document significantly higher stress levels in pregnant versus non-pregnant cohorts, our results serve to illuminate the particular susceptibility of this population to mental health issues, accentuating the importance of targeted interventions like BRT.[15]

Overall, the present findings corroborate existing literature advocating for the inclusion of relaxation techniques in prenatal care protocols.

CONCLUSION

This study concludes that Benson's Relaxation Technique (BRT) is a practical, low-cost, non-pharmacological method for reducing anxiety in women with high-risk pregnancies. Incorporating BRT into prenatal care can enhance maternal mental wellbeing and potentially improve pregnancy outcomes. Further

large-scale studies are recommended to confirm these results and establish standardized intervention protocols.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sadia Ahmad, Saqib Rabbani
Drafting or Revising Critically:	Samina Kausar, Aqela Sarwar
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Rhino-Orbital-Cerebral Mucormycosis During COVID-19 Pandemic Versus the Subsequent Period

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ABSTRACT

Objective: To compare the incidence of mucormycosis, its clinical features, and outcomes during the COVID-19 period versus the subsequent period.

Study Design: Retrospective study

Place and Duration of Study: This study was conducted in Basra between May 2020 and the end of June 2025.

Methods: This retrospective study was undertaken in Basra from the onset of the COVID-19 outbreak until the end of June 2025. Rhino-orbito-cerebral mucormycosis (ROCM) cases were classified into two groups according to infection time (during COVID-19 vs. post-COVID). The total number of patients who were evaluated in this study was 20.

Results: The incidence of ROCM during the pandemic was approximately six-fold higher than that of the post-COVID period. The predominant predisposing factor was Diabetes mellitus. ROCM was more common in males and its clinical features were approximately similar in both groups. However, during the COVID-19 period, one patient died, four required ocular enucleations, and one developed a cerebrovascular accident (CVA). In the post-COVID period, only one patient experienced bilateral visual loss, but none required enucleation, and no deaths or CVA were reported.

Conclusion: The incidence of ROCM during COVID-19 was higher than that of the subsequent period. However, the clinical presentations were approximately similar in both periods.

Key Words: COVID-19, Rhino-orbito-cerebral mucormycosis, Basrah-Iraq

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INTRODUCTION

Mucormycosis is a serious fungal infection that can lead to increase morbidity and mortality.[1]

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It is predominantly caused by a variety of Mucorales species, such as Mucor, Rhizopus, Rhizomucor, and Lichtheimia.[2] The rhino-orbito-cerebral region was the most commonly involved region, with 90% of cases were infected with Rhizopus species.[3-5] Different other organs can be affected, including the lungs, skin, gastrointestinal system and kidneys.[6,7] The process of infection typically begins when the fungal spores inhaled from the environment and then colonize the respiratory mucosa of the nasal cavity and paranasal sinuses. Subsequently, hyphae developed, permitting the fungus to spread rapidly to adjacent tissues. The ability of these organisms to invade blood vessels enhance its spread and invasiveness.[8]

Several determinants contribute to the increased vulnerability to Mucormycosis. Before the COVID-19 pandemic, the most predominant predisposing factor was haematologic cancer, followed by diabetes mellitus (DM).[8-12] Other conditions that supress immunity, such as prolonged corticosteroid use, and chemotherapy and chronic renal impairment, significantly increase the susceptibility of infection. COVID-19 has recently arisen as a significant predisposing influence for mucormycosis, especially in developing countries. This risk increased in hypoxic patients who received

corticosteroid therapy and iron supplements which together facilitate *Mucorales* proliferation and invasion.[13] Basra confronted two serious waves of COVID-19, the first wave began at the end of May 2020 and the second in late January 2021.[14] Up to the present time, no articles have demonstrated the COVID-19 behaviour in Basra beyond these two waves. At the national level, however, Iraq encountered two additional waves: the third began in March and ended in July 2021, and the Omicron wave peaked in January 2022. However, these waves were associated with markedly lower severity and fewer hospital admissions.

This study examines the patterns of Mucormycosis and its associated outcomes during the COVID-19 pandemic and the subsequent period.

METHODS

This study is a retrospective study of patients who were infected with mucormycosis in Basra, southern Iraq. It covers cases that occurred during the COVID-19 outbreak, including all four waves (from May 2020 to February 2022), as well as the post-COVID period up to the late June 2025. All suspected cases of mucormycosis in Basra were referred to and admitted at Basra Teaching Hospital. Diagnosis of rhino-orbito-cerebral mucormycosis was confirmed depending on multiple clinical criteria. These include features of sinusitis and its complications, such as facial pain, nasal obstruction, nasal discharge, periorbital swelling, proptosis, and vision loss. Radiological evaluation was performed using computed tomography (CT) and/or magnetic resonance imaging (MRI) of the nose, sinuses, and brain to detect areas of bone destruction or infiltration of the orbit or cerebral region. In addition, histopathological assessment of biopsied tissue from affected regions was performed to confirm the diagnosis. All patients underwent radical debridement of necrotic tissue in a single operative session and received liposomal amphotericin type B, at a dose of 10mh/kg before and after the surgery. Antifungal therapy was continued postoperatively for 4-6 weeks, until complete clinical and radiologic improvement was achieved. In this study, we assessed 15 patients during the COVID-19 period and 5 patients between February 2022 and June 2025.

RESULTS

During the COVID-19 period (21 months), 15 cases of mucormycosis were recorded, compared with 5 cases during the post-COVID-19 period (41 months). The crude incidence rate was 0.71 cases per month during COVID-19 and 0.12 cases per month post-COVID, corresponding to an incidence rate ratio of approximately 6. Poisson regression analysis, using months as the offset, confirmed this significantly higher incidence during the COVID-19 period ($p < 0.001$) (see Table 1).

Of the five cases identified in the post-COVID period, four were diagnosed with mucormycosis in 2022; none had confirmed SARS-CoV-2 infection by PCR testing. The fifth case occurred in February 2023, after which no further cases were recorded.

Table No. 1: Incidence of mucormycosis during COVID-19 *vs.* post COVID period

Period	Observation time (months)	Cases (n)	Incidence rate (cases/ month)	Rate ratio (95% CI)
COVID -19	20	15	0.71	≈5.9
Post COVID -19	41	5	0.12	1.0 (Reference)

P-value<0.001, n: number, CI: Confidence interval

Mucormycosis was more common in males during both periods, with a total of 12 male cases (60%) compared to 8 female cases (40%). However, this difference was not statistically significant.

It can be observed that the prevalence of diabetes mellitus among patients with mucormycosis was very high during both COVID and non-COVID period, with no statistically significant difference between them (p value= 0.544). Table (2) shows that 93.3% of mucormycosis patients in COVID period had diabetes mellitus, compared to 100% of patients in the post COVID period.

Table No.2: Distribution of diabetes mellitus among patients with mucormycosis during COVID and non-COVID periods

Periods	No DM	DM	Total
COVID	1(6.7%)	14 (93.3%)	15
Non-COVID	0 (0%)	5 (100%)	5
Total	1 (5%)	19 (95%)	20

P value: 0.554, DM: Diabetes Mellitus

Regarding symptoms, there were no significant difference between symptoms during COVID-19 era and the period after that. The most noticeable symptoms were as following: facial pain happened in all patients, headache and nasal obstruction were complained by 16 patients, olfactory problems; including anosmia, hyposmia or cacosmia, perceived in 15 patients, facial disfigurement (facial swelling and or periorbital swelling) developed in 13 patients. Nasal discharge was not a common feature, only in 10 patients and only one patient complained of nasal bleeding (see table 3).

On examination, eschar was the most common finding in both the COVID and non-COVID periods. All five non-COVID patients presented with this feature, and

only one patient in the COVID group lacked eschar. Mucormycosis involved the turbinates and lateral nasal wall in 60% of patients (12 in total) across both groups. The nasal septum was the second most affected site, observed in 55% of all patients (60% in the COVID group and 40% in the non-COVID group). The pyriform fossa was not involved in any patient during either period (see Table 4).

The majority of severe complications of ROCM were observed during the COVID-19 outbreak. During this period, four patients underwent orbital exenteration. In addition, one patient developed a cerebrovascular accident, and one patient died. In the post-COVID period, a female patient presented with bilateral blindness, which did not necessitate orbital exenteration and no patient died of ROCM.

Table No. 3: Symptoms of mucormycosis during COVID-19 *vs* post COVID-19

	Facial pain	headache	Nasal obstruction	discharge	Olfactory problems	Epistaxis	Facial disfigurement
COVID	15	11	12	6	11	1	10
NON-COVID	5	5	4	4	4	0	3
TOTAL	20	16	16	10	15	1	13

Table No. 4: Signs of mucormycosis during COVID-19 *vs* post COVID-19

SIGNS (Site of involvement)	NASAL WALL AND TURBINATES	NASAL FLOOR	SEPTUM	Palate	ORBIT	PYRI-FORM	LOSS OF SENSITIVTY	ESCHAR
COVID	9 (60%)	2 (13.3%)	9 (60%)	1(6.7%)	5 (33.3%)	0	8 (53.3%)	14 (93.3)
NON-COVID	3 (60%)	1 (20%)	2 (40%)	0 (0%)	2 (40%)	0	1 (20%)	5 (100%)
TOTAL	12 (60%)	3 (15%)	11 (55%)	1 (5%)	7 (35%)	0	9 (45%)	19 (95%)

DISCUSSION

ROCM is a life-threatening fungal infection that can lead to serious complications and the death rate of this disease is relatively high.[1] DM and haematological disorders are the major predisposing factors for this condition.[8,10] Other conditions that can reduce immunity, such as prolong consumption of prednisolone, chemotherapy and chronic renal failure increase the susceptibility to develop this illness.[13] The link between mucormycosis and infection with COVID-19 was reported in several countries, such as India and Pakistan. However, the confirmed cases in Europe were very low.[15]

This study found that every patient who developed ROCM had diabetes, except one non diabetic patient who was infected with COVID 19 and received steroid for around two weeks. One of COVID patients in addition to diabetes mellitus he had thalassemia. All COVID patients and three non-COVID patients received steroid before developing mucormycosis. The lack of a statistically significant difference between groups likely reflects the high baseline prevalence of diabetes among patients with mucormycosis, rather than a differential effect of COVID-19 status. The risk of infection increases with diabetes mellitus, particularly in diabetic ketoacidosis because hyperglycaemia supresses neutrophil chemotaxis, leukocyte phagocytosis and local inflammatory response. In addition, a glucose-rich environment associated with diabetes, as well as the ketone reductase system of *Rhizopus*, provide optimal conditions for the fungal growth and invasion.

Andreescu et al reviewed case reports and series depending on Google scholar database from October 2021 to November 2022 and they concluded that Diabetes Mellitus plays a vital role in mucormycosis growth and this is in consistent with our findings.[15]

Noticeably, incidence of ROCM is nearly six times in COVID era compared to the post COVID period, 15 cases over 21 months as compared to 5 cases in 41 months.

The surge in mucormycosis cases during the COVID-19 pandemic is closely linked to disease-related hypoxia, prolonged corticosteroid therapy, and frequent iron replacement, all of which facilitate fungal growth and tissue invasion.[14]

Most of patients in both periods were males, 60% in total. Majority of reports were in agreement with this finding, for example, Pal et al found that 78% of patients were males.[16] Andreescu et al mentioned that most of reviewed patients were males.[15]

Our study showed that all patients experienced facial pain. sixteen patients reported headache and nasal obstruction, while olfactory disturbances, including anosmia, hyposmia, or cacosmia, were noted in 15 patients. Facial disfigurement (facial or periorbital swelling) occurred in 13 patients. Nasal discharge was less frequent, observed in only 10 patients, and epistaxis was documented in a single case. A COSMIC study in India reported that orbital and facial pain as the most common symptom. However, this was complained

by only 23% of patients. Facial and orbital oedema presented in 21%. Nasal discharge and nasal obstruction were observed in 10% and 9 % respectively.[17]

On clinical examination, eschar was the most common finding in both COVID-associated and non-COVID patients. All five non-COVID cases developed eschar, whereas it was absent in only one patient in the COVID group. COSMIC study showed that 48% of patients demonstrated eschar formation. The turbinates and lateral nasal wall were the most commonly involved structures, affected in 60% of the total cases (12 patients) in both groups. The nasal septum was the second most frequently involved site, observed in 55% of all patients (60% in the COVID group and 40% in the non-COVID group). The pyriform fossa was documented in either group.

This study reveals that 27% of COVID-19 patients required eye exenteration to survive the disease. However, from the five patients, only one patient developed bilateral blindness but did not require enucleation. Sen et al observed that 16% of COVID-19 patients with mucormycosis required eye exenteration. However, 63% of patients lost their vision.[17]

Malek et al reported a case with acute bilateral blindness developed in a young patient with COVID-19 and rhino-orbito-cerebral mucormycosis.[18]

This study also showed that one patient developed cerebrovascular accident (CVA) after being infected with COVID-19 and mucormycosis. This patient was previously complaint of Thalassemia which could be a predisposing factor to develop CVA. A prothrombotic state in patients with Thalassemia has been linked with increasing risk of CVA.[19]

The mortality rate of ROCM varies from 30-90% of cases with cerebral involvement.[20,21] For cases associated with SARS-COV-2, the estimated mortality was 14-31% .[17,22] Our study showed that only 7% of the patients died during the pandemic and no patient died in the post COVID period.

CONCLUSION

ROCM was approximately six times more common during the COVID-19 period compared to the subsequent post COVID phase, with diabetes mellitus recognised as the predominant predisposing factor. The clinical manifestations were largely similar in the two periods; however, patients without COVID-19 demonstrated better outcomes than those with coexisting infection.

Limitations of the study: Because the disease is rare, the sample size was relatively small. In addition, articles covering ROCM are very limited worldwide, therefore, it was difficult to compare our results with other studies.

Author's Contribution:

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Original Article

Association Between Vitamin D Levels, miRNA Expression, and Sperm Parameters in Infertile Men

Impact of Vitamin D and Sperm miRNA on Sperm Parameters

Alaa Hachem[1] and Asaad Al-Shouk[2]

ABSTRACT

Objective: To examine the impact of vitamin D levels and the miRNA profiles of miR-34b, miR-19a-3p, and miR-122 on sperm parameters of men with male-factor infertility.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Urology, Teaching Hospital of Hilla, Hilla City, Iraq, between June 2024 and August 2025.

Methods: The current case control study was conducted involving 75 infertile and 75 fertile men. Semen samples were collected through masturbation after a time of abstinence and sperm analysis was performed on computer-assisted semen analysis (CASA). Serum vitamin D levels were measured by chemiluminescence, and RT-qPCR approach was used to investigate expression profiles of sperm-born miR-34b, miR-19a-3p, and miR-122.

Results: Infertile men were presented with significantly lower semen quality, including low semen volume, reduced sperm concentration, and lower motility. Infertile men were also presented with lower levels of vitamin D compared to control, ($p < 0.001$). MiRNA expression revealed miR-34b was reduced in infertile men, whereas miR-19a-3p and miR-122 levels were found upregulated. Examining correlation between vitamin D and miRNA expression revealed inverse correlation with miR-19a-3p ($r = –0.267$, $p < 0.001$), while no significant relationships were observed for miR-34b or miR-122. ROC analysis displayed moderate diagnostic potential for miR-19a-3p (AUC = 0.696, $p < 0.001$) and miR-122 (AUC = 0.614, $p = 0.015$).

Conclusion: Vitamin D deficiency and dysregulation of specific sperm miRNAs are closely linked to reduced sperm motility and viability. The inverse relationship between vitamin D and miR-19a-3p indicates a regulatory function through which vitamin D may control expression of miR-19a-3p and preserve normal sperm function. These results support the use of sperm-borne miRNAs as promising biomarkers for the evaluation of male infertility.

Key Words: Vitamin D, microRNA, sperm parameters, male infertility.

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INTRODUCTION

Male fertility mostly depends on the quality of the produced spermatozoa.[1] Sperm with good morphology, motility and viability could predict successful fertilization and pregnancy outcomes, while those with impaired sperm quality usually suffer from infertility and pregnancy loss.[2] Vitamin D was reported to play an important role in sperm cell formation and spermatozoa maturation.[3] Patients with vitamin D deficiency found with reduced semen quality and low pregnancy outcomes.[4] Vitamin D was also found with significant antioxidant properties against oxidative stress–induced sperm damage.[5] Higher reactive oxygen species (ROS) in semen reported to negatively impact the integrity of the sperm membrane, sperm proteins and DNA, as well as resulting in excessive lipid peroxidation, leading to male infertility through impacting sperm motility, viability, and morphology.[6] Recent findings suggest that higher ROS concentrations in semen, along with impaired sperm quality, are attributed to decreased serum levels of vitamin D.[5]

Spermatogenesis and sperm maturation processes are controlled by extensive sets of microRNAs (miRNAs), which regulate gene expression essential for sperm development.[7] Recent clinical studies associated abnormal miRNA expression profiles with reduced sperm functions and lower reproductive outcomes.[8] Several miRNAs were investigated, and many have been suggested as potential biomarkers for male fertility.[9] MiR-34b expression showed a positive association with sperm concentration and improved pregnancy outcomes.[10] MiR-19a/b-3p expression was

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found upregulated in patients with oligoasthenozoospermia, and higher miR-122 expression was associated with improved sperm concentration, which when downregulated resulted in impaired spermatogenesis.[11, 12] Since vitamin D and miRNAs are both recognized for their roles in maintaining male reproductive health and preventing infertility, their potential interaction in the context of male fertility remains mostly unknown. Therefore, this study was designed to examine the impact of vitamin D levels and expression profiles of miR-34b, miR-19a-3p, and miR-122 on sperm parameters of men with male-factor infertility.

METHODS

Patients and healthy volunteers' recruitment was conducted at the Department of Urology, Teaching Hospital of Hilla, Hilla City, Iraq, between June 2024 and August 2025. The protocol of the study was granted by the Ethics Committee of the College of Medicine, University of Al-Qadisiyah (Reference no. 77/315) and conducted in accordance with the Declaration of Helsinki. Each participant was informed about the study's objectives and informed consents were collected for these regards. In brief, 75 patients with male-factor infertility were selected according for being unable to achieve pregnancy for at least 12 months following regular unprotected intercourse, and presenting with at least one abnormality related to semen quality (low sperm count, reduced motility, or poor morphology), as defined by World Health Organization (WHO) reference values. The control group consisted of 75 men with proven fertility, normal semen parameters, and absence of history related to infertility or other diseases, such as sexually transmitted diseases, varicocele, cystic fibrosis, hormonal imbalance, obstructive azoospermia, or chemotherapy. Both groups were also selected based on the absence of significant female factor infertility, as well as the absence of systemic illnesses, endocrine disorders, prior medications or supplements that could impact levels of vitamin D or oxidative stress. To control any confounders, the two groups were paired in accordance with age and body-mass index.

Semen was collected through masturbation in a sterile container after three to five days of abstinence. After incubation for half an hour at 37 °C, sperm motility parameters were assessed using a computer-assisted semen analysis (CASA). In brief, (10 µL) of the semen sample was loaded into the counting chamber of the CASA apparatus (ASCEN technology, China), and percentages of progressive motility, non-progressive motility, as well as immotile sperm were reported for all samples.

For miRNA expression profile analysis, liquefied semen samples were centrifuged at 3000×g for 10 minutes to form a pellet of the sperm. The sperm pellet was then washed twice by resuspending it in phosphate-buffered saline (PBS) and centrifuging before adding 200 µL of QIAzol reagent (Qiagen, Germany). An equal volume of chloroform was then added to the solution and centrifuged to separate phases followed by collection of the upper RNA layer in fresh tubes and mixing with an equal volume of isopropanol, before centrifugation, removing supernatant, and washing the RNA pellet in 70% ethanol. RNase-free water was used to dissolve the RNA pellet, and total RNA yield and purity were assessed on a NanoDrop spectrophotometer (Thermo Scientific, UK).

Reverse transcription and complementary DNA (cDNA) production were performed with the use of Qiagen miScript II RT Kit (Qiagen, Germany), according to the manufacturer instructions. The reaction mix included 50 ng of total RNA, miScript HiSpec Buffer, nucleotides, reverse transcriptase, and a mix of oligo-dT and random primers. Tubes with the reaction mix were then incubated at 37 °C for 60 minutes to generate the cDNAs before incubation at 95 °C for 5 minutes to inactivate the reverse transcription enzyme. cDNA products were used as templates for quantitative PCR, which was carried out in 96-well plates on a real-time PCR system (Applied Biosystems StepOnePlus, USA), following instruction protocols of miScript SYBR Green PCR kit (Qiagen, Germany). The PCR thermal profile for SYBR Green assays was 15 minutes on 95 °C, 10 seconds on 95 °C (45 cycles), 30 seconds on 56 °C, then 30 seconds on 70 °C. miRNA's relative expressions were quantified at the end of the cycle.

For measurement of vitamin D, (5 mL) peripheral blood was collected in serum separation tubes. Serum samples were then aliquoted into labeled tubes followed by measuring 25-hydroxyvitamin D [25(OH)D] using a chemiluminescence immunoassay on an automated analyzer with a commercially available kit on Cobas e411 analyzer (Roche, Germany). All samples were run in duplicate and results were reported in nanograms per milliliter (ng/mL).

Statistical analysis was conducted on SPSS version 28 (IBM, USA). Independent sample t-test was used to compare variables between findings of groups of the study. Pearson correlation (r) was applied to examine relationships between miR-34b, miR-19a-3p, and miR-122 expression profiles, semen parameters, and serum vitamin D levels. Curve analysis (ROC) was used to assess the potential applications of these miRNAs as markers that could predict male infertility. Statistical significance was set at $p < 0.05$.

RESULTS

Infertile patients showed lower volume of semen (2.05 ± 0.92 ml) compared to the control (3.28 ± 1.18 ml; $p < 0.001$), lower sperm concentration (16.03 ± 5.78 million/ml) vs (53.84 ± 20.19 million/ml; $p < 0.001$) of the control, lower total sperm motility p

(25.85 ± 10.38%) compared to controls (62.91 ± 22.89%; $p < 0.001$), and lower progressive motility (15.15 ± 7.64% vs. 47.60 ± 15.55%; $p < 0.001$). Infertile patients were also found with reduced serum vitamin D (15.04 ± 5.75 ng/ml) compared to the control group (23.75 ± 7.92 ng/ml; $p < 0.001$). Table 1.

Table No.1: Comparison of semen parameters, serum vitamin D levels, and miRNA expression (miR-34b, miR-19a-3p, miR-122) between infertile patients and healthy fertile controls.

Characteristics	Healthy controls		Patients		P value
	Mean	S.D	Mean	S.D	
Age (years)	30.83	7.49	31.15	7.82	0.798
Volume (ml)	3.28	1.18	2.05	0.92	<0.001
Concentration (Million/ml)	53.84	20.19	16.03	5.78	<0.001
Total motility (%)	62.91	22.89	25.85	10.38	<0.001
Progressive motility (%)	47.60	15.55	15.15	7.64	<0.001
Vitamin D (ng/ml)	23.75	7.92	15.04	5.75	<0.001
miR-34b	2.70	1.53	1.08	0.65	<0.001
miR-19a-3p	0.80	0.43	1.29	0.71	<0.001
miR-122	1.38	0.70	1.69	0.87	0.017

Table No.2. Pearson correlation coefficients between microRNA expression levels (miR-34b, miR-19a-3p, miR-122) and semen parameters and serum vitamin D levels.

		miR-34b	miR-19a-3p	miR-122
Volume	r	0.219	-0.285	-0.165
	P-value	0.007*	<0.001*	0.043*
Concentration	r	0.480	-0.348	-0.171
	P-value	<0.001*	<0.001*	0.037*
Total motility	r	0.446	-0.310	-0.113
	P-value	<0.001*	<0.001*	0.167
Progressive motility	r	0.433	-0.286	-0.162
	P-value	<0.001*	<0.001*	0.048*
Vitamin D	r	0.128	-0.267	-0.082
	P-value	0.119	<0.001*	0.318

*Significant association

Table No.3: The table shows the Area Under the Curve (AUC) for the Receiver Operating Characteristic (ROC) analysis, p-values, and 95% Confidence Intervals (CI) for each microRNA was investigated.

	AUC	P value	95% CI	
			Lower Bound	Upper Bound
miR-34b	0.208	<0.001	0.134	0.283
miR-19a-3p	0.696	<0.001	0.610	0.781
miR-122	0.614	0.015	0.524	0.705

Results from MicroRNAs expression analysis showed significant differences in expression levels between groups. Infertile men exhibited significantly lower expression of miR-34b (1.08 ± 0.65) in comparison to controls (2.70 ± 1.53; $p < 0.001$). Conversely, miR-19a-3p expression was significantly higher in the infertile group (1.29 ± 0.71) than in controls (0.80 ± 0.43; $p < 0.001$). miR-122 was also significantly higher in infertile patients (1.69 ± 0.87) relative to fertile men (1.38 ± 0.70; $p = 0.017$). Table 1.

The association between miRNA expression and sperm parameters was also examined and showed a significant positive correlation between miR-34b expression and seminal volume ($r = 0.219$), concentration ($r = 0.480$), overall motility ($r = 0.446$), and progressive motility ($r = 0.433$). However, miR-19a-3p was inversely correlated with seminal volume ($r = –0.285$), concentration ($r = –0.348$), motility ($r = –0.310$), and progressive motility ($r = –0.286$). The inverse correlation was also evident in miR-122 expression profile, where miR-122 was negatively associated with volume ($r = –0.165$), concentration ($r = –0.171$), and progressive motility ($r = –0.162$). With respect to the correlation with vitamin D, neither miR-34b nor miR-122 was significantly associated with vitamin D levels. However, miR-19a-3p levels showed a significant inverse association with vitamin D ($r = –0.267$, $p < 0.001$). Table 2.

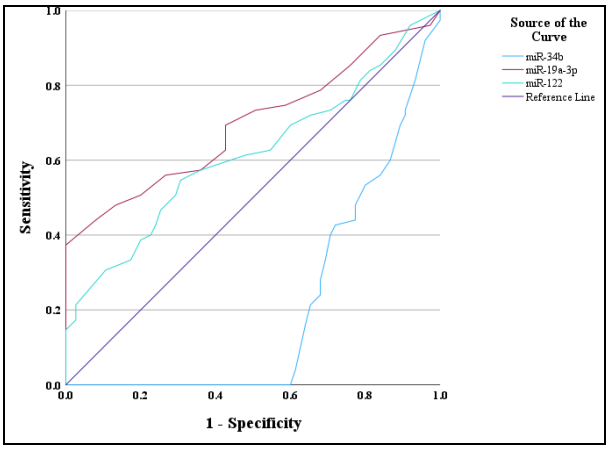

Figure No.1: Receiver Operating Characteristic (ROC) curves for miR-34b, miR-19a-3p, and miR-122 in distinguishing infertile men from fertile controls.

The diagnostic performance of the three microRNAs through the implementation of the ROC curve analysis showed the area under the curve (AUC) of 0.208 ($p < 0.001$) for miR-34b, miR-19a-3p with an AUC of 0.696 ($p < 0.001$), and miR-122 with an AUC of 0.614 ($p = 0.015$). The results suggest that these miRNA expression profiles may serve as molecular indicators of impaired sperm quality in infertile men. Table 3 and Figure 1.

DISCUSSION

In this study which included infertile men, we found that normal levels of vitamin D and semen miRNA can significantly maintain semen quality and male reproductive function. Studies linked vitamin D to better semen quality and normal androgen levels, while lower vitamin D levels were presented in cases with gonadal insufficiency, decreased sperm count, and infertility.[1] Vitamin D insufficiency was also associated with lower live birth rates and lower outcomes from assisted reproductive technologies.[4] Sufficient vitamin D levels are important to maintain the quality of semen parameters, and the observed inverse association, by the current analysis, between vitamin D and sperm parameters aligns with these reports.

Our infertile patients also displayed altered expression profiles of miRNA-19a/b-3p, miR-34b and miR-122. miR-19a-3p levels were shown to be higher than control, combined with reduced vitamin D levels. Those with higher miR-19a/b-3p levels in sperm cells were identified as infertile men with oligoasthenozoospermia, presenting with reduced sperm count, motility, and sperm morphology.[11] Our finding indicate an important role for miR-19a-3p in reproductive health where higher levels of miR-19a-3p could negatively impact male fertility.[7]

Our findings regarding miR-34b and miR-122 are in line with previous studies, showing significant reductions in male patients which were associated with reduced semen quality compared to healthy individuals. Abdolmabood et al. reported downregulation of miR-34b in infertile men, which was found to be indicative of impaired spermatogenesis.[13] Lower miR-34b levels were also linked to reduced sperm motility and sperm concentration.[14] Yeh et al. showed that higher miR-34b expression was positively correlated with implantation, pregnancy, and live-birth rates.[15] Similarly, studies have also associated the elevated miR-122 levels with enhanced male reproductive competence. Joshi et al.[9] provided evidence for a link between miR-122 and male infertility, and Mokánszki et al.[10] confirmed a positive correlation between miR-122 and sperm concentration, while Tomic et al.[12] correlated lower miR-122 levels to teratozoospermia in infertile men. However, serum levels of vitamin D did not show a significant correlation with seminal levels of miR-122, suggesting no impact of vitamin D levels on these miRNAs in sperm.

In contrast, the observed inverse relationship between vitamin D and miR-19a-3p by the current analysis holds considerable significance in the context of male reproduction. It may provide an insight into a novel mechanism where vitamin D directly or indirectly regulates expression of miR-19a-3p, limiting inhibitory actions of the miRNA on key regulatory genes which are controlling spermatogenesis and improving semen quality. Recent studies demonstrated a role for vitamin D in regulating expression of a wide range of miRNA profiles across various physiological and pathological contexts.[16] Although evidence regarding the relationship between vitamin D and miR-19a-3p is very limited in the literature, findings from a recent study point toward a possible regulatory mechanism of vitamin D supplementation on improving the clinical signs of allergic rhinitis and reduce miR-19a levels in B cells, an effect that was linked to lower IgE levels, reduced Th2 cytokines, and increased interleukine-10.[17] The impact of vitamin D on miR-19a expression levels is still not completely understood. However, recent evidence suggests that sufficient levels of vitamin D could restrain aberrant miRNA expression, thereby preserving normal physiological processes.[18] One of these suggested routes is through VDR signaling pathways, where VDR was shown to influence miRNA expression levels in various conditions.19 A study reported that supplementation of vitamin D in animal models with PCOS showed higher expression of specific miRNAs that are required for oogenesis while decreasing other aberrant miRNAs in the ovaries.[20] Those animal models were also presented with significant improvements in number of molecular markers related to ovarian dysfunction.[20] Additional studies described that certain miRNAs can also modulate expression of VDR, and low levels of miR-19a-3p could permit vitamin D to promote expression

of genes that are responsible for maintaining male fertility.[2] It is clear from our findings that sufficient vitamin D can favorably modify genetic expression in reproductive tissues to prevent infertility by creating an environment where normal expression profiles of pro-fertility genes are maintained.

Vitamin D is also known for its effective anti-inflammatory and antioxidative properties, which are widely recognized for regulating extensive cellular activities.[18] Hence, sufficient levels of vitamin D plus maintaining normal miRNA expressions could effectively alleviate excessive inflammatory or oxidation factors that generate apoptotic signaling in the sperm of infertile individuals.[3]

The findings first provide insight into the mechanisms that govern male reproductive health through endogenous levels of vitamin D and sperm miRNA expression profiles. Our findings align with recent reports supporting the roles of vitamin D and sperm miRNAs in maintaining normal sperm quality, in addition to the potential applications of sperm miRNAs and vitamin D levels as valuable biomarkers for male fertility. Larg cohort studies to validate these results are required, which could help in providing a better understanding of the molecular mechanisms that govern sperm pathophysiology.

CONCLUSION

The study showed that altered expression profiles of miR-34b, miR-19a-3p, and miR-122 were significantly associated with reduced normal sperm characteristics in infertile patients. The study also revealed an inverse relationship between vitamin D and miR-19a-3p, suggesting a mechanism that could contribute to enhanced male reproductive health through the regulatory actions of vitamin D on miR-19a-3p expression. The findings also described a potential application of miR-19a-3p, miR-34b, and miR-122 levels as viable biomarkers for assessing male fertility.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Alaa Hachem, Asaad Al-Shouk
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Final Approval of version:	All the above authors
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Original Article

Correlation of Thigh Length with Adult Human Stature

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ABSTRACT

Objective: To find correlation of thigh length with adult human stature.
Study Design: Cross sectional correlational study
Place and Duration of Study: This study was conducted at the Department of Forensic Medicine and Toxicology, King Edward Medical University, Lahore from 24 May 2024 to 24 August 2024.
Methods: Sixty medical students (30 males and 30 females) were enrolled, with informed consent obtained after explaining the study's purpose. Biodata, including name, age, gender, and city, was collected. Stature, inter anterior superior iliac spinal distance, and thigh length were measured per Krogman's procedures, with the measurement team trained to minimize observer bias.
Results: The mean age of participants was 20.48 years (SD 1.19), with 30 males and 30 females. Those under 20 years comprised 45% (n=27), while those over 20 made up 55% (n=33). The mean thigh length was 46.18 cm (SD 2.02) and mean stature was 169.48 cm (SD 8.35). The overall correlation between thigh length and stature was 0.203 (p=0.12). In males, it was -0.106 (p=0.579) and in females, 0.099 (p=0.60). For participants under 20, the correlation was 0.167 (p=0.40), while for those over 20, it was 0.252 (p=0.158).
Conclusions: This study evaluated thigh length as a predictor of adult stature, finding a weak to moderate correlation that varied by gender and age. Although it shows some potential, correlation is not strong enough to recommend it as a standalone measurement.
Key Words: Anthropometric measurement, Stature, Thigh length

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INTRODUCTION

Human identification has always been a key factor in establishing individuality of a person either in civil or criminal cases. This process of identification has multiple approaches towards addressing this issue which include physical parameters, ABO blood grouping, HLA markers, DNA analysis and Y-STR analysis etc. Among all these parameters is an anthropometric study which includes quantification of human size and shape along with its proportions. This analysis is used not only for clinical evaluation of a person assessing his health and ergonomics but also for forensic purposes to prove and settle an identity dispute in medicolegal cases.[1]

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Anthropometry can further be subdivided in multiple measurements of the body parts and the present study shall focus on long bones with specificity to measurement of the thigh i.e. femur bone. Multiple measurements of long bones is incorporated in mathematical statistics using regression analysis eventually reflecting a correlation among long bone like femur with overall height.[2] Although this physical analysis is an old school technique as compared to recent advancements to manifest identity yet as with all evolution of scientific scans, human stature estimation has also significantly metamorphosed to advanced technological approach for better anatomical comprehension.[3] This scientific approach has progressed due to improved regression model approach with more accurate results enabling stature evaluation even from partial bone fragments. Anthropometrists have delved deeper into physical measurements like Inter Anterior Superior Iliac Spinal Distance (IASISD) and Thigh Length (TL); TL measures the distance from the ASIS to the femur's medial condyle, while IASISD measures the distance between ASIS points.[4] On a comparative note IASISD is usually used in clinical scenarios for guidance in surgery whereas thigh length is a true leg length parameter which can give an accurate stature estimate in absolute least time consumption. This TL technique not only can be used as an evaluative tool in the living subjects but also has an archeological approach assisting identity

establishment as femur has the most significant correlative factor for height calculation.[5,6] In human population thigh length is dependable measure of human stature due to its steady proportional association with height reckoning around 26-27% of entire height across multiple populations.[7] This is a dependable method with accuracy in stature calculation in living subjects due to prominent anatomical landmarks indicative for precise measurements with least observer error which prompt application in regression analytical models. This technique is even most reliable in such calculative purposes to evaluate stature from skeletal remains either complete or partial.[8] Finally emphasizing on objectivity of this study it is elaborated that such a technique is extremely resourceful due to its accuracy, non-invasive approach with limited resources and technology in a third world country like Pakistan in assessing stature measurements in not only living subjects but also in post mortem examinations along with the fragmentary, mutilated remains procured by the police from either mass disasters or on exhumation to establish identification for various medicolegal purposes.[9]

METHODS

This cross-sectional correlational study was conducted at King Edward Medical University Lahore including students ranging from 1st year to final year MBBS, over three months after ethical approval from institutional review board vide letter number.56 /RC/KEMU dated 06th May 2024. A sample size of 60 cases was calculated with a 95% confidence level and a 5% margin of error, using non-probability consecutive sampling. Following approval from Ethical Committee, participants with inclusion criteria were enrolled, and written informed consent was obtained from participants. A detailed history and complete physical examination were conducted for all participants. For thigh length measurement, a measuring tape or anthropometer was used to measure from the greater trochanter, a bony prominence on the hip, to the lateral condyle of the femur, ensuring the thigh was fully extended and the knee was in a neutral position. This measurement was recorded to the nearest centimeter. Stature was measured using a stadiometer; participants stood erect with their backs against the device and heads positioned in the Frankfort plane. The measuring arm was lowered until it made contact with the participant's head, and the height was recorded to the nearest centimeter. A predesigned questionnaire was used to collect demographic information, including age, gender, and ethnicity/race, from each participant. The overall aim of the study was to assess the effectiveness of thigh length as a predictor of adult human stature, contributing valuable data for further research in this area.

Ethical Declaration: Ethical review board proceedings were conducted in Mayo Hospital Lahore under the Chairmanship of Prof. Muhammad Imran, Secretary Institutional Review Board, Chairman Department of Medicine, King Edward Medical University/Mayo Hospital, Lahore and project was approved vide letter no.**56 /RC/KEMU dated 06th May 2024.**

Inclusion Criteria: Study included individuals aged 18 to 24 years, with both males and females eligible for enrollment.

Exclusion Criteria: It included individuals younger than 18 or older than 25 years, those with stunted or enhanced bone growth, and those with a history of skeletal injury.

Data Analysis: Data was entered and analyzed through SPSS 26. Mean and standard deviation was calculated for quantitative variables like age, thigh length, and stature. Frequency and percentage were calculated for gender. Correlation analysis, such as Pearson correlation coefficient, was performed to examine the associations between thigh length and stature. Effect modifiers such as age and gender were controlled through post stratification and p-value <0.05 was considered significant.

RESULTS

The mean age of all patients was 20.48 years with a SD of 1.19 years. The mean thigh length was 46.18 cm with a standard deviation of 2.02 cm. The mean stature was 169.48 cm with a standard deviation of 8.35 cm as shown in Table-I.

The study involved 60 participants, evenly split between genders with 30 males and 30 females, each representing 50% of the total population. Participants were categorized into two age groups: 27 individuals (45%) were under 20 years old, while 33 individuals (55%) were over 20 years old as shown in Table-2.

The overall correlation between stature and thigh length in the study population (n=60) yielded correlation coefficient of 0.203 with a *p*-value of 0.12 as shown in Table-3.

Table No. I: Mean and standard deviation of Age, Thigh length and Stature (n=60)

Variable	n	Mean	SD
Age (Years)	60	20.48	1.91
Thigh Length(cm)	60	46.18	2.02
Stature (cm)	60	169.48	8.35

The correlation of stature with thigh length, when analyzed separately for males and females (n=60), showed differing results. For males (n=30), the correlation had an R value of -0.106 with a *p*-value of 0.579, indicating a weak negative and non-significant relationship. For females (n=30), the R value was 0.099 with a *p*-value of 0.60. The correlation of stature with thigh length, when analyzed according to age groups

(n=60), showed varying results. In participants aged <20 years (n=30), the correlation coefficient of 0.167 with a *p*-value of 0.40. For participants aged >20 years (n=30), the correlation showed an R value of 0.252 with a *p*-value of 0.158 as shown in Table-4.

Table No.2: Frequency of patients on the basis of gender and age (n=60)

Gender and Age Group	Frequency	Percentage
Male	30	50.0
Female	30	50.0
<20 years	27	45.0
>20 years	33	55.0

Table No.3: Correlation of stature with thigh length (n=60)

Variable	n	r	*p*-value
Over all Correlation	60	0.203	0.12

Table No.4: Correlation of stature with thigh length with respect to gender and age group (n=60)

Correlation	n	r	*p*-value
Male	30	-0.106	0.579
Female	30	0.099	0.60
<20 years	30	0.167	0.40
>20 years	30	0.252	0.158

DISCUSSION

Stature estimation is a crucial component in medico-legal investigations, particularly in the identification of unknown, fragmentary, or mutilated human remains.[10,11] Apart from other parameters of identification like age sex and ancestry accurately determining an individual's height can provide significant clues in forensic contexts, helping to establish identity and narrow down potential matches. This process becomes essential when dealing with incomplete or damaged skeletal remains where conventional identification methods may be insufficient.[12,13] In countries with limited resources, expertise in such simple procedures have paramount importance in identification of unknown fragmented, mutilated and skeletonized dead bodies. Other modern methods for objective identification require a detailed ante-mortem data and other sophisticated technology along with resources which in developing countries is limited. By applying anthropometric techniques and statistical models to the available skeletal elements, forensic experts can estimate the stature of the deceased, contributing valuable information to the investigative process.

In this study, correlation between thigh length and stature was determined utilized to explore the relationship. These statistical methods provide a robust framework for understanding how well IASISD can predict stature and the strength of their association. The correlation coefficient (r) obtained in this study was 0.927, indicating a very strong positive relationship.

In the present study overall correlation between stature and thigh length in study population was found to be 0.203, with a *p*-value of 0.12. This indicates a weak positive correlation that is not statistically significant, suggesting that while there is a slight association between thigh length and stature, it is not strong enough to be considered a reliable predictor of stature in this sample. The results suggest that while there is some degree of association between thigh length and stature, relationship is not strong enough to make thigh length a reliable predictor of stature on its own. This is consistent with findings from other studies which also report weak correlations between various anthropometric measurements and stature.[14,15] A study conducted by Arif Viqar et al. stated that a positive correlation was observed between the stature and the femur length and correlation was highly statistically significant. ($p<0.001$).

Another study finding aligns with other research where correlation between similar anthropometric measurements and stature has been shown to be both strong and significant, reinforcing reliability of these measurements for predicting stature.[16] These findings are well-supported by existing literature, which highlights the effectiveness of anthropometric measurements in predicting body dimensions.[17]

When analyzing the correlation between stature and thigh length separately by gender in the study population (n=60), the results varied significantly. For males (n=30), the correlation coefficient was -0.106 with a *p*-value of 0.579. This weak negative correlation suggests that, for males, thigh length has a negligible and statistically insignificant inverse relationship with stature. The high *p*-value indicates that this relationship is not statistically significant, and any observed association is likely due to chance.[18]

In contrast, for females (n=30), the correlation coefficient was 0.099 with a *p*-value of 0.60. This weak positive correlation implies that for females, thigh length shows a slight and statistically insignificant positive association with stature. Similar to results for males, high *p*-value signifies that observed correlation is not statistically significant; reinforcing that thigh length is not a strong predictor of stature in females either.

These gender-specific findings highlight that thigh length's predictive value for stature varies and is not robust across genders. Lack of significant correlation in both groups suggests that thigh length alone may not be a reliable measure for stature estimation. This is consistent with notion that anthropometric relationships can differ based on gender and other demographic factors, and underscores need for further research to

explore and validate more accurate predictors of stature in diverse populations. [19]

CONCLUSION

Statistical analysis of this study proved an insignificant correlation between thigh length and height of an individual especially of male gender. Weak correlative connection among the variable of thigh length with it as a predictive model of height estimation was established in this study as relationship varied by gender and age with an eventual conclusion that despite its potential, thigh length alone does not provide a highly accurate estimate of stature.[21]

Author's Contribution:

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Original Article

Evaluating the Diagnostic Accuracy of TI-RADS in Differentiating Benign and Malignant Thyroid Nodules Using Histopathology as the Benchmark Standard

Mehak Mohsin[1], Sehrish Salman[2], Sana Saleem Rana[1], Rahmat Javed[2], Motia Kanwal[3] and Amna Javed[1]

ABSTRACT

Objective: To evaluate the diagnostic accuracy of TI-RADS in differentiating benign and malignant thyroid nodules using histopathology as the benchmark standard.

Study Design: Cross-sectional analytical study

Place and Duration of Study: This study was conducted at the Watim Medical and Dental College, Rawalpindi from July 2024 to March 2025.

Methods: This study was conducted on 195 patients with thyroid nodules who underwent ultrasound evaluation followed by fine-needle aspiration cytology (FNAC) or thyroidectomy for histopathological confirmation. Nodules were classified according to the American College of Radiology TI-RADS (TR2–TR5) based on their sonographic features. Histopathological results were considered the gold standard.

Results: Of the 195 patients, 143 (73.3%) had benign and 52 (26.7%) had malignant nodules. The malignancy rate increased progressively with higher TI-RADS categories: 0% in TR2, 5.5% in TR3, 20.3% in TR4, and 80.8% in TR5. TI-RADS showed a sensitivity of 90.3%, specificity of 84.6%, PPV of 70.2%, NPV of 95.3%, and an overall diagnostic accuracy of 86.7%.

Conclusion: It is concluded that TI-RADS is a highly effective and reliable tool for differentiating benign from malignant thyroid nodules. Its strong correlation with histopathological outcomes confirms its diagnostic value. Routine application of TI-RADS in thyroid ultrasound practice is recommended to enhance diagnostic accuracy, standardize reporting, and minimize unnecessary invasive procedures.

Key Words: Thyroid nodules, TI-RADS, ultrasound, histopathology, diagnostic accuracy, thyroid malignancy.

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INTRODUCTION

Thyroid nodules are one of the most common endocrine disorders encountered in clinical practice, with their prevalence increasing markedly due to the widespread use of ultrasonography and other imaging techniques[1]. Studies estimate that palpable thyroid nodules occur in about 4–7% of the adult population, while incidental nodules detected on ultrasound can be as high as 19–68%[2]. Although the vast majority of these nodules are benign, a small but significant proportion (approximately 5–15%) harbor malignancy[3].

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The key clinical challenge lies in accurately identifying malignant nodules among the numerous benign ones to avoid unnecessary invasive procedures and ensure timely intervention for those with cancer[4]. This is particularly important considering the rising global incidence of thyroid cancer, largely attributed to increased detection of small papillary carcinomas through imaging[5]. The evaluation of thyroid nodules traditionally relies on a combination of clinical assessment, thyroid function testing, and imaging, with ultrasound being the first-line modality. Ultrasound plays a pivotal role in assessing nodule morphology, vascularity, margins, and the presence of suspicious features[6]. However, the interpretation of ultrasound findings has historically been subjective, varying widely between operators and institutions. This lack of standardization led to inconsistencies in recommendations for fine-needle aspiration cytology (FNAC), which has long been regarded as the diagnostic cornerstone. FNAC, though highly useful, is invasive, operator-dependent, and occasionally yields indeterminate or non-diagnostic results, leaving ambiguity in management[7]. Therefore, the need arose for a systematic, reproducible, and non-invasive method

to stratify the risk of malignancy based on ultrasound features[8]. In response to this need, the Thyroid Imaging Reporting and Data System (TI-RADS) was developed, inspired by the success of the Breast Imaging Reporting and Data System (BI-RADS) in radiology. TI-RADS provides a structured reporting system that classifies thyroid nodules into categories based on specific ultrasound characteristics[9]. The system assesses parameters such as nodule composition (solid, cystic, or mixed), echogenicity (hypoechoic, isoechoic, or hyperechoic), margin definition, shape (taller-than-wide versus wider-than-tall), and the presence of echogenic foci or microcalcifications. Each feature is assigned a point value, and the cumulative score places the nodule into a risk category ranging from benign (TI-RADS 1–2) to highly suspicious (TI-RADS 5)[10]. This structured approach aims to improve diagnostic consistency, reduce unnecessary FNACs, and guide clinicians toward evidence-based management pathways. Multiple iterations of TI-RADS have been introduced by various organizations, including the American College of Radiology (ACR TI-RADS), the American Thyroid Association (ATA guidelines), and the Korean Society of Thyroid Radiology (K-TIRADS)[11]. Among these, the ACR TI-RADS is the most widely implemented, providing clear recommendations regarding which nodules require FNAC based on both size and risk category. Studies have demonstrated that applying TI-RADS can significantly reduce the number of unnecessary biopsies while maintaining high sensitivity for detecting malignancy[12]. However, variations in diagnostic accuracy across populations have been observed, which may be attributed to differences in equipment, operator expertise, and the underlying prevalence of thyroid malignancy. Therefore, validation of TI-RADS in different clinical and demographic contexts remains essential[13]. Histopathological examination remains the definitive standard for diagnosing thyroid malignancy. Correlating TI-RADS classifications with histopathological outcomes provides critical insight into the reliability and clinical applicability of this ultrasound-based system[14]. Several studies have reported varying results, with sensitivity ranging from 70–95% and specificity between 60–90%. While TI-RADS has been praised for its ability to stratify nodules effectively and minimize unnecessary FNACs, some overlap in sonographic features between benign and malignant nodules persists, leading to potential misclassification. For example, follicular adenomas and carcinomas often share similar ultrasound appearances, making cytological or histological confirmation necessary for definitive diagnosis[15].

METHODS

This cross-sectional analytical study was conducted at Watim Medical and Dental College from July 2024 to March 2025. A total of 195 patients were included in the study. Non-probability consecutive sampling was employed to recruit participants who met the inclusion criteria. All adult patients (both male and female) aged 18 years and above presenting with thyroid nodules detected on clinical examination or imaging and referred for thyroid ultrasound followed by fine-needle aspiration cytology (FNAC) or surgical excision for histopathological evaluation were included. Patients with previously diagnosed thyroid malignancy, recurrent nodular goiter, incomplete ultrasound or histopathological records, and those who had undergone prior thyroid surgery or radiotherapy were excluded from the study.

Data Collection: After obtaining informed consent, all patients underwent a detailed thyroid ultrasound examination using a high-frequency linear transducer (7.5–12 MHz). Each thyroid nodule was evaluated according to the American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS) classification. Ultrasound features assessed included composition (solid, cystic, or mixed), echogenicity (hypoechoic, isoechoic, hyperechoic, or anechoic), shape (taller-than-wide or wider-than-tall), margin characteristics (smooth, lobulated, or irregular), and echogenic foci (microcalcifications or macrocalcifications). Based on these parameters, each nodule was assigned a TI-RADS score (TR1 to TR5) representing increasing suspicion of malignancy. Subsequently, patients underwent FNAC or thyroidectomy as clinically indicated, and histopathological findings were recorded as benign or malignant, serving as the gold standard for comparison. The histopathological evaluation was performed by experienced pathologists blinded to the ultrasound findings.

Data Analysis: All collected data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous variables such as age and BMI were expressed as mean ± standard deviation (SD), while categorical variables like TI-RADS category and histopathological diagnosis were presented as frequencies and percentages. Cross-tabulation was performed to compare TI-RADS categories with histopathological outcomes. Diagnostic accuracy parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were calculated using 2×2 contingency tables, taking histopathology as the benchmark. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the diagnostic performance and optimal cutoff value of TI-RADS for malignancy prediction. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Data were collected from 95 patients, the mean age was 42.6 ± 11.3 years, with a predominant female population (72.3%) compared to males (27.7%), consistent with the higher incidence of thyroid nodules

in women. The mean nodule size was 2.4 ± 1.1 cm. Most patients (65.1%) had solitary nodules, while 34.9% presented with multinodular goiter. Histopathological findings revealed that 143 nodules (73.3%) were benign and 52 (26.7%) were malignant, reflecting the expected distribution where the majority of thyroid nodules are non-cancerous.

Table No.1: Baseline Demographic and Clinical Characteristics of Patients (n = 195)

Variable	Category / Mean ± SD	n (%)
Mean age (years)	42.6 ± 11.3	—
Gender	Male	54 (27.7)
	Female	141 (72.3)
Mean nodule size (cm)	2.4 ± 1.1	—
Nodule type	Solitary	127 (65.1)
	Multinodular	68 (34.9)
Histopathological outcome	Benign	143 (73.3)
	Malignant	52 (26.7)

Among the 195 nodules, 15 (7.7%) were categorized as TR2, 54 (27.7%) as TR3, 74 (37.9%) as TR4, and 52 (26.7%) as TR5. The malignancy rate was 0% in TR2, 5.5% in TR3, 20.3% in TR4, and 80.8% in TR5, indicating a strong correlation between TI-RADS classification and histopathological malignancy.

Histopathological evaluation of the 52 malignant thyroid nodules revealed that papillary carcinoma was the most common type, comprising 84.6% of cases, followed by follicular carcinoma (9.6%), medullary carcinoma (3.8%), and anaplastic carcinoma (1.9%).

Table No. 2. Distribution of Thyroid Nodules by TI-RADS Category and Corresponding Malignancy Rate

TI-RADS Category	Total (n)	Benign (n)	Malignant (n)	Malignancy Rate (%)
TR2	15	15	0	0
TR3	54	51	3	5.5
TR4	74	59	15	20.3
TR5	52	10	42	80.8
Total	**195**	**135**	**60**	**30.8**

Table No. 3. Histopathological Spectrum of Malignant Thyroid Nodules (n = 52)

Type of Malignancy	Frequency (n)	Percentage (%)
Papillary carcinoma	44	84.6
Follicular carcinoma	5	9.6
Medullary carcinoma	2	3.8
Anaplastic carcinoma	1	1.9
Total	**52**	**100**

Solid composition, marked hypoechogenicity, irregular or spiculated margins, a taller-than-wide shape, and microcalcifications were highly predictive of malignancy ($p < 0.001$). Specifically, 88.5% of malignant nodules were solid, 78.8% were hypoechoic, 76.9% had irregular margins, 65.4% were taller-than-wide, and 71.1% exhibited microcalcifications.

Table No. 4. Association Between Sonographic Features and Histopathological Malignancy

Ultrasound Feature	Category	Malignant (n = 52)	Benign (n = 143)	p-value
Nodule composition	Solid	46 (88.5)	72 (50.3)	<0.001
	Mixed / cystic	6 (11.5)	71 (49.7)	
Echogenicity	Hypoechoic	41 (78.8)	56 (39.2)	<0.001
	Iso/hyperechoic	11 (21.2)	87 (60.8)	
Margins	Irregular / spiculated	40 (76.9)	32 (22.4)	<0.001
	Smooth / well-defined	12 (23.1)	111 (77.6)	
Shape	Taller-than-wide	34 (65.4)	27 (18.9)	<0.001
	Wider-than-tall	18 (34.6)	116 (81.1)	
Echogenic foci	Present (microcalcifications)	37 (71.1)	40 (28.0)	<0.001
	Absent	15 (28.9)	103 (72.0)	

Table No. 5. 2×2 Contingency Table Comparing TI-RADS and Histopathological Findings (n = 195)

TI-RADS Category	Histopathology Malignant	Histopathology Benign	Total
Positive (TR4 & TR5)	47 (True Positive)	20 (False Positive)	67
Negative (TR2 & TR3)	5 (False Negative)	123 (True Negative)	128
Total	**52**	**143**	**195**

Sensitivity TP / (TP + FN) = 90.3%
Specificity TN / (TN + FP) = 84.6%
Positive Predictive Value (PPV) TP / (TP + FP) = 70.2%
Negative Predictive Value (NPV) TN / (TN + FN) = 95.3%
Overall Diagnostic Accuracy (TP + TN) / Total = 86.7%

TI-RADS demonstrated a sensitivity of 90.3%, specificity of 84.6%, positive predictive value of 70.2%, negative predictive value of 95.3%, and an overall diagnostic accuracy of 86.7%. These results indicate that TI-RADS is a reliable, non-invasive tool for predicting malignancy in thyroid nodules.

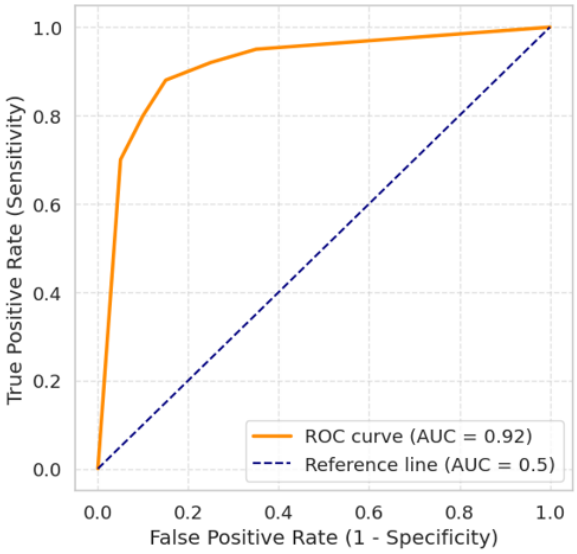

Figure No.1: Receiver operating characteristic (ROC) Curve for Ti-RADS

DISCUSSION

This study evaluated the diagnostic accuracy of the Thyroid Imaging Reporting and Data System (TI-RADS) in differentiating benign from malignant thyroid nodules using histopathology as the gold standard. A total of 195 patients were analyzed, with findings demonstrating that TI-RADS is a highly reliable, non-invasive tool for risk stratification of thyroid nodules. The overall sensitivity, specificity, and diagnostic accuracy of TI-RADS in this study were 90.3%, 84.6%, and 86.7%, respectively, with an area under the ROC curve (AUC) of 0.92, indicating excellent diagnostic performance. These findings confirm that higher TI-RADS categories correlate strongly with histopathologically proven malignancy, supporting its clinical utility in routine thyroid imaging practice. The demographic data in this study showed a mean patient age of 42.6 years and a marked female predominance (72%), which aligns with global epidemiological patterns of thyroid disorders, as females are approximately three to four times more likely to develop thyroid nodules compared to males[16]. This gender disparity is often attributed to hormonal influences, particularly estrogen-mediated thyroid stimulation. Most nodules in this study were benign (73.3%), consistent with existing literature reporting that 80–90% of thyroid nodules are non-malignant. The predominant histopathological type among malignant lesions was papillary carcinoma (84.6%), which is the most common thyroid malignancy worldwide, followed by follicular carcinoma, medullary carcinoma, and anaplastic carcinoma. The distribution of malignancy across TI-RADS categories in this study demonstrated a direct relationship between TI-RADS score and malignancy risk, with 0% malignancy in TR2, 5.5% in TR3, 20.3% in TR4, and 80.8% in TR5. These results mirror previous studies that reported a similar progressive trend in malignancy risk across increasing TI-RADS grades[17].

The sonographic features most strongly associated with malignancy in this study were solid composition, marked hypoechogenicity, irregular or spiculated margins, taller-than-wide shape, and microcalcifications. These features are well-documented indicators of thyroid malignancy, as they reflect tumor invasiveness and altered cellular architecture. Similar findings were reported by Ha et al. (2020), who identified microcalcifications and irregular margins as the most predictive indicators of malignancy, achieving comparable sensitivity and specificity levels. The presence of microcalcifications in particular is often linked with papillary carcinoma, reflecting psammoma body formation [18]. Compared with histopathology, TI-RADS showed excellent sensitivity (90.3%) and a high negative predictive value (95.3%), meaning that a low TI-RADS score effectively rules out malignancy, thereby reducing unnecessary FNAC procedures. The specificity (84.6%) and positive predictive value (70.2%) were also satisfactory, suggesting that higher TI-RADS categories are highly suggestive of malignancy [19,20]. Despite the strong performance of TI-RADS, several limitations must be acknowledged. Ultrasound evaluation remains operator-dependent, and image interpretation can vary among radiologists. Additionally, overlap in sonographic features between follicular adenomas and carcinomas may result in false-positive or false-negative classifications. Moreover, the study was conducted at a single center, which may limit generalizability. Including a larger multi-center cohort and incorporating inter-observer variability analysis in future studies would provide more comprehensive validation.

CONCLUSION

It is concluded that the Thyroid Imaging Reporting and Data System (TI-RADS) serves as a highly reliable, standardized, and non-invasive diagnostic tool for differentiating benign from malignant thyroid nodules. In this study, TI-RADS demonstrated excellent diagnostic performance, with a sensitivity of 90.3%, specificity of 84.6%, and overall accuracy of 86.7% when compared with histopathological findings, which served as the gold standard. The likelihood of malignancy increased progressively with higher TI-RADS categories, confirming its validity as a risk stratification method. Sonographic features such as solid composition, marked hypoechogenicity, irregular margins, microcalcifications, and a taller-than-wide shape were significantly correlated with malignancy, underscoring their diagnostic importance.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mehak Mohsin, Sehrish Salman, Sana Saleem Rana
Drafting or Revising Critically:	Rahmat Javed, Motia Kanwal, Amna Javed

Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Association Between the Difference in Age, Sex, and the Atherogenic Index of Plasma

Rithab Ibrahim Al-Samawi

ABSTRACT

Objective: This study aimed to examine the association between differences in both age and sex with AIP.
Study Design: Cross-sectional study
Place and Duration of Study: This study was conducted at the Department of Clinical Laboratory Sciences, College of Pharmacy, University of Al-Ameed, Karbala, Iraq, from 28 Aug 2024 to 30 March 2025.
Methods: Eight hundred participants were included, equally divided into two groups: males and females. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL) were examined by the colorimetric method, while the Friedewald equation estimated low-density lipoprotein cholesterol (LDL-c). AIP was determined using (log_{10} TG/HDL-c).
Results: The study reveals that females under 40 years tend to have a more favorable lipid profile, with lower levels of AIP, TC, TG, and LDL-c compared to males of the same age. Above 45years, both females and males have higher AIP and lipid levels. Among male groups, there are no significant differences between the younger and older groups. There was a statistical difference between the two female groups, with the younger one having a better AIP and lipid profile.
Conclusion: The study shows a significant association between age and sex with AIP among the study population.

Key Words: AIP; Lipid; Atherogenic; Females; Males

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INTRODUCTION

The AIP is an easily estimated value that depends on the lipid profile measured. Its calculation is based on the logarithmic ratio of TG to HDL-c[1]. When compared with the measurement of each lipid parameter, the AIP is considered a good indicator for cardiovascular risk. It is related to the distribution of small dense LDL particles and determines the rate of HDL-c esterification[2]. It also acts as a diagnostic index; it's more beneficial than a routine lipid profile due to its ability to identify when other risk lipid parameters appear normal. The AIP measurement refers to the zone of atherogenic risk[3]. Recent studies have revealed that AIP not only refers to the relationship between atherogenic and non-atherogenic lipoproteins but also acts as a vital marker of atherosclerosis and its related heart conditions[4,5].

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It has been found that AIP is associated with both age and sex, with higher AIP levels reflecting increased incidence of atherogenic cardiovascular diseases[6]. Studies also show that AIP is typically higher in males compared with females. Age is a significant factor, with older individuals generally showing higher AIP levels and thus a greater risk[7,8]. However, the relationship between age, sex, and AIP can be more complex and may vary depending on other factors. Therefore, this study aimed to investigate the relationship between age, sex, and AIP in a specific, well-defined group.

METHODS

The study involved 400 healthy males and 400 healthy females with a normal body mass index. The AIP, BMI, and lipid levels were estimated for every participant. Each group was categorized into two subgroups: one, ranging from 20 to 40 years, and the other, ranging from 45 to 65 years. Blood samples were collected after 12 hours of fasting to measure the Lipid parameters. Information concerning the lipid abnormality history, physical activity, and smoking behavior was collected through a questionnaire. The inclusion criteria for the study population were healthy participants, moderate physical activity, no evidence of chronic conditions, and the absence of liver, renal, thyroid, or other conditions, as well as the participant not being a smoker.

Estimation of Serum Lipid Levels: Lipid investigations were conducted on fasting participants. Colorimetric methods (Biolabo/France) were used to

calculate serum TC, TG, and HDL-c levels, whereas LDL-c levels were estimated using the Friedewald formula [9].

Statistical Tests: The data were summarised by mean ± S.D. and analyzed using IBM SPSS Statistics version 24. A t-test was applied to determine the relationship between AIP, lipid parameters, and different age groups. ANOVA test was used to compare the mean levels of lipid parameters in various sex groups. The results were statistically significant at p-value < 0.05. Lipid profiles were measured in mmol/l, while body mass index was in kg/m2.

RESULTS

The study revealed that the levels of AIP, TG, TC, and LDL-c were elevated significantly in males group when compared with females (P value < 0.05), (Table 1).

Table No. 1: Demographic and Biochemical Parameters for the study population

Parameters	Female n = 400	Male n = 400	P-value
Age (years)	41.988 ± 11.844	41.011 ± 10.701	NS
TC	4.247 ± 1.069	5.253 ± 1.472	< 0.00001*
TG	1.631± 0.730	2.204 ± 1.568	< 0.00001*
HDL-c	1.121± 0.311	1.167 ± 0.596	0.2763
LDL-c	2.110 ± 0.795	2.491 ± 1.245	0.0001*
BMI	24.640 ± 0.503	24.369 ± 1.111	NS
AIP	0.135 ± 0.225	0.210 ± 0.274	0.0023*

*P < 0.05, TC: total cholesterol, TG: triglyceride, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, BMI: body mass index, AIP: atherogenic index of plasma. NS : not significant

The study demonstrates that females under 40 years old have more favorable AIP and lipid profiles than males (Table 2).

Table No. 2: Comparison of AIP and Lipid Parameters between Younger Age Females and Males groups

Parameters (mmol/l)	Female n = 200 20-40 year	Male n = 200 20-40 year	P-value
TC	3.687 ± 0.563	4.572 ± 1.363	< 0.00001*
TG	1.363 ± 0.606	2.462 ± 1.454	< 0.00001*
LDL	1.907 ± 0.530	2.372 ± 1.053	0.00001*
HDL	1.150 ± 0.248	1.175 ± 0.667	0.19815
AIP	0.042 ± 0.205	0.195 ± 0.277	< 0.00001*

*P < 0.05, TC: total cholesterol, TG: triglyceride, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, AIP: atherogenic index of plasma.

Concerning females and males above 45 years, the study revealed that females also have a more favorable AIP and lipid profile than males (Table 3).

Table No. 3: Comparison of AIP and Lipid Parameters between Older Age Females and Males groups

Parameters (mmol/l)	Female n = 200 45-65 year	Male n = 200 45-65 year	P-value
TC	4.833± 1.115	4.894± 1.092	0.16432
TG	1.899± 0.746	2.344± 1.742	0.00328*
LDL	2.305± 0.953	2.588± 1.181	0 .041257*
HDL	1.092± 0.361	1.156± 0.298	0.180622
AIP	0.227± 0.207	0.238± 0.232	0.7098

*P < 0.05, TC: total cholesterol, TG: triglyceride, LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, AIP: atherogenic index of plasma.

Additionally, there was an increasing trend in AIP with advancing age, particularly after the age of 45 years, for both females and males; the results were significant for females, but not for males (Tables 4 and 5).

Table No. 4: Comparison of AIP and Lipid Parameters between Younger and Older Age Female Groups

Para-meters (mmol/l)	Female n = 200 20-40 year	Female n = 200 45-65 year	P-value
TC	3.660 ± 0.598	4.833 ± 1.115	< 0.00001*
TG	1.361 ± 0.607	1.899 ± 0.746	< .00001*
LDL	1.910± 0.531	2.305± 0.953	<0 .00001*
HDL	1.150 ± 0.248	1.092 ± 0.361	0.063455
AIP	0.0410± 0.203	0.227± 0.207	<0 .00001*

*P < 0.05, TC: total cholesterol, TG: triglyceride, LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, AIP: atherogenic index of plasma.

Table No. 5: Comparison of AIP and Lipid Parameters between Younger and Older Age Male Groups

Para-meters (mmol/l)	Male n = 200 20-40 year	Male n = 200 45-65 year	P-value
TC	4.666 ± 1.304	4.911 ± 1.089	0.192344
TG	1.982 ± 0.996	2.344 ± 1.742	0.092127
LDL	2.342 ± 1.053	2.582 ± 1.188	0.167189
HDL	1.176 ± 0.627	1.160 ± 0.295	0.838157
AIP	0.206 ± 0.269	0.237 ± 0.231	0.4405

TC: total cholesterol, TG: triglyceride, LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, AIP: atherogenic index of plasma.

DISCUSSION

The AIP is a crucial predictive parameter for atherosclerotic heart risk; it's measured based on the ratio of TG to HDL-c[10]. The study findings exhibit that both sex and AIP show a significant association, with males having higher mean AIP values than females Table 1. This agrees with previous literature indicating that males generally have a more atherogenic lipid

profile due to higher levels of TG[11]. The study shows that women under 40 tend to have a more beneficial lipid profile, with lower AIP, TC, TG, and LDL-c levels compared to men of the same age Table 2. On the other hand, there were no significant differences between the older female and male groups, except for TG and LDL-c Table 3.

The sex difference may be due to several factors. The decrease in estrogen concentration during menopause, hormonal changes, and lipid variance in females increases the risk of atherogenic lipid biomarkers[12]. In terms of age, AIP showed an increasing trend with advancing age, particularly after the age of 40. This relationship was clearer in the female population, particularly between younger and older women Table 4. Between the ages of 45 and 65 years, there was a decline in estrogen levels, which may lead to metabolic disorders, and this might explain the stronger relation between AIP and females' age[13]. However, the variation in the hormone and physiological cycles in females and males during their lives affects the mechanism of dyslipidemia[14]. Among Chinese postmenopausal women, a study revealed that AIP may be considered a strong indicator to reflect the risk of coronary artery disease[15]. This may reflect physiological variation in lipid metabolism and the accumulation of atherosclerotic risk factors with time. On the other hand, among male groups, there were no statistically significant differences, suggesting that other mediating factors may be involved Table 5. This direct association between the lipid levels and both sex and age might influence the levels of these parameters.

Numerous studies have revealed that AIP was related to atherosclerosis and severe cardiovascular stroke[16,17]. Additionally, several studies have referred to the associations between AIP and obesity, metabolic syndrome, and diabetes mellitus[18,19]. However, there is limited research on the association between sex, age, and AIP, with conflicting results. A study demonstrates that the association between AIP and hypertension differs by sex, with females having a significantly weaker relation than in males[20]. This study also revealed a significant association between atherogenic lipid parameters in males. Another study in Taiwan exhibits that the association between AIP and hypertension was stronger in males than in females[21]. The AIP was considered an important biomarker for plasma atherosclerosis and a diagnostic tool to suspect the risk of cardiovascular disease[22]. Moreover, several studies have shown an association between the AIP and the incidence of insulin-resistance-related metabolic diseases, as well as obesity[23,24]. This study demonstrates an association between higher AIP and old age in males and females. In human adipose tissue, TG is considered the most abundant lipid biomarker, which may contribute to the development of insulin resistance[25]. On the other hand, HDL-c is known to have anti-inflammatory properties in metabolic diseases due to its lipid and protein components[26]. AIP involves these two lipid biomarkers, TG and HDL-c, and consequently, it reflects dyslipidemia better than low HDL-c or high TG concentration alone.

These results highlight the crucial role of AIP as a strong biomarker for screening cardiovascular risk, especially in male and older populations. Routine investigations of AIP could enhance early detection and preventive strategies. Further studies should examine sex-specific associations with lipid metabolism, particularly among high-risk populations.

CONCLUSION

The findings of this study indicate that both age and sex are significant factors influencing the AIP. Males and older individuals tend to have higher AIP levels, which may contribute to a greater risk of developing cardiovascular diseases. AIP can be considered a simple, cost-effective tool for early risk assessment, particularly in preventive healthcare settings. Further longitudinal studies are recommended to confirm these associations and to explore additional influencing factors such as diet, physical activity, and overall health status.

Author's Contribution:

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Drafting or Revising Critically:	Rithab Ibrahim Al-Samawi
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Original Article

Frequency of Helicobacter Pylori Infection in Children presented with Abdominal Pain in a Tertiary Care Hospital

Helicobacter Pylori Infection in Children

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ABSTRACT

Objective: This study aimed to determine the frequency of H.pylori infection in children presenting with abdominal pain at tertiary care hospital in Pakistan.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Pediatrics, Abbas Institute of Medical Sciences Muzaffarabad Azad Jammu & Kashmir from January 2025 to June 2025.

Methods: This cross-sectional study was conducted among 317 children aged 5-15years who presented with abdominal pain at the pediatric outpatient department of the hospital. Data were collected using a structured questionnaire addressing demographic characteristics, socioeconomic background and potential risk factors. H.pylori infection was diagnosed using stool antigen test. Data was analyzed using SPSS 23.0 version and associations between risk factors and infection were assessed using chi-square tests, with a *p*-value <0.05 considered significant.

Results: Out of 317 participants 176 (55.5%) were males and 141(44.5%) were females with mean age of 9.8±3.1years. The overall frequency of H-pylori was 84 (26.5%). The infection was most common in the 11-15 year age group. A higher frequency was observed in low socioeconomic status(47.6%), those using tap water sources (32.5%), living in overcrowded conditions (31.6%) and practicing poor hygiene (34.5%) with statistically significant associations (p <0.05). No significant difference was found in genders (p>0.05).

Conclusion: This study concluded that H.pylori infection is highly prevalent among children with abdominal pain and is significantly associated with poor hygiene practices, overcrowding, unsafe drinking water and low socioeconomic status.

Key Words: Helicobacter pylori, Abdominal pain, Risk factor, Children, Stool antigen test.

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INTRODUCTION

Helicobacter pylori (H.pylori)is a gram negative rods bacterium that colonizes the gastric mucosa in the stomach triggering inflammation and mucosal damage. It has been reported as one of the cause of gastritis in children.[1] Although the infection most often arises in children the natural history, clinical presentation and implications of pediatric H-pylori infection differ from adults in important ways.[2]

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Globally the prevalence estimate for H-pylori in children vary depending on region, socioeconomic status, diagnostic methods and study population. The systematic review and large meta-analysis report revealed that the prevalence in pediatrics is approximately 20-35% worldwide but the rates are higher in low and middle income countries. The prevalence in these countries ranges from 50-70% and depending on age cohort and detection method. The most accurate diagnostic test to measure the activeness of infection are stool antigen and urea breathe test while serology tends to overestimate the current infection.[3,4]

The most common presentation of H-pylori infection in children are recurrent abdominal pain and dyspeptic symptoms although there is controversy in the strength and consistency and it remains debatable. Several studies on children from developing countries have reported a large proportion of positive H-pylori infection among children with recurrent or chronic abdominal pain compared to the controls which suggest that H-pylori may contribute to symptom burden in a subset of patients.[5] The public health and clinical significance of infection of H-pylori in children extends

beyond its potential role in causing abdominal pain. A meta-analysis revealed an association between infection of H-pylori and higher likelihood of iron deficiency anemia and randomized controlled trial revealed that eradication of H-pylori may improve iron parameters in some patients.[6,7] The risk factors in these patients reflect complex interactions among environmental, socioeconomic and familial determinants. Lower household income, overcrowding, lack of clean water, poor sanitation and high family density are associated with childhood acquirement. In lower middle income countries early age acquisition is common which results in higher prevalence in school-age children compared to children in high income settings.[4]

Accurate diagnosis of H-pylori requires attention to test performance features and to international guidelines recommendations. Recent studies from gastroenterology societies suggest that noninvasive tests i.e., urea breathe test and stool antigen testing, that detect active infection are preferred for diagnosing active infection in children with relevant indications. However, endoscopic biopsies should be reserved for those infections with alarming features or when endoscopy is clinically indicated. Furthermore, the increase prevalence of antibiotic resistance, the most recent guidelines advise that antimicrobial susceptibility testing (cultural or molecular methods applied to biopsy specimens) should guide eradication therapy whenever feasible. The above recommendations highlight the diagnostic complexities and need for local laboratory capacity when planning clinical management pathways for these children.[6,8]

The therapeutic strategies for pediatric H-pylori have evolved in response to resistance patterns and evidence regarding efficacy. Standard triple therapy regimens (amoxicillin, clarithromycin and a proton pump inhibitor) are the backbone for pediatric H-pylori eradication but clarithromycin resistance in many regions has decreases the empirical cure rates. Therefore, the therapeutic protocol for pediatric H-pylori depends on emphasizing on local resistance data or susceptibility tests. In addition, the decision to test and treat children is nuanced: blanket "test=and-treat" strategies for abdominal pain are not universally endorsed in children. Thus prevalence data from symptomatic pediatric populations are essential to guide rational testing policies at tertiary care centers.[9,10]

Several studies have assessed H-pylori association with abdominal pain in children. A study from Egypt where 100 children with recurrent abdominal pain were evaluated and did not found a strong relation of H-pylori with recurrent abdominal pain even though infection was present, after excluding other possible etiologies (parasite, helminthes etc.).[11] This suggest heterogeneity in presentations that H-pylori may not universally explain abdominal pain in children. Various local studies in Pakistan have revealed a higher frequency of H-pylori infection among children with abdominal pain. A cross-sectional study conducted in Multan on 148 children with age range from 4-12 years, 52.7% having recurrent abdominal pain were tested positive for infection of H-pylori through stool antigen test.[5] In another study at Nishtar Hospital, Multan among 185 children with recurrent abdominal pain (duration> 3months), 55.7% were having infection of H-pylori.[12] Another study using stool antigen test among 100 children aged 2-12 years with recurrent abdominal pain,38% children have H-pylori infection.[13] The frequency in Peshawar appears lower. In this study the detected prevalence was 24.9% in a total of 177 children presented with recurrent abdominal pain.[14]

These inconsistencies largely due to differences in diagnostic methods, study design and population characteristics. These differences highlight a major research gap in determining the true burden of active H-pylori infection among symptomatic pediatric patients. Moreover, limited data exist on demographic and environmental risk factors in the local context. Therefore, this study justified to generate evidence based, region specific data on the frequency of H-pylori infection among children presenting with abdominal pain in tertiary care hospital in Pakistan. The findings of this study will address current knowledge gaps, standardize local diagnostic approaches and contribute to improved clinical management and prevention strategies in pediatric gastroenterology.

The objective of this study was to determine the frequency of H-pylori infection among children presenting with abdominal pain in a tertiary care hospital.

METHODS

A descriptive cross-sectional study was carried out in the Department of Pediatrics, Abbas Institute of Medical Sciences Muzaffarabad Azad Jammu & Kashmir from January 2025 to June 2025.

Sample Size Calculation: A total of 317 sample size were selected (adding 10% to account for incomplete data or potential non-response). Using the World Health Organization (WHO) formula for a single population proportion the sample size was calculated;

$n = (Z^2 \times p(1 - p)) / d^2$

Inclusion Criteria

1. Children aged 5–15 years of either gender.
2. Patients presenting with abdominal pain of more than two weeks' duration.
3. Informed consent provided by children's parents/guardians.

Exclusion Criteria

1. Those who had received antibiotic therapy, proton pump inhibitors (PPIs), or bismuth compounds within the last four weeks.

2. Patients with known chronic gastrointestinal diseases such as celiac disease, inflammatory bowel disease, or chronic liver disease.
3. Children with systemic illnesses or immunocompromised states.

Data Collection Procedure: Ethical approval was obtained from the Institutional Review Board (IRB) and informed consent from parents/guardians was taken, data were collected using a structured questionnaire and clinical data form.

Each child underwent a detailed clinical examination, and relevant laboratory investigations were performed to confirm H. pylori infection. Children who tested positive for H. pylori infection were referred for appropriate medical treatment as per institutional protocols.

Diagnostic Method: H. pylori infection was diagnosed using the stool antigen test (HpSA), which is a non-invasive, reliable, and sensitive method suitable for pediatric populations. Stool samples were collected in sterile containers and tested using a commercially available enzyme immunoassay (EIA) kit.

Operational Definitions

- **Abdominal Pain:** Persistent or recurrent discomfort localized in the abdominal region lasting for two weeks or more, reported by the child or guardian.
- **Helicobacter pylori Infection:** Presence of H. pylori antigen detected in the stool sample using the enzyme immunoassay test, indicating current infection.
- **Socioeconomic Status:** Categorized based on monthly household income (low < PKR 50,000; middle 50,000–150,000; high > 150,000).
- **Overcrowding:** Defined as ≥3 persons per bedroom within the household.
- **Unsafe Water Source:** Consumption of tap or well water without boiling or filtration.
- **Poor Hygiene Practices:** Infrequent handwashing before meals or after defecation, and sharing of utensils among family members.
- **NSAID Use:** Regularly use within the last month.
- **Parental Education Level:** The highest educational qualification of either parent categorized as uneducated, primary, secondary, or higher.

All data was analyzed using SPSS version 23.0. Frequencies and percentages were used for categorical variables. Continuous variables were expressed as mean ± standard deviation (SD). The frequency of H. pylori infection was determined as the percentage of positive stool antigen tests among the total study population. Chi-square test was used for associations between H. pylori infection and risk factors. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 317 children aged 5-15 years presenting with abdominal pain were included in this study. Among them, 176 (55.5%) were males and 141(44.5%) were females resulting male to female ratio of approximately 1.2:1 as shown in figure 1. The mean age of participants was 9.8±3.1years. The majority of children 194(61.2%) were belonged to 5-10years age group, while 123 (38.8%) were aged between 11-15 years. In terms of socioeconomic status, 151(47.6%) of children were from low-income families, 120(37.9%) from middle-income families and 46 (14.5%) from high-income families.

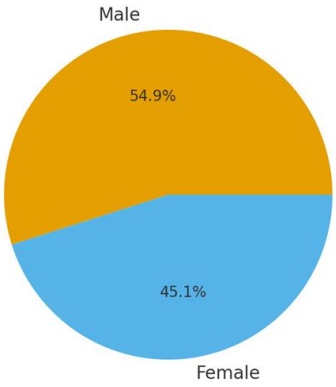

Figure No. 1: Gender Distribution.

Table No. 1: H-pylori infection by age and gender.

Variable	Category	Table(n)	Positive(n)	Percentage (%)	p-value
Gender	Male	176	49	27.8	0.48
	Female	141	35	24.8	
Age Group (years)	5-10	194	48	24.7	0.31
	11-15	123	36	29.2	

Table No. 2: Association of H-pylori infection with Risk Factors.

Risk Factors	Category	Total (n)	H-pylori positive (n)	% Positive	p-value
Socioeconimc Status	Low	151	50	33.1	**0.01**
	Middle	120	26	21.7	
	High	46	8	15.2	
Water Source	Tap	169	56	32.5	**0.004**
	Filtered	78	12	15.4	
	Bottled	33	5	12.1	
	well	37	11	29.7	

Overcrowding	Present	190	60	31.6	**0.02**
	Absent	127	24	18.9	
Poor Hygiene	Yes	174	60	34.5	**0.01**
	No	143	24	16.8	
Parental Education (Low)	Yes	143	47	32.9	**0.03**
	No	174	37	21.3	
NSAID Use	Yes	61	20	23.8	0.07
	No	256	64	15.0	
Family History of Gastritis/Ulcer	Yes	72	23	31.9	0.09
	No	245	61	24.9	

Out of total 317 participants, 84 (26.5%) were found to be positive for H-pylori infection based on stool antigen testing. The remaining 233(73.5%) tested negative. The infections were more prevalent amongst males 27.8% compared to female 24.8% though this difference was not statistically significant (p=0.48). Infection rates were slightly higher among older children (29.2% in 11-15 years) than younger (24.7% in 5-10 years) but the difference was also not significant (p=0.31) as shown in table 1.

A significant high prevalence of H-pylori infection was observed among children belonging to low socioeconomic status (33.1%) compared to middle (21.7%) and high (15.2%) income groups (p=0.01). Children consuming tap water 32.5% and well water 29.7% showed a significant higher infection rates than those using filtered 15.4% or bottled water 12.1% (p=0.004).

Similarly infection was more common among children from overcrowded households 31.6%, those with poor hygiene practices 34.5 % and parents with low education 32.9% compared their respective counterparts (p< 0.05). Use of NSAIDs and family history of gastritis or peptic ulcer disease were also associated with higher infection rates but the association was not significant (p=0.07 and 0.09 respectively). Table 2 summarize the distribution of H-pylori infection with respect to potential risk.

DISCUSSION

The present study aimed to determine the frequency of H-pylori infection in children presenting with abdominal pain in a tertiary care hospital in Pakistan. The findings revealed that a considerable proportion of pediatric patients had evidence of H-pylori infection, aligning with global and regional data that highlight the bacterium's high prevalence in developing countries. The observed infection rate of 26.5% corresponds closely with studies conducted in other South Asian populations, emphasizing similar environmental and socioeconomic factors influencing disease burden.

Comparing our findings with regional studies, Punhal et al[15] in Quetta reported a 32.5% prevalence among symptomatic children, Mahmud et al[13] revealed 38% in Rawalpindi, Afridi et al[14] observed 24.9% in Peshawar while Memon et al[16] documented 31% in Karachi. These findings are consistent with our results, suggesting a similar epidemiological pattern across various provinces of Pakistan. However, in a study by Khurshid et al[12] reported 55.7% cases, Ali Muhammadi et al[17] observed 58% cases of H-pylori in children with recurrent abdominal pain, Nadeem et al[18] reported 62% infection in children with H-pylori while Zeyrek et al[20] noted 49% from Turkey. These variations in literature could be due to the diagnostic tools, differences in sanitation and hygiene practices.

The current study revealed 55.5% males and 44.5% females. A Turkish study[19] reported 53% of male dominancy with recurrent abdominal pain. A study by Mahmud et al[14] 58% male gender while Khurshid et al[12] also revealed 54.6% males compared to females 45.6%. These studies are in consistent with this study. Alimohammadi et al[12] documented higher female predominance than male which opposes this study. The reason behind this trend remains unclear but may be related to behavioral or environmental exposure differences between genders.

The mean age presentation in this study was 9.8±3.1years. The mean age by Afridi et al[14] from Peshawar revealed 11.29 ± 2.74 years, khurshid et al[12] 7.57±1.93 years and Zeyrek[19] from Turkey found the mean age of 9 years. These studies are in comparable with this study. Therefore, early identification and treatment of infected children can have long-term benefits in reducing gastrointestinal morbidity in adulthood.

The current study also evaluated potential risk factors associated with H-pylori infection. A significant association was observed between H-pylori positivity and Low socioeconomic status, unsafe drinking water, overcrowded living conditions, poor hygiene and parental education. These findings align with previous literature suggesting that H-pylori transmission is predominantly fecal-oral or oral-oral, and thus closely related to hygiene and socioeconomic factors.[20] Sardar et al[21] highlighted that inadequate sanitation and contaminated drinking water were the strongest predictors of infection among children.

The strength of this is its focus on children presenting with abdominal pain in a tertiary care hospital, providing reliable local data on H-pylori infection in

Pakistan. It used laboratory-based confirmation of infection, minimizing diagnostic errors and included the key risk factors such as socioeconomic status, hygiene and water source for comprehensive analysis. The use of standardized methodology and an adequately calculated sample size enhanced the study's validity and allowed meaningful comparison with other regional and international studies.

The implications of these findings are significant for clinical practice and public health. Given that H-pylori infection is often asymptomatic but can contribute to long-term gastrointestinal disorders, routine screening of children with recurrent or unexplained abdominal pain may be warranted in endemic areas. Moreover, preventive strategies focusing on sanitation improvement, safe water supply, and public education are essential to curb transmission. Policymakers and healthcare providers must collaborate to ensure early detection, effective treatment, and preventive health education.

Limitations of the Study: The limitations of this study include its cross-sectional design, which limits causal inference, and the fact that it was conducted at a single tertiary care center, potentially affecting generalizability. Additionally, some risk factors were self-reported, which might introduce recall bias. Future studies involving larger, multicenter cohorts and incorporating molecular diagnostic methods could provide more comprehensive insights into transmission dynamics and bacterial virulence patterns in Pakistani children.

CONCLUSION

The findings of this study concluded that a significant high frequency of H-pylori among children presenting with recurrent abdominal pain. The infection was strongly associated with low socioeconomic status, poor hygiene practices, unsafe drinking water and overcrowded living conditions.

Author's Contribution:

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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Platelet-Albumin-Bilirubin (PALBI) Score to Predict Outcomes of Acute Variceal Bleed in Patients with Cirrhosis

Muhammad Mumtaz[1], Tanveer Hussain[2], Sadia Ahmed[3], Misbah Noureen[4], Anum Abbas[5] and Muhammad Umar[6]

ABSTRACT

Objective: The objective of this study was to determine whether the Platelet-Albumin-Bilirubin (PALBI) score outperformed the traditional Child-Pugh classification in predicting outcomes, such as deaths in hospitals and subsequent bleeding, in cirrhotic patients presenting with an acute variceal bleed.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the Gastroenterology Department, Holy Family Hospital, Rawalpindi from 1 July to 31 December 2021.

Methods: 68 cirrhosis patients who were admitted due to acute variceal bleeding were among them. Serum albumin, bilirubin, and platelet count were used to compute the PALBI score, and the Child-Pugh classification was also evaluated. The two main outcomes were re bleeding within four weeks and in-hospital death. Each scoring system's predictive accuracy was calculated using the Area Under the Receiver Operating Characteristic Curve (AUC).

Results: A total of 68 patients were enrolled in the study, with mean age 54.32 years. 63.2% (43) were male and 36.8% (25) were female. According to Child–Pugh classification, 5.9% were Class A, 27.9% Class B, and 66.2% Class C. while 14.7% were Grade 1, 38.2% Grade 2, and 47.1% Grade 3 PALBI score. Overall, in-hospital mortality was 16.17%, occurring only in Child–Pugh Classes B (5.26%) and C (22.22%), and in PALBI Grades 2 (7.69%) and 3 (28.13%). No deaths were observed in Child–Pugh A or PALBI Grade 1. Rebleeding occurred in 5.26% of Class B and 57.78% of Class C patients, and in 15.38% of PALBI Grade 2 and 71.88% of Grade 3 patients; no rebleeding occurred in Class A or PALBI Grade 1. PALBI grade showed a strong association with both mortality and rebleeding ($p < 0.001$). For predicting rebleeding, PALBI demonstrated high sensitivity (85.19%) and specificity (100%), with an excellent AUC of 0.926. Although not statistically superior to Child–Pugh (AUC difference 0.0944; $p = 0.0722$), PALBI showed better overall performance.

Conclusion: When predicting rebleeding and early mortality in individuals with acute variceal hemorrhage, the PALBI score is a trustworthy method.

Key Words: PALBI score, CTP, Variceal bleed, Mortality

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INTRODUCTION

Liver cirrhosis is a chronic, progressive disease characterized by extensive hepatic fibrosis and disruption of normal liver architecture, leading in the creation of regenerating nodules.

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The liver gradually loses its vital processes, such as protein synthesis, bile generation, and detoxification, as the illness progresses[1]. One of the most dangerous cirrhosis consequences is portal hypertension, which is characterized by increased resistance to blood flow via the fibrotic liver[2]. Elevated portal pressure can trigger the formation of varices, especially in the esophagus, which may rupture and produce acute variceal hemorrhage[3].

Acute variceal bleeding is a severe complication in cirrhotic individuals, representing a major source of morbidity and mortality in those with decompensated liver disease[4,5].

Cirrhotic patients presenting with variceal bleeding face not only the risk of an initial life-threatening bleed but also a significant risk of early rebleeding[6]. Rebleeding is a well-recognized and dangerous event that markedly increases mortality in this population[7]. Effective management of acute variceal bleeding therefore depends on early identification of patients at highest

risk for poor outcomes such as mortality and rebleeding.

Prognostic scoring systems have become essential tools in clinical practice, helping clinicians assess disease severity and predict patient outcomes[8]. These tools facilitate more personalized care and more efficient use of healthcare resources, especially in critical care settings[9].

The most extensively used scoring system for measuring liver function and prognosis in cirrhotic patients is the Child-Turcotte-Pugh (CTP) score[10]. Clinical factors like ascites and hepatic encephalopathy are combined with laboratory measurements including blood bilirubin, albumin levels, and prothrombin time in this approach[11]. Although the CTP score has shown promise in the treatment of cirrhosis, it has significant drawbacks. Ascites and encephalopathy are subjectively graded, and several crucial physiological indicators, including platelet count, are excluded[12].

The Model for End-Stage Liver Disease (MELD) score, which is based on laboratory data and has been demonstrated to predict survival in recipients of liver transplants, is another popular tool[13]. Nevertheless, MELD does not adequately represent the complexity of cirrhosis and its sequelae, much as the CTP score[14].

METHODS

This study included 68 patients with confirmed liver cirrhosis who presented with acute variceal bleeding—defined by the presence of hematemesis (vomiting of blood) or melena (black, tarry stools)—within 24 hours of hospital admission. Inclusion criteria required that patients have documented cirrhosis and evidence of variceal hemorrhage. Patients with non-variceal sources of gastrointestinal bleeding were excluded from the study. All participants provided informed consent. Data collection involved detailed assessment of demographic characteristics, medical history, and laboratory results. Key variables recorded included age, gender, platelet count, serum albumin, bilirubin levels, prothrombin time, and Child-Turcotte-Pugh (CTP) score. Both PALBI and CTP scores were calculated for each patient.

RESULTS

Demographics Analysis

This research study analyzed 68 patients of an acute variceal bleed, which presented valuable data as illustrated below. Table 1 shows the descriptive statistics that provide a detailed glimpse of the study sample characteristics.

Descriptive Analysis: Hematological parameters observed during the post rebleeding event depict significant findings concerning the recovery of the patients after acute variceal bleeding. The average hemoglobin (Hb) level on rebleeding was 6.27 g/dL (+/- 1.37) that is considerably lower than the normal hemoglobin range (12-16 g/dL in women and 13-17 g/dL in men).

Table No. 1: Summary of Demographics

Characteristics	N	Mean	Std. Deviatio	Minimum	Maximum
Age	68	54.32	10.34	26.00	78.00
Albumin(g/L)	68	29.37	4.25	18.00	36.00
APTT	68	37.38	3.04	27.66	48.00
Bilirubin(micromole/L)	68	26.25	17.79	5.47	90.97
Creatinine	68	1.69	0.72	0.50	4.10
Hb(g/dL)	68	7.22	1.95	3.30	13.80
HCT	68	22.45	5.94	10.70	43.20
INR	68	1.52	0.49	0.81	2.50
K	68	4.07	0.57	3.00	7.10
Na	68	134.84	5.55	122.00	145.00
CTP Score	68	9.10	1.54	6.00	12.00
PALBI Score	68	-2.06	0.42	-2.96	-1.09
Urea	68	40.75	23.87	11.00	144.00
WBC	68	6.19	2.00	2.60	14.60
PLT	68	113.74	32.13	42.00	193.00
Duration of admission	68	3.04	0.21	3.00	4.00

Table No. 2: Descriptive Statistics

Hematological Parameters	N	Mean	Std. Deviation	Maximum	Minimum
Hb (g/dL) after rebleed	39	6.27	1.37	9.2	3.3
HCT (%) level after rebleed	39	19.44	3.93	30.2	11.7

This is an indication that a lot of patients were still suffering from anemia as a result of losing blood during the variceal bleeding. The noted hemoglobin level range of 3.3 to 9.2 g/dL is quite variable and there were patients with severe anemia and those with moderately low levels. This observation is in line with the clinical expectations, because patients presenting with variceal bleeding can lose significant amount of blood, which results in reduction of hemoglobin levels. The standard deviation value of 1.37 indicates a moderate level of variation or difference in the severity of anemia amongst the patients.

Analysis of Mortality and Rebleeding Incidents According to Child- Pugh Classes: There is evident connection between the classifications of Child-Pugh and mortality and rebleeding outcomes of the patients with cirrhosis of liver who have presented with an acute bleeding of variceal disease. Child-Pugh scoring system groups patients with an elevated degree of liver disease in three classes (A, B and C), in the order of severity. No cases of either in-hospital mortality or rebleeding were observed in a Class A (n=4), which indicates an excellent prognosis in patients with the mildest form of liver dysfunction. The risks were moderate in Class B (n=19) patients, with the rate of mortality and rebleeding equal to 5.26%. This implies a relatively high risk that is nevertheless quite noticeable in comparison with the extreme category. The prognosis of patients with Class C (n=45) was much worse: 22.22% of the patients passed away in the hospital, and 57.78% had rebleeding. These findings are found in close correlation of the worst clinical outcomes with the highest Child-Pugh class.

Table No. 3: Analysis of Mortality and Rebleeding Incidents According to Child- Pugh Classes

Child Class		In hospital mortality				Rebleed			
		Yes		No		Yes		No	
	Total N	N	N %	N	N %	N	N %	N	N %
A	4	0	0.00%	4	100%	0	0.00%	4	100%
B	19	1	5.26%	18	94.74%	1	5.26%	17	89.47%
C	45	10	22.22%	35	77.78%	26	57.78%	9	20.00%
Chi square	Constant					0.00			

Table No.4: Patient Outcomes by PALBI Grade Regarding In-Hospital Mortality and Rebleeding Incidents

PALBI Grade		In hospital mortality				Rebleed			
		Yes		No		Yes		No	
	Total N	N	N %	N	N %	N	N %	N	N %
"Grade 1"	10	0	0.00%	10	100%	0	0.00%	10	100%
Grade 2	26	2	7.69%	24	92.31%	4	15.38%	20	76.92%
Grade 3	32	9	28.12%	23	71.88%	23	71.88%	0	0.00%
Chi square	Constant					0.00			

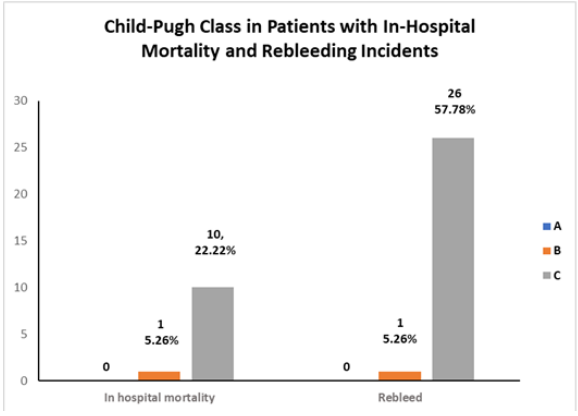

Figure No.1: Child-Pugh Class in patients with in-hospital mortality and rebleeding incidents

The chi-square testing of the statistical analysis proved this distribution to be very important (p = 0.000) and this strengthens that the severity of a child is directly correlated to the likelihood of development of complications. This confirms the fact that the Child-Pugh classification can be used as a prognostic tool when patients bleed varices. Finally, Table No. 3 has stressed on the value of risk stratification early during Child-Pugh classes. The good results of Class A patients, moderate risks of Class B and high risks of mortality and rebleeding of Class C patients all remain the same as before. These outcomes have demonstrated the importance of increasing the monitoring and, perhaps, aggressive treatment plans among the patients of Class C.

Patient Outcomes by PALBI Grade Regarding In-Hospital Mortality and Rebleeding Incidents: The statistics support the obvious relation between PALBI grade and the patient outcomes pertaining to in-hospital mortality and the rebleeding cases. Both the mortality and rebleeding rates were 0 percent in PALBI Grade 1. On the other hand; PALBI Grade 2 patients experienced 7.69 percent mortality rate with 15.38 percent experiencing rebleeding due to considerably lower values of 28.12 percent and 71.88 percent experiencing

mortality and rebleeding respectively in PALBI Grade 3 patients. The result of a statistical test reveals that there is a great degree of significance between adverse outcomes and PALBI grade as indicated by the Chi-square outcome. Although in the analysis the result of the Chi-square is recorded as Constant 0.00, it is most likely that it is a wrong coding of the output and the accurate outcome is lower than 0.001. What makes the point is that this low p-value gives credence to the fact that the differences in mortality and rebleeding as seen in the PALBI grades are not likely to arise out of chance. So, the connection between higher PALBI grades and poor clinical outcomes is strongly evident and in that regard, the prognostic value of PALBI grading supports its application in clinical practice

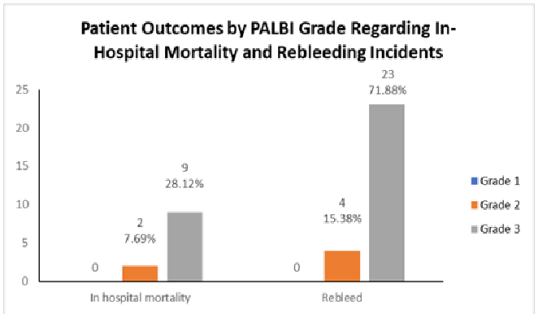

Figure No.2: Patient outcomes by ALBI grade regarding in-hospital mortality and rebleeding incidents.

DISCUSSION

The Platelet-Albumin-Bilirubin (PALBI) score is an objective laboratory-based measure for assessing liver function and portal hypertension severity in cirrhosis patients. Unlike older scoring systems like the Child-Pugh classification, the PALBI score is based purely on routine, quantifiable parameters platelet count, serum albumin, and bilirubin levels which eliminates subjective clinical characteristics. By using platelet count as a proxy marker of portal hypertension, the PALBI score provides a more nuanced and reliable assessment of liver failure, making it a viable risk-stratifying tool in patients with cirrhosis and acute variceal bleeding.

The purpose of the research was to compare the efficacy using the widely utilized Child-Pugh classification as a PALBI score to anticipate clinical consequences, which includes deaths in the hospital and bleed again, with cirrhosis and acute variceal bleeding. Our results show that the severity of liver dysfunction, as evaluated by both the Child-Pugh and PALBI scores, is highly related with the probability of unfavorable outcomes in patients experiencing re bleeding[15].

The Child-Pugh score has long been considered a cornerstone for assessing prognosis in cirrhotic patients and continues to hold clinical value[16]. In our study, patients classified as Child-Pugh Class C showed markedly higher in-hospital mortality (22.22%) and rebleeding rates (57.78%)[17]. In contrast, there were no mortality or rebleeding events among Class A patients, with intermediate outcomes observed in Class B[18]. These results are consistent with existing literature showing that patients with decompensated cirrhosis (Class C) are at significantly increased risk of complications such as bleeding and death[19]. The findings reaffirm the role of the Child-Pugh classification as a validated risk stratification tool in cirrhosis, particularly in the context of variceal hemorrhage.

An advantage of the PALBI score lies in its simplicity and objectivity, combining platelet count, serum albumin, and bilirubin levels. In our study, the PALBI score demonstrated superior predictive accuracy compared to the Child-Pugh score, with an AUC of 0.926 versus 0.831. This suggests the PALBI score may be more effective in predicting mortality and rebleeding, given its reliance on objective and easily obtainable laboratory parameters[20]. These results align with previous studies highlighting the PALBI score's utility in predicting survival and complications among patients with cirrhosis and hepatocellular carcinoma.

It is noteworthy, therefore, that the difference in prediction performance between the two scores was not statistically significant ($p = 0.0722$). This implies that even though the PALBI score had a greater AUC, the study did not conclusively show the difference[20,21]. One possible explanation for this finding is the small sample size, which may have hampered the capacity to detect a statistically significant difference. To ascertain whether the PALBI score actually performs better than the Child-Pugh classification in predicting mortality and rebleeding among cirrhotic patients, larger, prospective trials are necessary[22]. The PALBI score offers an objective, reproducible, and practical approach for assessing liver function in cirrhotic patients with acute variceal bleeding. Unlike the Child-Pugh score, which includes subjective clinical variables such as ascites and encephalopathy, PALBI relies solely on routine laboratory tests, making it more standardized and consistent. Moreover, by incorporating platelet count, the score better captures aspects of portal hypertension, offering a more nuanced evaluation of liver dysfunction than either the Child-Pugh or MELD scores. Its simplicity, low cost, and clinical relevance position it as a promising tool for risk stratification in this patient population. Nonetheless, robust validation through larger, multicenter, prospective studies is essential to confirm its clinical utility and to explore potential integration with other biomarkers or imaging modalities for even better prognostic accuracy.

CONCLUSION

PALBI score offers superior predictive ability compared to the traditional Child-Pugh classification and provides a more objective, reproducible measure of liver function. Its dependence on routine, easily obtainable laboratory parameters and its high predictive accuracy make it a practical option for clinical use, potentially supporting improved risk stratification and more personalized treatment strategies.

Suggestions: Nevertheless, while the PALBI score shows considerable promise, our results also highlight the need for further validation through larger,

multicenter, prospective studies. Such research would help confirm its comparative advantage over existing prognostic models, evaluate its integration with other diagnostic tools, and establish its role in routine clinical practice. Ultimately, the PALBI score may become an important component of cirrhosis management, helping clinicians identify high-risk patients who would benefit from timely and intensive interventions, thereby improving outcomes and cost-effectiveness in the treatment of acute variceal bleeding.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muhammad Mumtaz, Tanveer Hussain, Sadia Ahmed
Drafting or Revising Critically:	Misbah Noureen, Anum Abbas, Muhammad Umar
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Nurse-Led Preventive Interventions Regarding Hepatocellular Carcinoma Knowledge, Complications and Quality of Life among Chronic Liver Disease Patients

Nimra Saleem, Azeem Kaleem and Madiha Mukhtar

ABSTRACT

Objective: To evaluate the effectiveness of a nurse-led preventive intervention on refining quality of life, disease-related knowledge, and complication lessening among patients with prolonged liver illness associated with hepatitis B, C, and Non-alcoholic fatty liver disease.

Study Design: Quasi-experimental pre–post study

Place and Duration of Study: This study was conducted at the Allama Iqbal Teaching Hospital, Dera Ghazi Khan from 1st July 2025 to 30th September 2025.

Methods: The link between the nurse led preventive education and health improvement was assessed among patients. A total of 24 chronic liver disease patients aged 28–55 years were registered. The data were collected using the Prolonged Liver Illness Questionnaire, the SF-36 Quality of Life Scale, and the HCC Knowledge Assessment Scale.

Results: Males are more with chronic liver disease than females which indicates that they are more exposed to the disease, mostly married**.** 83.3% improvement was seen among patients' symptoms due to following the preventive intervention. Mean difference has been reduced from 14.72 to 3.60. The systemic domain showed the highest mean difference (3.35). Fifteen (62.5%) have a good knowledge level, 8(33.3%) participants have a fair knowledge level, and only 1(4.2%) has a poor knowledge level. A Pearson correlation analysis was performed to check the association between the SF-36 Items. Mean and CLDQ 6 items Mean in which All SF-36 subscales correlated significantly with all scores of CLDQ subscales ($p< 0.05$).

Conclusion: Nurse-led preventive education has a significant influence on patients' chronic health conditions. Organized patient education is a key policy within hepatology facilities to improve long-term predictive markers and avoid the onset of hepatocellular carcinoma.

Key Words: Chronic liver disease, Hepatocellular carcinoma, Nurse-led education, Quality of life

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INTRODUCTION

Chronic liver disease (CLD) and its related problems, including irreversible scarring of the liver and hepatoma, are leading to increased mortality, morbidity, and financial burdens. Hepatitis ranks among the top causes of death, positioned 7th globally[1], and is an increasingly pressing health alarm in Asian nations. Chronic active hepatitis, in terms of the global incidence, has categorized Pakistan as the second.[2]

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There are about 10 million people in the country who are chronically infected with hepatitis.[3] Hepatitis viral hepatitis is one of the major causes of high mortality and morbidity in developing nations.[4]

A study by the Pakistan Medical and Research Council in 2007-2008 found that 13 million Pakistanis are affected by HBV and HCV, with 2.5% being HBV carriers and 4.8% HCV carriers.[5] It is well-known that most viral hepatitis carriers remain unaware of their infection due to the disease's asymptomatic nature, which later contributes to a significant increase in disease burden.[6] In a report, 45% to 85% of virus-related hepatitis cases go unknown despite the availability of diagnostic laboratories. The focal reasons cited for this are money, painful testing environments, and the inconvenient locations of these test centers.[7]

Ranked as the seventh maximum prevalent malignancy and the additional leading reason of cancer-related deaths globally, hepatocellular carcinoma (HCC) is a significant health concern.[8] Histologically, HCC represents of all liver cancer cases are 75%. Worldwide,

hepatitis B virus (HBV) and hepatitis C virus (HCV) are responsible for 80% of HCC cases. In the Far East and Black Africa, 85% of cases occurring with HCC predominantly affect individuals in low- and middle-income countries.[9] The highest frequency rate is in Asia, with China accounting for 47% of the global load. The highest expected prevalence rate is in Mongolia, while the lowest is in Nepal and Morocco, according to GLOBOCAN's 2018 global survey.[10]

A reduction in HBV incidence has been observed in Asian countries (11). In the United States, the increase in numbers is associated with a new rise in HCV dominance, exacerbated by widespread narcotic use.[12]

However, screening rates are suboptimal, ranging from 11% to 64%, due to obstructions at the individual, preventive measures, and healthcare system.[13] The principal liver cancers are Malignant hepatoma and Intrahepatic bile duct carcinoma, with fewer types including liver cancer with soft tissue liver tumor.[14] Approximately 90% of these cases are hepatocellular carcinoma (HCC), the main public form of liver melanoma. A study on HCC death movements from 1990 to 2014 exposed that as in a study[15] "the Nordic countries" experienced an proliferation in HCC deaths, whereas Southern Europe saw a decline. However, even after consistent HCV clearance, cirrhosis patients are still considered at high risk for HCC development.[16]

While significant efforts are undoubtedly necessary to develop initial revealing and treatment of HCC, primary prevention plans designed at decreasing the prevalence of Type 2 diabetes, obesity, and limiting mycotoxin growth are equally critical.[8] In developed countries, chronic liver diseases are predominantly caused by alcoholic liver disease, steatotic liver disease (SLD), iron-overloaded, and lasting viral hepatitis, including hepatitis B and C.[17] The combination of factors such as a lack of disease awareness, insufficient medical knowledge, frequent illness episodes, and ongoing consent to therapeutic health education has become a modern method for medical professionals to diagnose patients' conditions and assess their health and treatment effectiveness.[18] Authorized by this knowledge, they can well cross the complications of healthcare, work in collaboration with their treatment team, and develop their probabilities of survival. Policies to progress education, increase vaccine accessibility, and support public arrangements should be focused on rural and lower-socioeconomic areas, where the needs are most noteworthy. The state of knowledge, complications, and quality of life for adults in Dera Ghazi Khan with prolonged liver illness caused by hepatitis B, C and to inform the creation of directed public health interventions.[19]

METHODS

This quasi-experimental study was conducted in the Allama Iqbal Teaching Hospital, Dera Ghazi Khan from 1st July 2025 to 30th September 2025 vide letter No. 70144891 dated June 30, 2025. The link between nurse-led preventive education and health improvements among patients with chronic liver disease and all its outcomes was observed. A total of 24 patients diagnosed with hepatitis B, C, and non-alcoholic fatty liver disease and age 28-55 years were included. Both male and female individuals were involved and patients who are diagnosed with chronic liver disease for <3 months and primary source of CLD is hepatitis B, hepatitis C were included. Participants with end-stage liver disease, individuals undergoing treatment for HCC or those who have received treatment within the past six months and cognitive impairments or mental health conditions that affect their ability to understand or engage with the educational content were excluded.

An overall of four tools consisted of the socio-demographic, which includes age, sex, education, level of education, family members, and marital status, and the HCC knowledge calculation scale that measures the level of knowledge regarding the hepatocellular carcinoma, Questionnaire Items (SF-36) measures the quality of life with 8 domains separately, and the Chronic Liver Disease Questionnaire used for the symptoms were used for data collection. Qualified participants were advanced during their routine hospital visits. Before the learning intervention, they accomplished a structured pre-intervention questionnaire that involved informed consent, demographic information (age, gender, marital status, education level, language, and family size), and baseline assessment tools. The data was entered and analyzed through SPSS-23. A combined sample T-test was practical to compare the marks of pre- and post-data of knowledge related to HCC, CLDQ, and of 8 domains of excellence of life, i.e. general health, psychological health and social relations. P-value ≤ 0.05 was statistically significant**.**

RESULTS

There were males with age 36-45 years are more affected by chronic liver disease (Tables 1-2). The Shapiro-Wilk normality test was used, in chronic liver disease of the pre- and post-intervention group was found to be statistically significant, $p<0.05$ (Tables 3)

Table No. 1: Demographic characteristics of chronic liver disease patients (N=24)

Variable	No.	%
Age (years)		
28-35	6	25.0
36-45	10	41.7
46-55	8	33.3
Gender		
Male	18	75.0
Female	6	25.0

Marital status		
Married	19	79.2
Unmarried	3	12.5
Education		
Primary	2	8.3
Matric	3	12.5
Intermediate	7	29.2
Children		
3	8	25.0
5-6	18	75.0

The majority of the patients 21(87.5%), have a Poor knowledge level, 1(4.2%) participants have a fair knowledge level, and 2(8.3%) participants have good knowledge in the pre-intervention group. After the intervention, participants' knowledge levels improved, with 15 (62.5%) having a good knowledge level, 8 (33.3%) having a Fair knowledge level, and only 1 (4.2%) having a poor knowledge level (Table 4). The Shapiro-Wilk normality test was used, hepatocellular carcinoma of pre-intervention was found to be statistically significant, $p<0.05$ (Table 5).

The percentage of nurses led preventive classified as Low severity level dropped significantly from 87.5% to 4.2% with an average increase of +14.72 points in low severity. However, the Moderate severity level of participants was from 8.3-12.5% and increased significantly by +29.33.However, the percentage of patients with high severity rose from 4.2-83.3%. The low gain of +3.60 points recorded by the high severity participants indicates that the patients have a moderate severity level (Table 6).

A Pearson correlation analysis were performed to check the association between SF 36 Items Mean and CLDQ 6 items Mean in which All SF-36 subscales connected meaningfully with all marks of CLDQ subscales ($p< 0.05$) (Table 7).

Table No. 2: Mean of different domains of the SF-36 Item Health Survey pre- and post (N=24)

Scale	Pre-intervention	Post-intervention	Difference
General health	25.17±6.44	78.12±6.17	52.95
Physical functioning	38.54±6.16	77.08±7.64	38.54
Role Functioning/Physical	11.45±16.45	84.37±16.17	72.92
Role Functioning Emotional	11.11±18.82	83.33±19.65	72.22
Social functioning	30.20±11.00	77.08±12.03	46.88
Pain	25.83±14.42	65.00±18.17	39.17
Energy/fatigue	44.37±10.96	66.04±9.88	21.67
Emotional well being	38.50±9.49	75.16±7.91	36.66

Table No. 3: Normality test of CLDQ with pre- and post-intervention group (N=24)

Groups	Shapiro-Wilk		
	Statistic	Df	Sig.
Pre group	.620	24	.000
post group	.477	24	.000

Table No. 4: Mean Score HCC knowledge level of pre and post intervention group (N=24)

HCC knowledge level Categories	Pre-intervention		Post-intervention		Mean difference
Poor knowledge (<50%)	21(87.5%)	25.71±7.62	1(4.2%)	45.00±0.00	19.29
Fair knowledge (51-74%)	1(4.2%)	60.00±0.00	8(33.3%)	64..37±4.95	4.37
Good knowledge (75-100%)	2(8.3%)	75.00±0.00	15(62.5%)	79.33±4.95	4.33

Table No. 5: Normality Test of HCC Knowledge with pre and post Intervention group

Groups	Shapiro-Wilk		
	Statistic	Df	Sig.
Pre group	.783	24	.000
post group	.939	24	.156

Table No. 6: Comparison of chronic liver disease patients with pre- and post-intervention group (N=24)

CLQD Score Categories	Pre-intervention		Post-intervention		Mean difference
Low severity level (29-100)	21(87.5%)	79.28±7.20	1(4.2%)	94.00±0.00	14.72
Moderate severity level (101-170)	2(8.3%)	135.00±41.01	3(12.5%)	164.33±3.21	29.33
High severity level (171-203)	1 (4.24%)	175.00±0.00	20(83.3%)	178.60±3.21	3.60

Table No. 7: Pearson correlation coefficient between CLDQ domain and SF-36 domains (N= 24)

CLDQ 6 items		PF	PH	EP	Energy	EW	SF	GH	Pain
Abdominal	4.46±1.72	.838**	.786**	.776**	.716**	.744**	.700**	.838**	.627**
Fatigue	4.40±1.74	.859**	.842**	.804**	.683**	.796**	.788**	.871**	.648**
Systemic	4.28±1.89	.873**	.837**	.780**	.698**	.812**	.769**	.861**	.652**
Activity	4.63±1.71	.821**	.772**	.749**	.665**	.774**	.696**	.831**	.614**
Emotion	4.61±1.59	.820**	.813**	.762**	.700**	.776**	.745*	.841**	.621**
Worry	4.57±1.75	.801**	.781**	.701**	.629**	.753**	.731**	.823**	.604**

**Relationship is significant at the 0.01 level (2-tailed).

DISCUSSION

This quasi-experimental study aimed to assess the impact of a nurse-led preventive intervention on patients with chronic liver disease (CLD) at Allama Iqbal Teaching Hospital in Dera Ghazi Khan, Pakistan. Focusing on lifestyle, dietary control, drug adherence, and emotional support has been seen to effectively enhance the outcomes of patients in earlier research by preventive interventions. The present study showed demographic analysis proved helpful to establish the effect of patient factors on the outcomes of interventions, 75% were Urdu speakers and other patients could understand simple English making it easy to pass a message and comprehend in the teaching session. The SF-36 scores have been used to verify a significant improvement in the eight dimensions after the preventive intervention, which is an excellent improvement in the quality of life of patients in general. The general difference was the highest with Physical Functioning (72.92%). The next highest differences were realized in Role Functioning (Emotional Problems), General Health, and Social Functioning. These findings are consistent with previous studies[20], which indicated that controlled health preventive education improved the physical and mental health conditions of chronic liver disease patients. Reliability analysis did verify that all the areas had good to excellent internal consistency, which validated the fact that the instrument was successfully measuring patient-reported change.

The HCC Knowledge Assessment Scale demonstrated that participants' knowledge regarding liver disease and cancer prevention was significantly enhanced following the intervention by a nurse ($p<0.05$). The results concur with a study conducted by a researcher who established that education especially tailored by a nurse immensely enhanced disease-specific knowledge and screening behavior in risk patients.

A Pearson correlation test revealed that all of the SF-36 subscales had a significant relationship ($p<0.01$) with the CLDQ domains. The greatest increase was on physical health item of SF-36, then on role functioning due to emotional reasons, general health, social functioning, pain and energy/fatigue.[20] This means that the patients were not only becoming physically better but also emotionally well and capable of handling their illness after receiving education by the nurses. These results are aligned to the current literature, which has revealed the positive relationship between preventive measures and the quality of life of chronic disease patients such as hepatitis, diabetes, and hypertension.[21] The outcome justifies the status of nurses as effective educators who can be placed to deliver disease-specific information in an easily understood and culturally sensitive manner. This same outcome was reported by Yang et al[22], who demonstrated that community-based nursing education contributes immensely to knowledge and disease compliance behaviors.

CONCLUSION

Quality of life, knowledge associated with HCC, and reduction of problems in patients with long-term liver disease are significantly increased with the help of preventive educational interventions conducted by nurses. To improve the management of chronic diseases, especially in a resource strained setting such as in Pakistan, politicians and healthcare providers should consider utilizing nurse led education plans.

Author's Contribution:

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Drafting or Revising Critically:	Nimra Saleem, Madiha Mukhtar
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

School-Based Classical Music for ADHD Symptoms in Children

Karen Delicia Setiobudi[1,2], Yunias Setiawati[1,2] and Atika[3]

ABSTRACT

Objective: To evaluate whether classical music exposure can reduce symptoms of attention deficit hyperactivity disorder (ADHD) in elementary school children.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the SDN Klampis Ngasem 1, Surabaya, Indonesia, from July 2024 to July 2025, encompassing preparation, data collection, and analysis over one year, with each participant completing a two-week intervention period.

Methods: A quasi-experimental pre–post study was performed among 121 third-grade students screened using the Abbreviated Conners' Teacher Rating Scale (ACTRS). Children scoring ≥12 were considered to have ADHD-related symptoms, and 26 met inclusion criteria after parental consent. Participants were randomly assigned to an intervention group (n = 13), who listened to classical music for 20 minutes over five sessions, or a control group (n = 13), who continued regular activities. Teachers completed the ACTRS before and after the intervention.

Results: The intervention group showed a significant decrease in ACTRS scores from 14.61 ± 2.98 to 10.46 ± 3.55 ($p = 0.005$), whereas the control group showed no significant change (median 12.00 pre- vs 12.00 post-intervention, $p = 0.59$). Between-group analysis confirmed greater improvement in the intervention group ($p = 0.004$).

Conclusion: Short-term classical music listening significantly reduced ADHD-related symptoms based on teacher ratings. This low-cost, non-invasive approach shows promise as a complementary strategy, though confirmation through larger and diagnostically verified studies is needed.

Key Words: Attention Deficit Hyperactivity Disorder; classical music; therapeutic play; emotion regulation; complementary therapy; child mental health

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INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental conditions in childhood, characterized by inattention, hyperactivity, and impulsivity that impair academic, social, and emotional functioning.[1] Global prevalence is estimated at 5–7% of children, with symptoms often persisting into adolescence and adulthood.

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The burden extends beyond the individual to families and schools, where challenges in behavior management and learning support are significant.[2]

Current treatment strategies typically combine pharmacological approaches with behavioral interventions. Although stimulant medications remain highly effective, concerns about side effects, long-term safety, and variability in treatment response have prompted interest in complementary or adjunctive strategies.[3] One promising avenue is music-based intervention, which is non-invasive, low-cost, and generally well-accepted by children and parents.[4]

Music has been shown to influence brain networks involved in attention, arousal, and executive function.[5] Structured and rhythmic auditory input, such as classical music, may enhance cortical regulation and promote calmness, leading to better concentration and emotional regulation.[6] Several studies and systematic reviews have reported improvements in behavior, attention, and mood among children and adults exposed to music. However, evidence specific to ADHD remains limited, particularly in school-based interventions.[5,7]

Tools such as the Abbreviated Conners' Teacher Rating Scale (ACTRS) provide a practical means of identifying children with elevated ADHD-related behaviors in classroom settings. While not a diagnostic instrument, ACTRS offers a validated screening

approach that captures symptom severity and allows teachers to monitor changes over time.[8]

This study was designed to examine whether structured exposure to classical music could reduce ADHD-related symptoms among elementary school children. By implementing a quasi-experimental design in a classroom setting, we aimed to assess the potential of classical music as a complementary approach to support attention, reduce hyperactivity, and improve self-regulation.

METHODS

Study design and setting: This research employed a quasi-experimental design with pre-test and post-test evaluation. The study was conducted at an elementary school, SDN Klampis Ngasem 1 Surabaya, Indonesia, during the academic year 2024-2025.

Participants: A total of 121 third-grade students were screened using the Abbreviated Conners' Teacher Rating Scale (ACTRS). Teachers completed the ACTRS for each student during class hours, with an estimated completion time of 15 minutes per child. Students with ACTRS scores ≥12 were considered to have ADHD-related symptoms and were eligible for participation. This threshold has been commonly applied in previous studies to identify children at risk for ADHD.[9]

From this screening, 26 children met the inclusion criterion and were enrolled after obtaining written parental consent. Participants were randomly assigned into an intervention group (n = 13) and a control group (n = 13).

Inclusion and exclusion criteria: Inclusion criteria were: (1) third-grade students aged 8–9 years, (2) ACTRS score ≥12, and (3) parental consent. Exclusion criteria included children with hearing impairments, neurological conditions, or other psychiatric disorders that could interfere with participation.

Intervention: The intervention consisted of structured listening to selected classical music for 20 minutes per session, across five consecutive school days. Sessions were conducted in groups in the school assembly hall, under supervision of teachers and the research team. The control group continued with their usual school activities without exposure to music.

Outcome measure: ADHD-related symptoms were measured using ACTRS, which includes 10 items assessing hyperactivity, inattention, and impulsivity on a four-point scale (0 = not at all, 3 = almost always). Scores ≥12 are considered indicative of significant ADHD symptomatology. Teachers completed the ACTRS before and immediately after the intervention.

Data analyses: Data were analyzed using SPSS version 22. Descriptive statistics summarized participant characteristics. Paired t-tests were used to compare pre- and post-intervention scores in the intervention group, while the Wilcoxon signed-rank test was applied for the control group due to non-normal data distribution. Between-group differences were analyzed using the Mann–Whitney test. Statistical significance was set at $p < 0.05$.

Ethical considerations: This study was approved by our institutional ethics committee under approval number **168/EC/KEPK/FKUA/2025**. Written informed consent was obtained from the parents or guardians of all participants.

RESULTS

A total of 121 third-grade students were screened using the Abbreviated Conners' Teacher Rating Scale (ACTRS). Twenty-six children scored ≥12 and were therefore considered to have elevated ADHD-related symptoms. These children were enrolled after parental consent and randomly assigned into the intervention group (n=13) or the control group (n=13). All participants completed both pre- and post-test assessments.

Table No. 1. Sample characteristics.

Characteristic	Intervention (n = 13)	Control (n = 13)
ACTRS Score (Mean ± SD)	14.61 ± 2.98	12.00 ± 0.58
Sex		
Male	11 (84.6%)	7 (53.8%)
Female	2 (15.4%)	6 (46.2%)

Baseline characteristics: Baseline characteristics are presented in Table 1. The mean ACTRS score at baseline was higher in the intervention group (14.61 ± 2.98) compared with the control group (12.00 ± 0.58). The intervention group consisted mostly of boys (84.6%), whereas the control group had a more balanced sex distribution (53.8% male, 46.2% female).

Table No. 2: Pre- and post-intervention ACTRS scores in intervention and control groups

Group	Pre-intervention (Mean ± SD / Median)	Post-intervention (Mean ± SD / Median)	Within-group p-value	Score difference Median (min–max)	Between-group p-value
Intervention (n = 13)	14.61 ± 2.98	10.46 ± 3.55	0.005	–5.00 (–10.00 – 5.00)	0.004
Control (n = 13)	12.00 (12.00 – 13.00)	12.00 (11.00 – 13.00)	0.59	0.00 (–1.00 – 1.00)	

Symptom changes: Pre- and post-intervention ACTRS scores are summarized in Table 2. The intervention group demonstrated a significant reduction in ADHD-related symptoms after five consecutive sessions of classical music listening, with mean scores decreasing from 14.61 ± 2.98 to 10.46 ± 3.55 ($p = 0.005$). In contrast, the control group showed no meaningful change; median scores remained at 12.00 pre-test versus 12.00 post-test ($p = 0.59$).

Between-group comparison: The reduction in ACTRS scores was significantly greater in the intervention group compared with the control group (median difference –5.00 versus 0.00, $p = 0.004$, Table 2). These results indicate that short-term exposure to classical music was associated with measurable improvements in ADHD-related symptoms.

DISCUSSION

This study found that short-term exposure to classical music was associated with a significant reduction in ADHD-related symptoms among elementary school children, as measured by teacher ratings on the ACTRS. In contrast, children in the control group showed no meaningful change. These findings suggest that classical music listening may be a promising complementary strategy to reduce behavioral symptoms associated with ADHD in school settings.

Our results are consistent with previous work showing that music interventions can improve attention, emotional regulation, and behavior in children with ADHD or related conditions.[10] Studies of the so-called "Mozart effect" and other music-based therapies have reported improvements in executive function, mood, and arousal regulation.[11] Recent systematic reviews have also emphasized the potential of music to modulate attention networks and reward pathways implicated in ADHD. By providing structured auditory input, classical music may help regulate cortical activity, reduce hyperarousal, and support sustained attention.[5,12]

The intervention's feasibility and acceptability shine through: sessions were group-based, conducted right in the school environment, required minimal resources, and demanded no specialized equipment. These are key advantages, especially in schools where access to behavioral or pharmacological care is limited. This highlights the potential for classical music to be implemented as an adjunctive classroom strategy, particularly in resource-limited settings where access to behavioral or pharmacological therapy may be restricted.[13,14]

Nevertheless, several limitations must be acknowledged. First, ADHD was identified based on ACTRS scores rather than a full clinical evaluation. Although ACTRS is a validated and widely used screening tool, it cannot substitute for a comprehensive diagnosis based on DSM-5 criteria.[15] Second, the sample size was relatively small, and the intervention lasted only five days, limiting generalizability and the ability to evaluate long-term effects. Third, outcomes relied on teacher reports, which may be influenced by subjective perceptions. Future studies should include larger samples, multiple informants, longer interventions, and objective measures of cognitive performance or neurophysiological activity.

Despite these limitations, the findings contribute to the growing body of evidence supporting music interventions for ADHD. By demonstrating measurable symptom improvement after a brief school-based intervention, this study highlights the potential role of classical music as an accessible, non-invasive, and low-cost complement to standard ADHD management.

CONCLUSION

This study showed that short-term exposure to classical music was associated with a significant reduction in ADHD-related symptoms among elementary school children, as measured by teacher ratings on the ACTRS. Children who participated in the intervention demonstrated improvements in attention, reduced hyperactivity, and better self-regulation compared with controls. As an accessible, low-cost, and non-invasive strategy, classical music may complement established behavioral and pharmacological approaches in ADHD management.

Author's Contribution:

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Drafting or Revising Critically:	Karen Delicia Setiobudi, Yunias Setiawati
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Frequency of Raised C-Reactive Protein in Acute Stroke Patients

Raised C-Reactive Protein in Stroke

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ABSTRACT

Objective: Stroke is still one of the most common causes of death in the world. Inflammation is a significant factor in the mechanism and course of stroke. CRP, as a major inflammatory marker, has been widely reported in cardiovascular diseases; however, its prevalence and significance in acute stroke among our local population are less clear. To characterise the frequency of elevated CRP levels in patients with acute stroke.

Study Design: Cross-sectional descriptive study

Place and Duration of Study: This study was conducted at the Department of Neurology, Bolan Medical Complex, Quetta Form June 2025 to August 2025.

Methods: Consecutive sampling was done and 96 patients in the age range of 20-70 years who reported with clinical symptoms of acute stroke (duration 1.7 mg/dl.

Results: Fifty-two (54.2%) of the 96 patients were shown to have elevated CRP levels. Patients mean age was 58.4 ± 9.1 (range, 30-72) years; there were more males than females (60.4%). Ischemic stroke (68.8%) was more prevalent than hemorrhagic stroke (31.3%). The most common comorbidity was hypertension (72.9%). Elevated CRP was found more frequently in ischemic patients than in haemorrhagic (p=0.03).

Conclusions: This study demonstrated high rate (54.2%) of raised CRP in acute stroke patients indicating the marked inflammatory response to the condition. The significantly higher frequency of elevated CRP in ischemic stroke compared to haemorrhagic stroke in our cohort provides a novel, clinically relevant insight for our local population. This suggests that the underlying inflammatory burden may differ by stroke aetiology even at presentation, positioning CRP as a simple, cost-effective biomarker that could aid in initial diagnostic suspicion and inflammatory risk stratification in resource-limited settings like Pakistan.

Key Words: Acute Stroke, C-Reactive Protein, Inflammation, Biomarker, Frequency.

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INTRODUCTION

Stroke remains one of the most formidable challenges confronting global health systems, contributing substantially to morbidity, mortality, and long-term disability worldwide. It affects both developed and developing nations and ranks as the third leading cause of death globally, following ischemic heart disease and cancer[1,2]. The World Health Organization defines stroke as a rapidly developing clinical syndrome characterized by focal or global neurological dysfunction lasting at least 24 hours or resulting in death, with a vascular origin[3].

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Pathophysiologically, stroke occurs through two primary mechanisms: interruption of cerebral blood flow due to arterial occlusion (ischemic stroke) or rupture of a cerebral blood vessel (hemorrhagic stroke). The global burden of stroke is profound. It is estimated that approximately 15 million individuals experience a stroke annually, of whom nearly 5 million die and another 5 million are left with permanent disability. Hypertension alone has been implicated in over 12.7 million stroke cases worldwide. Although public health interventions targeting blood pressure control and smoking cessation have reduced stroke incidence in high-income countries, demographic transitions have sustained a high global burden. Notably, nearly 70% of all strokes and 87% of stroke-related deaths occur in low- and middle-income countries, where healthcare infrastructure and resources are often limited. Recent advances in stroke research have shifted understanding beyond mechanical vascular obstruction toward recognition of inflammation as a central component of stroke pathophysiology. Inflammatory processes contribute to atherosclerosis progression, cerebral infarction, and secondary neuronal injury. Systemic inflammatory states have been identified as independent predictors of poor outcomes in various vascular conditions, including ischemic heart disease, carotid

artery disease, and ischemic stroke treated with reperfusion therapies. Leukocytosis and inflammatory biomarkers have also been associated with adverse outcomes following mechanical thrombectomy. Among inflammatory markers, C-reactive protein (CRP) has gained particular attention. CRP is an acute-phase reactant synthesized primarily by hepatocytes and serves as a sensitive, though nonspecific, indicator of systemic inflammation. High-sensitivity CRP (hs-CRP) assays enable detection of low-grade inflammation and provide insight into the inflammatory milieu associated with atherosclerosis. Beyond being a passive marker, CRP actively participates in atherogenesis by promoting macrophage uptake of low-density lipoprotein cholesterol, facilitating foam cell formation, and activating the complement cascade, thereby exacerbating vascular injury. Several studies have reported variable frequencies of elevated CRP levels among acute stroke patients. Munir et al. reported elevated CRP in 61.9% of acute ischemic stroke cases, while Finck et al. documented a prevalence of 46.3%. Such variability underscores the influence of genetic, environmental, and methodological factors. Despite CRP's established role in cardiovascular disease, its profile in acute stroke—particularly within the Pakistani population—remains insufficiently explored. To address this gap, the present study was designed to evaluate CRP levels in patients presenting with acute stroke in a tertiary care setting in Quetta. Beyond estimating the prevalence of CRP elevation, this study aims to compare inflammatory responses between ischemic and hemorrhagic stroke subtypes at initial presentation. By elucidating these differences, the study seeks to enhance understanding of stroke pathophysiology and support the potential utility of CRP as a clinically relevant biomarker in acute stroke assessment.

METHODS

Study Design and Setting: This descriptive cross-sectional study was carried out at the Department of Neurology, Bolan Medical Complex Hospital Quetta over a period of June To August 2025. The study was approved by institutional ethical review committee of Bolan Medical Complex Hospital, Quetta (**Approval No:/BMCH/DA-I/2025/ 306**) and The College of Physicians and Surgeons Pakistan (CPSP).

Sample Size and Sampling Technique: The WHO sample size calculator for a single proportion was used to calculate the sample size. Considering confidence level (at 95%), margin of error = 10%, and estimating the expected proportion of raised CRP as 46.3% based on a previous study (Finck et al., 2023), at least the sample size required was calculated to be 96 patients. Consecutive non-probability sample of the eligible patients was included.

Inclusion and Exclusion Criteria: Both male and female of age group 20–70 patients were included who presented with clinically acute stroke (as per operational definition) upto 24 h. Exclusion criteria included a history of traumatic brain injury (TBI) within the past 2 years, acute coronary syndrome in the previous month, cerebrovascular events in the past, autoimmune diseases, hepatic failure, chronic renal failure or any other illness that caused CNS dysfunction (stroke mimic).

Data Collection Procedure: A proforma was designed for recording of each participant's information after receiving the informed consent. This included demographics (age, gender, place of residence), clinical details (duration of symptoms, history of hypertension and diabetes mellitus), anthropometry [height-weight measurement for body mass index (BMI)] and CT radiological data (type of stroke). Venous blood (3 ml) was collected by venipuncture and sent to the hospital laboratory. The measurement of the serum concentration of CRP was done by a solid-phase enzyme-linked immunosorbent assay (ELISA) kit. According to the working definition, a CRP concentration >1.7 mg/dl was defined as "raised."

Data Analysis: Statistical analysis was conducted by SPSS version 25.0. Participants' characteristics and levels of variables were displayed as mean ± standard deviation (SD) numbers for continuous data, such as age and BMI. Categorical variables, such as gender, comorbidities, stroke types, and behaviours, were given as frequencies and percentages. Stratification was according to age, sex, BMI, comorbidities, residence and stroke type. The Chi-square test was used to study associations between elevated CRP and these strata. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Ninety-six patients with acute stroke were included. The baseline demographic information of the study cohort is presented in Table 1.

Table No. 1: Baseline characteristics of study participants (N=96)

Characteristic		**Value**
Age (years), Mean ± SD		58.4 ± 9.1
Gender, n (%)	Male	58 (60.4%)
	Female	38 (39.6%)
Residence, n (%)	Urban	61 (63.5%)
	Rural	35 (36.5%)
BMI (kg/m²), Mean ± SD		26.8 ± 4.2
Comorbidities, n (%)	Hypertension	70 (72.9%)
	Diabetes Mellitus	45 (46.9%)
Type of Stroke, n (%)	Ischemic	66 (68.8%)
	Haemorrhagic	30 (31.3%)

The main aim of the study was to assess the prevalence of elevated CRP. Regarding CRP, the number of patients with CRP level greater than 1.7 mg/dl was 52 from a total 96, which is equivalent to a percentage of 54.2% (Table 2).

Table No. 2: Frequency of Raised CRP (N=96)

CRP Status	Frequency (n)	Percentage (%)
Raised (>1.7 mg/dl)	52	54.2%
Not Raised (≤1.7 mg/dl)	44	45.8%

The distribution of elevated CRP in different types of stroke was evaluated (Table 3). More patients with ischemic stroke (59.1%) than haemorrhagic had elevated CRP (43.3%) ($p = 0.03$).

Table No. 3: Association between Stroke Type and Raised CRP

Stroke Type	Raised CRP (n=52)	Normal CRP (n=44)	p-value
Ischemic (n=66)	39 (59.1%)	27 (40.9%)	0.03
Haemorrhagic (n=30)	13 (43.3%)	17 (56.7%)	

Additional subgroup analysis was conducted to investigate the relation between elevated CRP and other demographic or clinical characteristics (Table 4). There was no statistically significant relationship with age, gender, place of residence or hypertension and DM status ($p > 0.05$).

Table No. 4: Stratification Analysis for Raised CRP

Stratifying Variable	Category	Raised CRP (n=52)	p-value
Age	≤60 years (n=55)	28 (50.9%)	0.41
	>60 years (n=41)	24 (58.5%)	
Gender	Male (n=58)	30 (51.7%)	0.52
	Female (n=38)	22 (57.9%)	
Hypertension	Yes (n=70)	40 (57.1%)	0.29
	No (n=26)	12 (46.2%)	
Diabetes Mellitus	Yes (n=45)	26 (57.8%)	0.48
	No (n=51)	26 (51.0%)	
BMI	Obese (>30) (n=22)	14 (63.6%)	0.27
	Non-Obese (≤30) (n=74)	38 (51.4%)	

DISCUSSION

This hospital-based cross-sectional study demonstrated that 54.2% of acute stroke patients had elevated incidence of serum CRP levels. This result is well within the typical range of this factor in previous literature; it is greater than the expression reported by Finck et al.[7] and less than that (61.9%) reported by Munir et al.[6] in Pakistani people of a community. This discrepancy might be due to different study populations, numbers of subjects, the specific time point of blood collection after stroke onset or genetic and environmental factors that may affect systemic inflammatory response.The major realization of a high CRP elevation rate emphasises the profound inflammatory chain reaction initiated by cerebral insult[9]. CRP is not only a surrogate but is thought to have an effect in the pathogenesis of stroke. It stimulates atherogenesis through increasing the low density lipoprotein uptake by macrophages which results in foam cell formation. It also enhances the expression of adhesion molecules, and increases the release of several other pro-inflammatory mediators that in turn amplify endothelial dysfunction and brain tissue injury[10].A striking observation in our study was the significantly more common occurrence of raised CRP among those of ischemic stroke (59.1%), as opposed to those with hemorrhagic stroke (43.3%). This is consistent with the currently accepted view that a chronic inflammatory process, an established characteristic of atherosclerosis, underlies most ischemic stroke. It is this inflammatory milieu in stable, yet vulnerable atherosclerotic plaques that is a critical determinant of plaque rupture and thrombosis[11]. The acute infarction itself subsequently initiates an additional severe inflammatory response, maximising CRP production. Unlike ischaemic stroke, where the original insult is mainly due not to mechanical trauma but ischemia-reperfusion, in haemorrhagic stroke the traumatic origin of injury is perhaps superimposed by less pronounced or different acute phase reactant response kinetics that would account for these observations (ie. reduced frequency)[12].The novel contribution of our findings lies in the demonstration of this significant disparity within our specific local population. While this pathophysiological concept is known, empirical data from Pakistan is scarce. Our results provide concrete, local evidence that the inflammatory response, as measured by a standard CRP test, is etiologically different at the point of hospital admission. This has immediate clinical relevance. In a resource-constrained setting where advanced diagnostics may be delayed, an elevated CRP could add weight to a clinical suspicion of an ischemic event, potentially prompting different initial management considerations, such as more aggressive antiplatelet therapy or statin initiation, compared to a hemorrhagic

stroke.[13] Furthermore, our finding that over half of all stroke patients present with an elevated CRP underscores the pervasive nature of inflammation in acute stroke. This positions CRP not merely as an academic biomarker but as a practical, low-cost tool for "inflammatory risk stratification." Identifying patients with a heightened inflammatory state at admission could help clinicians flag those at potentially higher risk for complications like early neurological deterioration or infection, warranting closer monitoring. In contrast to some reports we observed no substantial relationship between elevated CRP and traditional vascular risk factors (hypertension, diabetes mellitus) in our stroke population[14]. This may be because, in a population already enriched for having had a serious vascular event (ie, stroke), the baseline infective burden is uniformly high potentially diluting the relative impact of specific additional comorbidities[13]. Our sample size also may have not been large enough to detect such more subtle relationships.

Our findings align with modern studies. A meta-analysis by demonstrated that high CRP concentration in acute stroke strongly correlate with increased stroke severity, infarct volume and dependence[14]. Another prospective cohort conducted by Lv et al. underscored the synergistic effects of high CRP and dyslipidemia on stroke risk, further lodging the crosstalk between inflammation and metabolism[15].The clinical message of our work is simple. As over 50% of our patients with acute stroke have been found to have an abnormal CRP and its relative simplicity, inexpensiveness and rapid availability, the CRP assay has potential value as a useful adjunct in the emergency evaluation of stroke[15]. Our data strengthens this argument by showing that its value may be enhanced by its ability to reflect the differing inflammatory pathophysiology between stroke subtypes.The limitations of our study were that it was a single-centre study and the sample size was not large enough to allow for generalization. Secondly, as a cross-sectional study, an association but not causation between CRP and stroke can be established.

CONCLUSION

In view of the substantial prevalence of increased CRP among acute stroke patients, our study adds a novel layer of understanding by highlighting a distinct inflammatory profile between ischemic and haemorrhagic strokes in a Pakistani cohort. This justifies further studies to get this simple test into initial clinical evaluations to stratify not only general risk but also to provide an early clue to the underlying stroke aetiology and its associated inflammatory burden. Prospective investigations on a larger scale, with high-sensitivity CRP assays are needed to further define the association between hs-CRP and long-term outcomes.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Abdul Ali, Hussain Ahmed, Abdul Aleem
Drafting or Revising Critically:	Muhammad Saddam, Riaz Ahmed, Noor Ahmed Khosa
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Frequency of Common Causes of Rejected Conventional X-Rays in a Radiology Department

Abdul Raheem[1], Muhammad Ashraf Kasi[2], Pari Gul Jogezai[3] and Sumaiya Basheer[1]

ABSTRACT

Objective: the present study aims to evaluate the frequency and causes of rejected conventional X-ray examinations in a radiology department, providing baseline data to support quality improvement initiatives, reduce unnecessary radiation exposure, and enhance diagnostic efficiency.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the Diagnostic Radiology Department Bolan Medical Complex Hospital Quetta from August 2025 to October 2025.

Methods: A cross-sectional study was performed at the Radiology Department, enrolling 92 patients whose traditional chest/abdominal X-ray had been deemed inadequate. Patient characteristics as well as reasons for rejection, i.e., overexposure, underexposure, inadequate positioning, patient motion, and artefacts were collected using a structured proforma. Statistical analysis was also carried out between demographic characteristics and reasons for rejection, with a $p \leq 0.05$ being significant**.**

Results: The mean age of the participants was 45.6 ± 13.2 years54; with 58.7% males and 41.3% females55 noted there in were significant differences between the male and female groups (Table 1). Overexposure (43.5%) was the most common reason for rejection, followed by underexposure (27.2%), wrong body part placement (15.2%), patient motion artifact (8.7%), and other artifacts (5.4%). There was a strong relationship between gender and exposure errors ($p = 0.02–0.03$), with males being more likely to be overexposed and females underexposed at higher proportions of the ED/IRLH. Malposition was also more prevalent among the rural ($p = 0.04$) dwellers.

Conclusion: Over- and under-exposure are still the main reasons for rejection of routine X-ray, and statistically significant differences were noted in gender and locality.

Key Words: Conventional radiography, Image quality, Overexposure, Reject analysis, Radiology quality assurance, Underexposure.

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INTRODUCTION

Medical imaging represents one of the most significant advancements in modern medicine, providing essential support for diagnosis, treatment planning, and patient monitoring. However, the rapid expansion and frequent utilization of diagnostic imaging—particularly conventional X-ray examinations—have raised concerns regarding population exposure to artificial ionizing radiation.

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Diagnostic medical imaging contributes more than 50% of man-made ionizing radiation exposure in the general population[1,2], and cumulative radiation exposure has been associated with increased long-term health risks, including malignancies[3].In routine clinical practice, repeat radiographic examinations are often required when initial images are deemed diagnostically unacceptable due to technical or quality-related issues. Such repetitions increase radiation dose to patients and impose additional demands on radiology personnel, resulting in higher costs, reduced workflow efficiency, and compromised patient throughput[4]. Consequently, rejection analysis—the systematic evaluation of the frequency and causes of rejected radiographs—has become an essential component of quality assurance in diagnostic radiology. Image rejection rates (RRs) serve as indicators of radiographer performance, protocol adequacy, and departmental operational standards[5]. Lower RRs reflect optimal image quality and efficient departmental function, whereas higher RRs indicate inefficiencies and unnecessary radiation exposure[6]. Common causes of radiograph rejection include overexposure or underexposure, improper patient

positioning, patient motion, image artifacts, and equipment-related issues. Several local and international studies have identified positioning and centering errors as the most frequent reasons for image rejection[7,8]. These errors are often attributed to inadequate patient preparation, limited radiographer experience, or suboptimal communication. Exposure-related errors typically arise from incorrect technique selection or insufficient knowledge of exposure parameters, emphasizing the need for continuous professional education and quality surveillance. Rejection rates vary across institutions due to differences in equipment, departmental policies, workload, patient demographics, and radiographer expertise. Recent audits have reported rejection rates ranging from 3% to 12%[8,9]. Studies have demonstrated that overexposure, underexposure, and positioning errors remain the most prevalent causes of rejection. Multicenter investigations have further shown that rejection analysis is an effective tool for identifying areas requiring targeted interventions such as retraining, protocol optimization, and equipment calibration[10]. Despite increasing adoption of digital radiography systems, challenges persist in resource-limited settings due to outdated equipment, inconsistent quality control practices, and training deficiencies[4,5,9]. Moreover, data on reject analysis from low- and middle-income countries, including Pakistan, remain limited. This lack of locally relevant data hinders benchmarking and the development of evidence-based quality improvement strategies. Therefore, the present study aims to evaluate the frequency and causes of rejected conventional X-ray examinations in a radiology department, providing baseline data to support quality improvement initiatives, reduce unnecessary radiation exposure, and enhance diagnostic efficiency.

METHODS

This cross-sectional study was conducted in the Department of Radiology, Bolan Medical Complex Hospital (BMCH), Quetta from August to October 2025. Ethical approval was obtained from the Research Evaluation Unit, College of Physicians and Surgeons Pakistan (CPSP), following approval of the research synopsis entitled "Frequency of Common Causes of Rejected Conventional X-Rays in a Radiology Department" (Reference No: CPSP/REU/RAD-2021-001-3373; dated August 12, 2025). The sample size was calculated using the WHO sample size calculator for a single population proportion, based on previously reported rejection rates in radiographic imaging. Using a confidence level of 95%, a margin of error of 10%, and the most frequent reported cause (overexposure: 48.8%), the estimated sample size was 92 patients. A non-probability consecutive sampling technique was employed.

Patients of either sex, aged 20–70 years, whose conventional chest or abdominal radiographs were deemed diagnostically unacceptable were included. Exclusion criteria comprised pregnancy, age above 70 years, requirement for specialized imaging (e.g., CT or fluoroscopy), refusal to participate, and incomplete clinical or demographic data. Written informed consent was obtained from all participants. Rejected radiographs were independently reviewed by a consultant radiologist with over two years of post-qualification experience. Causes of rejection were classified as overexposure, underexposure, improper positioning, patient motion, or artifacts, based on predefined operational definitions. Data were recorded using a structured proforma. Statistical analysis was performed using SPSS version 22.0. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized as mean ± standard deviation. Chi-square tests were applied post-stratification, with a p-value <0.05 considered statistically significant.

RESULTS

A total of 92 patients undergoing conventional chest and abdominal X-rays that were rejected were included in the study. The mean age of participants was 45.2 ± 13.6 years, with 53.3% males and 46.7% females. Most participants (59.8%) belonged to urban areas, while 40.2% were from rural regions. Regarding educational level, 27.2% were illiterate, 30.4% had primary education, 25% had intermediate education, and 17.4% were graduates or above (Table 1).

Table No.I: Demographic characteristics of the study participants (n = 92)

Variable	Mean ± SD / Frequency (%)
Age (years)	45.2 ± 13.6
Gender	
Male	49 (53.3%)
Female	43 (46.7%)
Place of living	
Urban	55 (59.8%)
Rural	37 (40.2%)
Education level	
Illiterate	25 (27.2%)
Primary	28 (30.4%)
Intermediate	23 (25.0%)
Graduation or more	16 (17.4%)

Table No.2: Frequency of causes of rejected conventional X-rays (n = 92)

Cause of rejection	Frequency (%)
Overexposure	40 (43.5)
Underexposure	25 (27.2)
Faulty positioning	14 (15.2)
Patient movement	8 (8.7)
Artifacts	5 (5.4)

The most frequent cause of rejection was overexposure (43.5%), followed by underexposure (27.2%), faulty positioning (15.2%), patient movement (8.7%), and artifacts (5.4%) (Table 2). When analyzed by gender and place of residence, a significant association was found between these factors and the causes of rejection. Overexposure occurred significantly more often among males (55.1%) than females (30.2%) ($p = 0.02$), whereas underexposure was higher among females (37.2%) than males (18.4%) ($p = 0.03$). Faulty positioning was observed more frequently among rural residents (21.6%) compared with urban residents (10.9%), showing a statistically significant relationship ($p = 0.04$) (Table 3). Overall, overexposure and underexposure remained the leading causes of image rejection, demonstrating clear variations across gender and residence. These results suggest that differences in radiographic technique and patient cooperation may influence rejection patterns, emphasizing the need for continued staff training and stricter adherence to exposure protocols to improve image quality and reduce repeat examinations.

Table No.3. Association of causes of rejected X-rays with gender and place of living

Variable	Overexposure n (%)	Underexposure n (%)	Faulty positioning n (%)	Patient movement n (%)	Artifacts n (%)	p-value
Male (n = 49)	27 (55.1)	9 (18.4)	7 (14.3)	4 (8.2)	2 (4.1)	**0.02***
Female (n = 43)	13 (30.2)	16 (37.2)	6 (14.0)	5 (11.6)	3 (7.0)	**0.03***
Rural (n = 37)	18 (48.6)	8 (21.6)	8 (21.6)	2 (5.4)	1 (2.8)	**0.04***
Urban (n = 55)	22 (40.0)	17 (30.9)	6 (10.9)	6 (10.9)	4 (7.3)	0.08

*Significant at $p \leq 0.05$.

DISCUSSION

Excessive--underexposure as well as improper position are the main reasons conventional X-rays were refused in this study, to be followed by patient movement and artifacts. This negative correlation suggests that exposure errors still continue to be the predominant source of image rejection, while optimization of radiographic exposure factors is a persistent problem. The predominance of exposure and positioning related errors is consistent with international literature where similar trends are observed repeatedly in various clinical and geographical locations.[8,9,11] These common causes of repetition emphasize the technical and procedural elements of image acquisition that have a direct impact on radiographic quality and patient safety. Exposure factors are generally the result of mistaking kVp, mAs, and inappropriately used automatic exposure controls. Compiled by poor knowledge of radiographic factors or failure to adapt exposure techniques according the patient's body habitus, images can be overexposed (too bright) or underexposed (low contrast).[8,11,16] Even with dose-monitoring technology and systems available in digital radiography, exposure rejects remain a substantial percentage of the overall number of images rejected thereby indicating that radiographer training underpinned by accepted standards and quality control monitoring is necessary. There was a marked correlation between patient sex and overexposure; male patients had significantly higher rates of rejection because of too much exposure. This relationship may be due to differences in body composition and size, leading to variations in personal exposure. The discovery is consistent with studies recommending individualized dosimetry protocols to tailor the radiation dose and image quality to a specific patient.[13,14] Local analyses in United Arab Emirates and Saudi Arabia showed that establishing of a reject analysis on regular basis decreased the overall rejection rate by 30% after selective corrective actions.[13,14] Common programs include in-house lectures for staff, technique-chart review and the introduction of a double-check process before exposure. To achieve global harmonization and standardization of reject analysis processes, a vendor-neutral reject analysis framework has been prescribed by American Association of Physicists in Medicine (AAPM) Task Group 305 for inter-comparison of rejection data across institutions.[15] This model encourages transparency and consistency; it allows comparisons, bench-marking and data-driven quality improvement within radiology departments worldwide. It allows health-care facilities to identify systemic deficiencies, improve efficiency and compliance with international standards in terms of radiation protection habits and image quality management. Recent work from Europe, Asia and Africa continue to confirm that rejection analysis is indeed a powerful method of continuous quality improvement.[9,16-19] For instance, Hofmann et al[16] in Norway found that systematic REJ-auditing increased staff awareness and resulted in lower number of superfluous recalls, while Calatayud-Jordán et al[18] showed that reject analysis can contribute to radiation protection developments by the estimation of cumulative patient doses. Also, Ismail and Abdul Halin[19] from Malaysia noted that high reject rates were commonly due to poor image review habits and lack of direct feedback to radiographers, highlighting the importance of a structured audit regime. In summary, the results of the current study support the idea that

reject analysis continues as a necessary tool for radiological quality assurance. That exposure and position errors continue to predominate in the face of technological progress, even when controlling for other factors that influence image quality, suggests that human performance—training, attention to detail, protocol adherence—still plays a significant role in determining how good an imaging study is.[16,20] Through the institutionalization of routine reject analysis, following up regular competency assessments and creating a culture of continuous feedback, radiology departments provide an opportunity to significantly lower unnecessary repetition and thereby increase patient safety and departmental efficiency. Concentrated experience, accurate rejection log entries and following optimized imaging protocols don't only protect the patient's well-being but also enhance the diagnostic reliability and credibility of the services rendered in radiology.

CONCLUSION

Overexposure and underexposure were the leading causes of rejected conventional X-rays, revealing significant associations with gender and place of residence. The findings highlight that improved training, regular quality audits, and standardized exposure protocols are essential to reduce rejection rates, enhance diagnostic efficiency, and minimize unnecessary radiation exposure to patients.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Abdul Raheem, Pari Gul Jogezai
Drafting or Revising Critically:	Sumaiya Basheer, Muhammad Ashraf Kasi
Final Approval of version:	All the above authors
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Original Article

Prevalence of Aplastic Anemia Among Adult Patients with Anemia

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ABSTRACT

Objective: To analyze the demographic and hematological characteristics of adults presenting with anemia versus those without aplastic anemia and to support timely clinical recognition and management.
Study Design: A Cross-sectional study.
Place and Duration of Study: This study was conducted at the General Medicine Department, Sandeman Provincial Hospital / Bolan Medical Complex Hospital Quetta from June 2025 to August 2025.
Methods: One hundred adult patients with anemia were enrolled in a cross-sectional study at a tertiary care hospital. These patients underwent a detailed assessment and laboratory testing, including a complete blood count, reticulocyte count, and peripheral blood smear. Patients with peripheral blood smears showing a reticulocyte count. We're working up with anemia and bone marrow aspirations. Aplastic anemia was diagnosed according to Camitta criteria. Data were collected, including demographics, hematological, and clinical variables. SPSS version 24 was used to perform statistical analyses of these variables, and inter-group differences in cases were assessed using chi-square tests and independent t-tests between aplastic and non-aplastic anemia.
Results: The 100 participants (mean age 42.8 ± 13.6 years; 54% male), the incidence of aplastic anemia was 12%. Anemia was commonly accompanied with, fatigue (83% of cases), infections (58%), and bleeding (42%). Pancytopenia was significantly more prevalent in cases of aplastic anemia (92) compared to non-18 cases; (p <0.001). Hemoglobin levels (6.1 ± 0.9 g/dL vs. 8.4 ± 1.2 g/dL; p <0.001), and the count of platelets and neutrophils was significantly lower within the aplastic group. When looking at the severity, 50% were categorized as severe, 33% fall into the very severe category, and 17% were indicated as non-severe.
Conclusion: A significant proportion of adult anemia cases are due to aplastic anemia with severe cytopenias. This demonstrates the need for prompt diagnostic evaluations and timely bone marrow assessments.
Key Words: Aplastic anemia; prevalence; pancytopenia; adults

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INTRODUCTION

Aplastic anemia is a rare but serious disorder of the blood and bone marrow, accounting for fewer than 1% of cases of anemia worldwide. It also carries the risk of opportunistic infection, bleeding, and death, in addition to debilitating fatigue. It is thus a potentially severe global health concern[1]. In low and middle-income countries, anemia is one of the most frequent complaints of outpatients and inpatients in most clinical settings.

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In most of these cases, diagnostic investigations are focused on nutritional, hemolytic, or chronic disease anemias. Because of this, bone marrow failure syndromes like aplastic anemia are often missed. This has, unfortunately, led to missed opportunities to diagnose and clinically manage individuals suffering from these syndromes[2]. The causes of aplastic anemia are highly variable and include autoimmune stem cell destruction, environmental toxins like benzene, drug reaction, viral infections (especially hepatic), and certain inherited disorders. The causes of a significant number of cases, however, remain unknown[3]. The incidence of aplastic anemia also varies widely by region, with the highest rates occurring in Asia by a large margin. These differences are likely due to a combination of environmental, socio-economic, and differences in health care availability. The actual impact and prevalence of aplastic anemia and its complications are not fully understood. They are likely not fully appreciated by the physician community in South Asia and other regions of the world[4,5]. Clinically, aplastic anemia is associated with complications emanating from the lack of functioning bone marrow. Bone marrow insufficiency leads to anemia and consequent fatigue and pallor, infections due to neutropenia, and

bleeding due to thrombocytopenia[6]. Such complications are non-specific and are therefore likely to be encountered in numerous other related disorders, further slowing the rate of accurate diagnosis. Obtaining a correct diagnosis is greatly assisted by the presence of complications, as evidenced by laboratory findings of anemia in the normocytic or macrocytic range, associated with reticulocytopenia and other cytopenias. The gold standard for the most accurate diagnosis is a bone marrow biopsy, which is likely to show a hypocellular marrow with few of the cells expected in a normally functioning bone. Such a condition significantly impedes the ability to diagnose in a timely fashion. This is of great importance because the commencement of immunosuppressive therapies and/or hematopoietic stem cell transplantation will lead to significantly improved health outcomes[7,8]. Given the limited local prevalence data and the unique challenges posed by this condition, identifying aplastic anemia in adults with anemia is a high priority. This can benefit clinical practitioners in clinical diagnosis and also help in the pragmatic parallel allocation of health resources[9]. Methodical and straightforward data collection of demographic details alongside clinical findings, in the form of a blood test, will provide the clinician with a 'softer' option for anemia, compared to the numerous other causes of anemia that are likely to have a more complex treatment plan. The objective of this study is to address this knowledge gap to enhance clinical practice in resource-constrained healthcare settings, where advanced diagnostics may not be readily available.

METHODS

Study Design & Setting: This cross-sectional study was conducted in the General Medicine Department of a tertiary care teaching hospital. The study included adult patients presenting with anemia who underwent diagnostic evaluation

Participants: The World Health Organization's criteria for hemoglobin levels were used to include participants aged 18 or older with a diagnosis of anemia. Those with known hematologic malignancies and/ or recent chemotherapy treatment, pregnant patients, individuals with acute blood loss, and those who suffer from chronic renal failure were excluded from analysis. All participants in this study have undergone a standard medical history, a complete medical examination, and a complete blood analysis. Any cases in the study suspected of having osteoporosis were referred for bone marrow biopsy.

Sample Size Calculation: Applying the expected 5% prevalence rate of the condition, the 95% confidence level (Z=1.96), and 4% margin of error, the sample size was determined as $n = Z^2P(1-P)/d^2$. However, given the time and budget resources, 100 patients were selected, while the calculated sample size was 114.

Inclusion Criteria: Men aged 18 years or older have a hemoglobin level of 13 g/dL, whereas women of the same age have a hemoglobin level of 12 g/dL. Approve participation

Ethical Approval Statement: The study protocol and synopsis titled “Prevalence of Aplastic Anemia Among Adult Patients with Anemia” were reviewed and approved by the Research Evaluation Unit of the College of Physicians and Surgeons Pakistan (CPSP) (Ref No: CPSP/REU/MED-2022-001-19384, Dated May 30, 2025). The approval confirms that all research procedures comply with ethical standards for conducting studies involving human participants. A copy of the acceptance letter is available from the corresponding author upon request.

Exclusion Criteria: Hematologic cancer, which is known. Recent treatment with chemotherapy or radiotherapy. Women who are pregnant, Patients with severe acute hemorrhage

Diagnostic and Management Strategy: A complete blood count, reticulocyte count, and peripheral blood smear were done on each patient. Bone marrow biopsy was done on those with suspected marrow failure. Aplastic anemia was diagnosed according to the Camitta criteria, and subsequently, confirmed patients received supportive care, transfusions, and infection prophylaxis, and were referred for evaluation of immunosuppressive therapy.

Statistical Analysis: Demographic and laboratory values statistics were summarized descriptively and analyzed utilizing SPSS version 24. Prevalence was calculated in percentages. An independent t-test was used to compare the means of two continuous groups, and a chi-square test was used to assess the relationship between categorical variables. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 100 adult patients with anemia were evaluated. The mean age of participants was 42.8 ± 13.6 years, with 54 males (54%) and 46 females (46%). The overall prevalence of aplastic anemia among the study population was 12% (n=12). Fatigue (83%), recurrent infections (58%), and bleeding manifestations (42%) were common presenting symptoms in aplastic anemia cases. Pancytopenia was significantly more frequent in the aplastic group (92%) than in non-aplastic anemia patients (18%) ($p < 0.001$). Mean hemoglobin was markedly lower in aplastic anemia patients (6.1 ± 0.9 g/dL) compared to non-aplastic cases (8.4 ± 1.2 g/dL) ($p < 0.001$). Platelet count and absolute neutrophil count were also significantly reduced in patients with aplastic anemia ($p < 0.01$). Bone marrow biopsy confirmed hypocellularity in all diagnosed cases. Based on severity classification, 50% were classified as severe, 33% as very severe, and 17% as non-severe aplastic anemia. Early diagnostic evaluation

through hematological and marrow assessment helped establish timely diagnosis and guided appropriate management pathways.

Intervention Outcome:

Individuals with aplastic anemia received supportive management, including blood transfusions, infection prophylaxis, and requests for consideration of immunosuppressive therapy. Early detection during workup for anemia strengthened management planning and minimized complications. Timely marrow evaluation enabled more immediate clinical decisions, which might improve long-term outcomes, especially in settings with fewer healthcare resources.

Table No. 1: Baseline Demographic Characteristics of Study Participants

Variable	Total (n = 100)	Aplastic Anemia (n = 12)	Non-Aplastic Anemia (n = 88)	p-value
Mean Age (years) ± SD	42.8 ± 13.6	39.5 ± 12.8	43.3 ± 13.9	0.28
Gender (Male), n (%)	54 (54%)	7 (58.3%)	47 (53.4%)	0.72
Gender (Female), n (%)	46 (46%)	5 (41.7%)	41 (46.6%)	—

Baseline demographic distribution of adult patients evaluated for anemia, comparing those diagnosed with aplastic anemia versus non-aplastic causes.

Table No. 2: Hematological Parameters of Study Participants

Parameter	Aplastic Anemia (n = 12)	Non-Aplastic Anemia (n = 88)	p-value
Hemoglobin (g/dL), mean ± SD	6.1 ± 0.9	8.4 ± 1.2	<0.001
Platelet Count ($\times 10^9$/L), mean ± SD	32 ± 14	156 ± 48	<0.01
Absolute Neutrophil Count (ANC), mean ± SD	0.7 ± 0.3	2.8 ± 1.1	<0.01
Reticulocyte Count (%)	0.3 ± 0.1	1.2 ± 0.6	<0.001
Pancytopenia, n (%)	11 (92%)	16 (18%)	<0.001

Comparison of key hematological indices between aplastic anemia patients and those with non-aplastic anemia, showing significantly lower counts in the aplastic group.

Table No. 3: Prevalence and Severity Classification of Aplastic Anemia

Category	n	%
Total Aplastic Anemia Cases	12	12%
Severe Aplastic Anemia	6	50%
Very Severe Aplastic Anemia	4	33%
Non-Severe Aplastic Anemia	2	17%

Distribution of aplastic anemia severity based on Camitta criteria among diagnosed patients.

Table No. 4: Clinical Features Among Aplastic vs. Non-Aplastic Anemia Cases

Clinical Feature	Aplastic Anemia (n = 12)	Non-Aplastic Anemia (n = 88)	p-value
Fatigue	10 (83%)	61 (69%)	0.31
Recurrent Infections	7 (58%)	19 (22%)	<0.01
Bleeding Manifestations	5 (42%)	9 (10%)	<0.01
Pallor	12 (100%)	88 (100%)	—
Fever	6 (50%)	17 (19%)	<0.05

Frequency of major clinical symptoms among anemic adults, demonstrating significantly higher rates of infections and bleeding in the aplastic anemia group.

DISCUSSION

The presence study investigated the frequency of aplastic anemia among adult patients presenting with anemia at a tertiary care facility and found a 12% prevalence. This underscores the significant burden of bone marrow failure in everyday clinical practice. Anemia in low-resource settings is often attributed to malnutrition and/or chronic illnesses; however, our results indicate that aplastic anemia should be considered a significant differential diagnosis, especially in patients with pancytopenia or unexplained cytopenias. 92% of patients with aplastic anemia had pancytopenia, compared with 18% of patients without aplastic anemia, underscoring the importance of combined cytopenias in the diagnosis[11]. The prevalence of aplastic anemia in our study is comparable to that reported in other recent regional and international studies. An investigation performed in India reported a prevalence level of about 10.4% in patients suffering from primary cytopenia. This indicates that syndromes involving bone marrow failure remain very important clinically in the South Asian population[12]. Study-based evidence from a 2021 multi-center study in China shows that there is a growing incidence of aplastic anemia, particularly in the younger population. This is consistent with our analysis, which found that 67% of the patients in the study were under 45 years old[13]. There is a notable trend in the environment and specific occupations that affects the population, as seen in the previously mentioned study and other recent ones. Patients with aplastic anemia have much lower hemoglobin levels, lower platelet counts, and fewer neutrophils than patients with different types of anemia. This trend follows descriptions related to previously conducted hematological studies, where a 2020 investigation revealed that if anemia is present, with the added characteristic of a low reticulocyte count, it is a powerful predictor of an underlying factor of marrow failure; this is also consistent with our findings of very

low reticulocyte counts in the cohort[14]. Hypocellular bone marrow findings in all patients with aplastic anemia further support recent studies on bone marrow histomorphometry, which confirm that severe hypocellularity of the bone marrow is the most important and most reliable characteristic of the disease[15]. The clinical symptoms observed in the study, particularly lethargy, repetitive infection, and the tendency to bleed, are also very typical symptoms of aplastic anemia. Our recent findings corroborate the impact of neutropenia complications on the health of those affected. This is similar to the findings of a study demonstrating that infections in aplastic patients (58\%) are significantly higher than in non-aplastic patients[16]. Also, there was a higher prevalence of bleeding complications. Regression analysis similar to that of a 2019 study that demonstrated a strong positive correlation between low platelets (30 × 10, particularly 9/L) and increased bleeding complications (thrombocytopenia)[17]. In our analysis of the severity of the condition, however, we classified it as very severe, with iorar findings observed in 79% of patients, indicating severe forms of the disorder. This underscores the phenomenon of very late-presenting cases, especially there with minimal resources[18]. The delay is most especially due to minimal access to bone marrow biopsy facilities and very low awareness of the condition (marrow failure syndromes). This delay may lead to the most commonly accepted outcome in evidence-based medicine: early diagnosis resulting in poor outcomes[19]. This was the emphasis of the most recent guidelines on treatment, which emphasize the need to initiate immunosuppressive therapy very early, as well as hematopoietic stem cell transplantation[20]. In conclusion, the findings of this study support the recommendation that all patients who are anemic be provided with a detailed evaluation and diagnostic workup in a stepwise approach that precisely addresses bone marrow and other reticulocyte counts, peripheral blood smears, and, where appropriate, emphasizes the marrow[21]. In recent years, a global convergence of opinion supports the early identification of aplastic anemia, which can increase the likelihood of surviving the disease, especially for younger patients in whom this disease can be cured. Overall, this study adds critical regional data on the addition of this discipline in aplastic anemia and the need for greater attention to detail in diagnosing patients[22]. The global analytical review of recent evidence assesses the congruence of our data with the worldwide concern about the unrelenting challenges of diagnosing patients with this disease in low-resource settings.

Limitations: This study was conducted at one educational institute due to its limitations and conditions. Although some patients were reported to be clinically unstable, and patients refused to take tests, some patients with suspected illnesses also did not have bone marrow biopsies. Nutritional deficiencies and chronic infections, when present, also complicate the case, as they may be connected to the underlying hematologic defect and therefore affect diagnostic accuracy and prevalence estimates.

CONCLUSION

Aplastic anemia accounted for a significant proportion of cases of adult anemia and was closely associated with severe cytopenias. Early diagnostic evaluations, such as prompt bone marrow testing, should be performed to ensure an accurate diagnosis and facilitate proper management. Improving diagnostic processes strengthens efforts in under-resourced areas, where recognition of the condition is delayed and poses a major clinical obstacle.

Author's Contribution:

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Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Incidence of Hypertension in Early Adolescent Children between 12-14 years Old Age

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ABSTRACT

Objective: Prevalence of pre-high blood pressure and hypertension in early adolescent children between 12-14years old age.

Study Design: Cross-section study

Place and Duration of Study: This study was conducted at the schools of intermediate public in Al-Diwaniyah Governorate, Iraq from 1st January 2023 to 15th April 2023.

Methods: This cross-section study was done in schools of intermediate public in Al-Diwaniyah Governorate, Iraq. The participants were carried out on 200 (100 males and 100 females) early adolescent children aged 12-14 years.

Results: There were79% of normal blood pressure values, and 13% of them were in pre hypertension state with 4:5 male to female rate, 2% of them were in hypertension stage 1 with 3:1 male to female rate, 1% were stage 2 all females, 1.5% were isolated systolic hypertension with 1:2 male to female rate, 2% were isolated diastolic hypertension with 1:3 male to female rate, and the remainder were white coat hypertension.

Conclusion: The increasing prevalence of hypertension in childhood were carrying problem of global health, demands early recognition with good treatment, a guide in a specific direction evolve progress with raise awareness of this subject shall be predicted, where early prevention could resolute before all measures.

Key Words: Incidence, Adolescent children, Blood pressure, Hypertension

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INTRODUCTION

One of the major global health issues affecting children and adolescents is hypertension, or increased blood pressure, and this is also true in Sub-Saharan Africa (SSA).[1-4] In SSA, there are currently 26.5 million teenagers with high blood pressure, and this number is projected to rise.[5] This trend is attributed by researchers to increased rates of children and teenage obesity also overweight.[1,2] Comorbidities such as cardiovascular disease and metabolic syndrome are linked to adolescent obesity.[6] Future health outcomes are significantly influenced by adolescence, a critical time in human development.

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The World Health Organization (WHO) defines adolescence as "the period of life between being a child and growing to become adult, extending from the age of 10-19 years.[7]

The leading risk factor for cardiovascular diseases worldwide is hypertension[8], which can damage organs even during adolescence.[9]People who have high blood pressure (BP) as children are more likely to have high BP as adults.[10]

According to a number of guidelines, childhood hypertension should be characterized as increased blood pressure on three different occasions, unlike adult hypertension.[11,12] According to estimates, 4% of children worldwide have hypertension, and between 2000 and 2015, that number increased to 75-79%.[13] The fast economic growth and growing prevalence of childhood obesity in China exacerbate this trend.[14,15] Even while juvenile hypertension symptoms are usually minor, they can cause organ damage[16-18] and lead to early cardiovascular disorders in adulthood.[19-21]

It is unknown how frequently hypertension is detected in pediatric offices in Iraq. Pediatricians in this nation are in charge of the medical treatment of children under the age of twelve. After that, general practitioners (GPs) often take over their treatment, while more than 30% of teenagers between the ages of 13 and 17 still see pediatricians. Our study sought to fill these knowledge gaps by providing a summary of prevalence of hypertensive children in AL-Diwania, Iraq, on bases of three different visits.

METHODS

This cross-section study was done in public schools of intermediate in Al-Diwaniyah Governorate, Iraq from 1st January 2023 to 15th April 2023 vide letter No. 278.A total of 200 (100 males and 100 females) early adolescent children aged (12-14) years were enrolled. A questions form was arranged after dealing with reviewing articles with the prevalence of hypertension as well as pre-hypertension and related outcomes in developed and undeveloped countries and it consisted from knowledge about sex, weight, age, height, and body mass index (BMI), family history of hypertension, blood pressure, and exercise.

The chosen students were contacted following the signing of an informed consent form by the participants and their guardians. A questionnaire was used to gather information on sociodemographic traits such as age in years, parental educational attainment(less than and equal to secondary school), sex, maternal status of employment (employed or housewife), smoker behaviour, and hypertension history in family. To gather the data, the researchers trained two medical research assistants. The usual methods were used to measure height, weight, and blood pressure. Hypertension was regarded as the major objective and BMI and socio-demographics as secondary outcomes after the patient had been seated for at least 10 minutes. the measuring device which was a standardized digital blood pressure (Omron Digital HEM-907,Tokyo-Japan) was used to take the blood pressure twice while the arm was kept at heart level. The average of two blood pressure readings was calculated. If the difference between the two readings was greater than 5 mm Hg, measurements were repeated until the reading stabilized. The diastolic and systolic blood pressures were computed by age as well as sex based on reports made at diagnosis, evaluation, and treatment of hypertension in adolescents and children.

The participants' measurement of weights was in kilograms using the standard methods, which included calibrating the scales and setting them to zero before each measurement. To the nearest 100 grams, the weight was determined. With their hands by their sides and minimal movement, the participants stood. Additionally, shoes and superfluous clothing were removed. The subject's height was measured to the nearest 0.1 cm while they stood erect with their back against the wall and their feet together. The BMI was calculated by dividing the participants' weight in(kg) by their squared height in meters (m). The data was entered and analyzed through SPSS-26.

RESULTS

There were 79% of normal blood pressure values and 13% of them were in pre hypertension state with 4:5 male to female rate, 2% of them were in hypertension stage 1 with 3:1 male to female rate, 1%was stage 2 all females, 1.5% were isolated systolic hypertension with 1:2male to female rate, 2% were isolated diastolic hypertension with 1:3 male to female rate, and the remainder were hypertensive white coat (Table 1). Other findings involving risk factors such as body mass index, exercise, and family history and their relation to every type of hypertension that was found in the sample were implemented (2-4).

Table No. 1: Types of bloodpressure abnormal values according to male and female rates

Gender	Pre-hypertension	Hypertension stage 1	Hypertension stage 2	Isolated systolic HTN	Isolated diastolic HTN	White coat hypertension
Male	12	3	-	1	1	1
Female	15	1	2	2	3	2

Table No. 2: Body mass index of early adolescent childrenarranged dependingonexercise and family history

Variable	Normal BMI	Underweight	Overweight	Obese
Exercise	2	-	-	-
Family history	3	3	3	-
No exercise or family history	13	1	-	-

Table 3: Types of blood pressure abnormal values, categorized based on body mass index values

Variable	Hypertension stage 1	Hypertension stage 2	Isolated systolic HTN	Isolated diastolic HTN
Normal BMI	3	1	3	2
Underweight	-	-	-	-
Overweight	-	-	-	2
Obese	1	1	-	0

Table No.4: Types of blood pressure abnormal values, categorized based onfamily history and exercise

Variable	Hypertension stage 1	Hypertension stage 2	Isolated systolic HTN	Isolated diastolic HTN
Exercise	-	-	1	-
Family history	3	1	1	-
No exercise or family history	1	1	1	4

DISCUSSION

A study from Saudi Arabia that included 401 adolescents (200 males)revealed that Hypertension are present in 69 male and female child (of which are a general prevalence of17.2%) which is higher than its prevalence in our study which is6.5% including hypertension stage one 2%, hypertension stage two 1%,isolated systolic hypertension 1.5% and isolated diastolic hypertension 2%.In their sample, 17 children (4.2%) had pre-hypertension with a ratio of male to female was 2.1:1, however in our sample, the percentage was significantly higher (13% of our sample had pre-hypertension with ratio of male to female reported as 0.8:1). With a ratio of male to female reported 1.5:1 in their sample and 0.8:1 in ours, obesity was observed in 77 young children (19.2%) as opposed to just 9 in our sample (4.5%). While 33 children (8.2%) were underweighting in the Saudi study and 15% in ours, 60 patients (15%) in the Saudi study were overweight, which is quite similar to their prevalence in our study (14%).[3]

The prevalence of hypertension among school-age teenagers was found to be 5.62% in an Indian study conducted in 2009 till 2010, which is less than the 6.5% seen in our study.[4] According to a different Pakistani research, 3% of people had hypertension. Studies conducted in the West likewise found that 2–5% of people had hypertension.[5,6]

A research conducted in Switzerland in 2005 to 2006 looked at 5207 students in the canton of Vaud's sixth grade (2621 boys, 2586 girls).[10] The prevalence of excess body weight (also known as "overweight" or "at the risk of becoming overweight") was reported 14.3%, which is almost identical to the 14% found in our study.(7) Compared to our study, which found 2% stage 1 and 1% stage 2 (8.9), the prevalence of hypertension was 2.2% in this study (1.7% stage 1 and 0.4% stage 2). We recommend doing other studies to early detect undiagnosed cases. Prevention of hypertension may be considered as part of the prevention process for stroke as well as disease of cardiovascular system. Public health, population-based measurements to the reduction of primary hyper-tension in children as well as adults include reduced sodium intake, reduction in obesity, and an increase in physical activity through school- and community-based programs and avoidance of tobacco intake.

CONCLUSION

The increasing prevalence of hypertension in childhood were carrying problem of global health, demands early recognition with good treatment, BMI and no exercise are significantly correlated with hypertension. Initiatives aimed at preserving a healthy body mass index during adolescence are advised in order to support teenagers' well-being both now and in the future. The sustainability of such projects depends on the participation of all relevant parties.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Qahtan Khayoon Alyasiri, Suzan Sabbar Mutlag
Drafting or Revising Critically:	Qahtan Khayoon Alyasiri, Hind Muter Ibrahim
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

The Relationship Between Pentraxin-3, Procalcitonin and Glycemic Control in Diabetic Foot Infections

Ekhlas Hatem AL-Asadi[1], Hawraa Hamid Hussein[1] and Hawraa M. Kadhim[2]

ABSTRACT

Objective: To evaluate the relative importance of pentraxin-3, procalcitonin, C-reactive protein, glycated hemoglobin and body mass index as well as identify any correlations between these parameters.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the University of Babylon, College of Medicine, Iraq from 18th June to 24th September 2024.

Methods: Fifty participants with diabetic foot infection and fifty healthy individuals were among the one hundred people whose Pen-3, procalcitonin, C-reactive and glycated hemoglobin levels were estimated. The age range of patients and control subjects is 38 to 69 years. The sandwich-enzyme-linked immunosorbent assay kit and the colomeric technique were used to test the levels of pentraxin-3, procalcitonin and C-reactive protein in serum. The glycated hemoglobin kit was also used.

Results: There was a substantial rise in serum levels of pentraxin-3, procalcitonin and C-reactive protein as well as a significantly elevated glycated hemoglobin ($p<0.001$). However, glycated hemoglobin was a major relationship between pentraxin-3 and procalcitonin.

Conclusion: There is a substantial link between diabetic foot and variability, pentraxin-3 and procalcitonin among patients with diabetes.

Key Words: Glycated hemoglobin (HbA1c), C-reactive protein (CRP), Procalcitonin

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INTRODUCTION

Diabetic foot it is considered one of complications of besides disturbing problems of diabetes, defined by way of a foot usual by ulceration that is consistent with neuropathy and peripheral arterial disease of the minor associate in diabetes patient. The vascular complications of DM can be pretentious by some microangiopathy or macroangiopathy.[1] The macroangiopathy in T2DM is a form of earlier atherosclerosis worrying carotid, coronary in adding to peripheral arteries. This cause big vessels growth of diabetic foot ulcer in DM patient.[2] Diabetic foot is the important cause of lower end amputation in diabetic patient, known to stretch a poor prognosis to the patient.[3] Wound healing a procedure that arises subsequent rupture of the skin wall and usually eased by cytokines and growth factors out by particular cell stimulate through immuneresponse, counting fibroblasts, endothelial cell, keratinocytes and platelets. Cytokines and growth factors are significant group of the molecular method complex in make cutaneous wound healing probable.[4]

An adequate technique of analysis, treatment of the illness to prevent recurrence, and the amputation of affected limbs are all strategies that have been researched and put into practice in an effort to reduce the number of persons diagnosed with DF. A major consideration in allocating resources and deciding on treatment procedures is the accurate assessment of DF severity.[5] Additional indicators of illness severity are required to reestablish clinical choices since infection symptoms in diabetics might be hard to identify and the disease can advance quickly. Pentraxin-3 (PTX-3), C-reactive protein (CRP), and procalcitonin (PCT) are the most appropriate inflammatory indicators for this purpose out of the many that have been researched extensively.[6]

Several cells at the site of inflammation, including as adipocytes, endothelial cells, fibroblast granulosa, mesangial cells, and mono-nuclear phagocytes, generate PTX-3, a soluble gratitude receptor. After that

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point, it may be able to directly reproduce a more advanced form of vascular inflammation. Angiogenesis and restenosis are inhibited by PTX-3, an endothelial controller in ischemic vascular disease and thrombosis that binds to angiogenic fibroblast growth factor-2.[7] One of the acute-phase reactants linked to CRP is PTX-3. Their family tree is quite similar to that of PTX-3. The antimicrobial response is greatly impacted by PTX-3, in addition to its function in cell debris clearance.[8] Emerging from the body's natural inflammatory response and infection defense systems, PTX-3 may be an improved biomarker for DFU compared to other systemic indicators.[9] Cancer, cardiovascular disease, asthma, sepsis, and PTX-3 are just a few of the illnesses and disorders linked to improved health and longer life expectancy. Scientists have looked at the PTX-3 DFU.[10] Understanding the role of PTX-3 as a biomarker for DF infection, limb amputation level prognosis, and patient survival was the main goal of this study. In connection to the diagnosis of DF, the secondary aims are to assess Hba1c, C-reactive protein, procalcitonin, and duration of diabetes.[11]

A 116-amino-acid biomarker that has recently gained popularity for application in infection detection is polypeptide C-reactive tissue (PCT), which is released by thyroid C-cells, lung, liver, and kidney parenchymal cells. An influential person in this field is the brains behind PCT. If you're looking for an indicator of bacterial infection, it may be more dependable than C-reactive protein (CRP), according to some writers.[12] Virus infection and non-specific inflammatory disorders are associated with relatively modest PCT residues. This research evaluated PCT's diagnostic accuracy in respect to other inflammatory markers, such as C-reactive protein.[13]

A important indicator of diabetes mellitus is an elevated HbA1c. Instead than only checking blood sugar levels at one point in time, it alerts you to the possibility of persistent glycaemia. 50% of HbA1c is finished in the month before to sample, with 25% completed in the month before[14], and it gives a combined measure of glycaemia over the whole 120-day lifetime of the red blood cell. However, during this 120-day period, fresh glycaemia has the major influence on the HbA1c score. The purpose of this research was to examine the relationship between and variations in Pentraxin-3 level, PCT, CRP, and HbA1c in individuals diagnosed with DFI.

METHODS

This case-control study was conducted at University of Babylon, College of Medicine, Iraq from 18th June to 24th September 2024 vide letter No. 4545/QM/Approval/3FG533 dated March 01, 2024. The equation for the Daniel sample size technique was used to determine the sample size. One hundred Iraqi subjects participated in this review; fifty of them had diabetic foot ulcers. All patients had their medical histories taken, which included information on where they lived, how old they were, whether or not they smoked, any relevant family history, and any treatments that may have an impact on the outcomes. Fifty healthy subjects were used as a control group. The participants' ages ranged from 38 to 69. The modification did not include individuals with type 1 diabetes mellitus, any accompanying acute or chronic inflammatory disorders, or cancer.

Immediate analysis of serum concentrations of Pentraxin-3, PCT, and CRP using the sandwich-ELIZA kit. The micro-ELIZA plate in this kit (from Bioassay Technology Laboratory) was pre-coated with an antibody that specifically targets the Pentraxin-3 level, Procalcitonin. ROCK's COBAS INTEGR is a fully automated analyzer that assessed glycated hemoglobin A1c (HbA1c). Meters squared divided by kilos of body weight are the formula for a person's body mass index (BMI). Body mass index = Weight (kg)/Square Height (m^2). For the statistical analysis, SPSS-20 was used. P values were considered significant when they were less than 0.05.

RESULTS

The age distribution of the control and DF groups did not vary significantly (P=0.59). The removal of disparities in the findings of the analyzed parameters that were caused by large variation in age is facilitated by the similarity in age.

Table No. 1: Comparison of demographic and biochemical data of study and control groups

Variable	Control groups (n=50)	DF Group (n=50)	P value
Age (years)	49±15.22	51±10.77	>0.071
BMI (kg/m^2)	25.14±1.91	26.63±2.52	>0.063
HbA1c	5.6±0.3	7.2±0.412	<0.03
CRP (mg/dl)	33.34±20.1	125.7±88.3	<0.04
PCT (ng/ml)	0.41±0.03	1.13±0.3	<0.032
Pentraxin-3 (Pg/ml)	1130.7±730.4	3150.8±1530.9	<0.049

$p<0.05$ (Significant)

Table No. 2: Correlation coefficient between different parameter in diabetic foot groups

Variables	Diabetic Foot Group	
	r	P
Pentraxin-3 vs HbA1c	0.71	0.05
Pentraxin-3 vs CRP	0.52	0.01
Procalcitonin vs HbA1c	0.83	0.05
Procalcitonin vs CRP	0.77	0.03

Correlation is significant at $p< 0.05$

There was no statistically significant difference in body mass index (BMI) between the control group and the DF (P=0.63). The increase concentration of HbA1c, CRP, PCT and Pentraxin-3 levels, and in DF patients related with control with significant ($p<0.05$) mean changes among them (Table 1).

A significant positive relationship was detected between Pentraxin-3, PCT and HbA1c concentration. On the other hand, Pentraxin-3 level, PCT, and CRP have also significant correlation in DF patients (Table 2).

DISCUSSION

The general concept of diabetes mellitus is associated with chronic high blood sugar and also with a group of metabolic disorders. The condition worsens when complications of the disease occur. These contain large vascular difficulties e.g. cardio-vascular disease besides cerebrovascular disease. As for micro- vascular complications, they are, nephropathy retinopathy as well as neuropathy, especially distal peripheral neuropathy and complications of blood vessels in the foot that main to diabetic foot ulcers diabetic foot ulcers.[15] Health care costs, patient satisfaction, and the need for lower limb amputations are all negatively impacted. In addition to potentially leading to mortality, diabetic foot infection (DFI) often necessitates hospitalization of the patient.[16] Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Streptococcus spp., and Enterococcus spp. were the most often reported bacteria found in patients with DFI. The identification of the causative agent has a pivotal role in surgical management including the avoidance of amputation and the prevention of infection spread.[17] Unique regulations pertaining to the structure of DFI were accessible last year. The management of diabetic foot infections was helped by these strategies.[18] In reaction to inflammation and infection, especially bacterial infections, the acute-phase protein C-reactive protein (CRP) increases. Compared to healthy subjects, diabetes individuals have a higher CRP level.[19] Recently PCT has gained interest as a diagnostic tool for infected DF. In response to infection or inflammation, the thyroid C cell, liver, lungs, and kidneys stimulate the production of this peptide.[20] While clinical findings are the primary basis for an IDFU diagnosis, evaluation of inflammatory markers like C-reactive protein (CRP) and, more recently, positron emission tomography (PET) may aid in the diagnosis of infection when clinical signs are inadequate. Currently, there are only a small number of studies that have examined PCT's usefulness in diagnosing both systemic and contained bacterial infections.[21]

PTX-3 was intended to be a death and re-operation rate analyzer in DFU patients. That might be because PTX3 is already known to be an indicator of metabolic syndrome and vascular disease, both of which are prevalent in diabetic individuals. Pentraxin-3 showed a statistically significant alteration among DF besides control this might groups due to the overall inflammatory state of people through diabetes besides could be related through infection besides following sepsis in certain DF patients, Previous study , originate lesser level of PTX3 a analyst for infection besides amputation related toward control.[22]

Patients with DF had significantly higher levels of Pentraxin-3, PCT, CRP, and HbA1c compared to the control group in this research. The current research on diabetic foot found a strong association between glycosylated hemoglobin (HbA1c) and serum Pentraxin-3 and PCT. Serum pentraxin-3 and PCT were also shown to be strongly linked with C-reactive protein.

CONCLUSION

Changes in the levels of pentraxin-3, procalcitonin, C-reactive protein, and glycated hemoglobin in patients with diabetic foot infections may suggest a strong association between the two conditions.

Author's Contribution:

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Drafting or Revising Critically:	Ekhlas Hatem AL-Asadi, Hawraa M. Kadhim
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

The Relationship Between Vitamin D and Glucose Indices and Lipid Profile in Obese Diabetic Patients

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ABSTRACT

Objective: To examine the relationship between vitamin D levels, glycemic control values, and lipid profiles of obese diabetic patients.

Study Design: Descriptive study

Place and Duration of Study: This study was conducted at the College of Nursing, University of Basrah, Iraq from 1st January 2025 to 31st March 2025.

Methods: Ninety males of 60 diabetic patients and 30 healthy controls were enrolled. Serum levels of vitamin D, HbA1c, glucose, and the elements of the lipid profile (total cholesterol, triglycerides, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, and very low-density lipoprotein-cholesterol) were measured in addition to anthropometric measurements.

Results: The diabetic patients had significantly higher body mass index, Inflammation, immunology, the endocrine system, cardiovascular disease, and dyslipidemia. These are just a few of the numerous areas where vitamin D appears to play a regulatory function. Pearson correlation analysis revealed that the level of vitamin D had a significant negative relation with both HbA1c and glucose concentrations. Non-significant or weak correlations between vitamin D and lipid parameters were, however, found.

Conclusion: The possibility of poor glycemic control in obese diabetics being a factor of deficiency of vitamin D and draw attention to the possible advantages of vitamin Dmonitoring and supplementation in the treatment of diabetes.

Key Words: Vitamin D, Obesity, Diabetes mellitus, Lipid profile, Glycemic control

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INTRODUCTION

Obesity prevention is one of the most pressing issues in modern medicine, as the rate at which obesity incidence is rising throughout the world suggests a pandemic. Obesity and type 2 diabetes mellitus are significant international health issues affecting a significant proportion of the population with a high rate of prevalence and a high correlation with cardiovascular morbidity and mortality. Obesity leads to insulin resistance, chronic low-grade inflammation, and dyslipidemia, which are core factors in the emergence and progression of T2DM and its complications.[1]

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Vitamin D has long been recognized to perform extra-skeletal functions; however, of late, there has been attention on its non-skeletal extra-bone functions. There is also emerging evidence indicating this vitaminis involved in the process of glucose metabolism, insulin secretion, and insulin sensitivity by affecting pancreatic 0- cell activity and peripheral insulin sensitization and response.[2]

The effect of environmental factors and genetic predisposition on diabetes is enhanced by obesity, which overlaps with each other in its genetic as well as environmental aspects. The consequences of metabolic imbalance due to the accumulation of excess adipose tissue and food are insulin resistance, inability to autophagically break down body cells, and low-grade systemic inflammation. This causes a progressive increase in blood glucose levels and an accelerated loss of functioning β-cells.[3,4]

Recent research has shown a connection between T2DM and a lack of this vitamin.[5-7] It was pointed out that there is a connection between the diagnosis of T2DM and a lack of this vitamin. Hypovitaminosis D may contribute to insulin resistance and Diabetes Mellitus, shedding light on the disease's pathophysiology.[8] An increase in the size of the vitamin D depot and a decrease in the blood's circulating

calcidiol content can result from obesity. A lack of vitamin D is very widespread in the case of obesity and T2DM patients. It is believed that excess adipose tissue stores vitamin D, making it less biologically available, and that little sun exposure and poor diet also contribute to deficiency among this population.[9]

Vitamin D insufficiency and diabetes are both extremely common illnesses across the world.[8] Research points to a connection between the diagnosis of T2DM and a lack of this vitamin. Vitamin D can also affect lipid metabolism besides its impact on glucose metabolism. Experimental and clinical evidence indicate that vitamin D may be able to regulate lipid profiles through its ability to impact the lipoprotein lipase activity, inflammation, and the concentration of parathyroid hormone involved in lipid regulation. Low vitamin D has been linked with increased lipid profile; however, the results of studies are conflicting.[10] In low- and middle-income countries, a portion of the global population is susceptible to vitamin D deficiency because they don't eat enough foods high in vitamin D and don't get enough ultraviolet B radiation from sunshine.[11] Interest has grown significantly in recent years, with new studies concentrating on the function of vitamin D in the body.[12]

There is a need for further clinical and experimental studies on the pathophysiology of these disorders, and an attempt to understand the common mechanisms that work to develop them and increase their complications, and thus try to avoid the damage resulting from obesity and diabetes to increase the safety and efficacy of both existing and recently developed medicines. With the high rate of patients with T2DM having vitamin D deficiency, obesity, and dysmetabolic states, it is of particular clinical significance to learn how vitamin D status is linked to glucose indices and lipid profiles. The clarification of these associations can aid in the creation of prevention and treatment methods that can achieve a better metabolic outcome for obese diabetic patients. Consequently, the proposed study intends to examine the relationship between vitamin D and the glucose indexes and lipid profile among obese diabetic patients.

METHODS

The study was conducted at College of Nursing, University of Basrah, Iraq from 1st January 2025 to 31st March 2025 vide letter No. 199/5/203 dated 2/2/2025. Ninety samples in all, ranging in age from 25 to 50 years were enrolled. Before the sample was collected, the participants were given a brief explanation. The direct interviews were conducted to gather patients' medical histories and other data. Group I (control) included 30 healthy men and Group II included 60 women with diabetes mellitus. Body weight and height were among the anthropometric measurements that were taken. Using the individuals' height and weight, we calculated their BMI. We used a weight scale to determine the weight. The height was measured with a measuring stick on the weight scale.[13,14]

A serum separation tube was filled with roughly 5 milliliters of venous blood. Give it ten minutes or so to develop a clot. To extract the serum, the samples were then centrifuged for ten minutes at room temperature at 3500 rpm. The serum was kept at -20°C in a deep freezer. The COBAS INTEGRA 400 plus was used in an enzymatic colorimetric test to measure the serum. The data were analyzed using SPSS-22 and comparisons between means using least significant differences ($P \leq 0.05$). The correlation among parameters was analyzed by bivariate (Pearson) correlation.

RESULTS

The second group's body mass index (BMI) value was considerably higher than that of the control group (Table 1). The results showed that the second group's HbA1c and glucose concentration levels were noticeably higher than the control group. The findings revealed that the two groups' vitamin D levels had significantly dropped (Table 2).

Lipid profile values were significantly increased, according to the data. On the other hand, the high-density lipoprotein HDL score significantly decreased (Table 3). The associations between body mass index (BMI), glucose, triglycerides, lipid profile, and glycosylated hemoglobin were investigated using a Pearson correlation analysis. Table 4 provides a summary of the findings indicated that increasing levels of these metabolic indicators are linked to higher BMI. They demonstrated a significant positive connection with HbA1c, glucose, TG, LDL, and VLDL. Notably, vitamin D3 and BMI had a negative but non-significant correlation. Concerning glucose, TG, TC, HDL, and VLDL, HbA1c showed strong positive relationships. Moreover, HbA1c and vitamin D3 had a negative association, which means that glycaemic control and vitamin D level could be negatively related. Both TC and TG were positively correlated with glucose levels, and HDL and vitamin D3 were negatively correlated. These results are conducive to a trend of dyslipidemia related to hyperglycaemia. HDL had a high negative correlation with the majority of risk factors, such as BMI, HbA1c, and TG, in line with its cardiovascular protective effect. Vitamin D3 was inversely correlated with HbA1c and glucose, suggesting lower levels in subjects with poorer glycaemic indices, although its correlation with lipids was weak or non-significant (Table 4).

Table No. 1: Frequency of BMI level in patients and healthy individuals

Group	Mean±Standard Error
Group I (Control, N= 30)	29.83±0.85
Group II (Patients, N= 60)	36.35±0.53
P value	0.000

Table No. 2: Glycemic control comparison between groups

Group	HbA1c (%)	Glucose (mg /dL)	Vitamin D (kg/m$^{2)}$
Group I (Control)	5.85±0.08	99.11±4.42	8. 85±1.41
Group II (Patients)	9.99±0.26	255.39±13.86	3.82±0.29
P value	0.000	0.000	0.000

Table No. 3: Concentration of lipid profiles in the patients and healthy individuals

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group I (Control)	169.23±6.64	104.20±6.89	45.97±1.76	106.42±4.73	20.84±1.38
Group II (Patients)	196.36±5.18*	205.27±14.29*	24.95±1.56*	123.88±3.76*	41.06±2.86*
P value	0.002	0.000	0.000	0.007	0.000

*Significant (P≤0.05)

Table No. 4: Correlations between BMI, HBA1c, Glucose, TG, TC, HDL, LDL, VLDL, and D3

Variable	Correlation	BMI	HBA1c	Glucose	TG	TC	HDL	LDL	VLDL	D3
BMI	Pearson Correlation	1	.509**	.396**	.269*	.155	.224*	.224*	.269*	-.098
	Sig. (2-tailed)		.000	.000	.010	.146	.035	.035	.010	.357
	N	90	90	90	90	90	89	89	90	90
HBA1c	Pearson Correlation	.509**	1	.618**	.287**	.275**	.291**	.291**	.287**	-.390**
	Sig. (2-tailed)	.000		.000	.006	.009	.006	.006	.006	.000
	N	90	90	90	90	90	89	89	90	90
Glucose	Pearson Correlation	.396**	.618**	1	.269*	.344**	.182	.182	.268*	-.219*
	Sig. (2-tailed)	.000	.000		.010	.001	.087	.087	.011	.038
	N	90	90	90	90	90	89	89	90	90
TG	Pearson Correlation	.269*	.287**	.269*	1	.282**	.268*	.268*	1.000**	-.205
	Sig. (2-tailed)	.010	.006	.010		.007	.011	.011	.000	.052
	N	90	90	90	90	90	89	89	90	90
TC	Pearson Correlation	.155	.275**	.344**	.282**	1	.277**	.277**	.282**	-.186
	Sig. (2-tailed)	.146	.009	.001	.007		.009	.009	.007	.079
	N	90	90	90	90	90	89	89	90	90
DL	Pearson Correlation	-.582**	-.624**	-.336**	-.345**	-.172	-.354**	-.354**	-.345**	.344**
	Sig. (2-tailed)	.000	.000	.001	.001	.105	.001	.001	.001	.001
	N	90	90	90	90	90	89	89	90	90
LDL	Pearson Correlation	.224*	.291**	.182	.268*	.277**	1	1	.268*	-.058
	Sig. (2-tailed)	.035	.006	.087	.011	.009			.011	.591
	N	89	89	89	89	89	89	89	89	89
VLDL	Pearson Correlation	.269*	.287**	.268*	1.000**	.282**	.268*	.268*	1	-.205
	Sig. (2-tailed)	.010	.006	.011	.000	.007	.011	.011		.052
	N	90	90	90	90	90	89	89	90	90
D3	Pearson Correlation	-.098	-.390**	-.219*	-.205	-.186	-.058	-.058	-.205	1
	Sig. (2-tailed)	.357	.000	.038	.052	.079	.591	.591	.052	
	N	90	90	90	90	90	89	89	90	90

**Significance at0.01 level

DISCUSSION

The expression of vitamin D receptors (VDRs) and vitamin D-activating enzymes in pancreatic 8-cells, adipose tissue, and skeletal muscle suggests that vitamin D status can have a mechanistic relationship with metabolic regulation.[15,16] In this study, statistical comparisons demonstrated that the mean BMI of the

second group was notably higher than that of the control group, indicating that the condition diabetes associated with the second group may have influenced body weight significantly.

There are many pathophysiological pathways and complex correlations between type 2 diabetes and obesity.[17,18] This enhances the confirmed correlation between obesity and T2DM, in which adipose tissue hyperplasia supports resistance to insulin, persistent low-grade inflammation, and 8-cell dysfunction. Ruze et al[2] traced the pathophysiological role of obesity in the progression of T2DM, and its mechanisms include metabolic inflammation and ectopic fat deposition.

The findings are aligned with the existing studies that show that there are complex associations between vitamin D, obesity, glycemic control, and lipid metabolism.[19,20]

The research found that the level of glucose and HbA1c is high among diabetic patients and is accompanied by a pronounced decrease in the level of serum vitamin D. They agree with the literature that has proposed that hypovitaminosis D can affect the insulin secretion and sensitivity, worsening the glycemic control.[6] They emphasize that the absence of vitamin D influences systemic inflammation and pancreatic 8 cells and can cause the appearance of diabetes.[21-23]

Further, there were negative relationships among the vitamin D, HbA1c, and glucose levels. These negative correlations support the hypothesis that vitamin D may have some protective effect on metabolism. Wang et al[5] indicates that when vitamin D is taken, it is known to regulate insulin receptor expression and glucose transporter activity, which are known to promote insulin sensitivity and glycemic homeostasis. Also, the patients were found to have a high level of dyslipidemia. BMI and glycemic indices had a strong association with these changes. Interestingly, the correlation of HDL-C with BMI, HbA1c, and TG showed a negative correlation, which proves the inverse correlation of HDL-C with cardiometabolic risk.

Interestingly, it was observed that the weak correlations between vitamin D and lipid markers were still negative; however, the negative association between vitamin D and glycemic markers could mean that vitamin D effects are more directly exerted on glucose metabolism than on lipid profiles. This is in agreement with the findings of Klahold et al[7], who established that vitamin D supplementation was more efficient in enhancing insulin sensitivity than lipid parameters in diabetic patients. All these results are indicative that vitamin D deficiency is not only an outcome of obesity but also a possible contributing factor to the pathogenesis of T2DM and metabolic derangements related to it. This advocates the recommendation to screen and correct vitamin D, particularly in populations at risk of diabetes and obesity, as supported by the world guidelines.

CONCLUSION

A combination of unfavorable glycemic control and lipid profiles in obese diabetic patients indicates that the consideration of vitamin D status in the therapeutic management of diabetes is necessary. Further interventional studies are needed to determine whether correcting hypovitaminosis D can contribute to improved metabolic outcomes in this patient population.

Author's Contribution:

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Drafting or Revising Critically:	Duha T. Al-Taie, Zahraa Mahmoud Hussain Al-Hejaj
Final Approval of version:	All the above authors
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Original Article

Health-Related Quality of Life in Women Battling Breast Cancer

Duaa Saeed Obaid[1] and Wafaa Ahmed Ameen[2]

ABSTRACT

Objective: To assess quality of life in women with breast cancer by examining their sociodemographic and health profiles and determining the relationship between these factors and quality of life.

Study Design: Cross-sectional descriptive study

Place and Duration of Study: This study was conducted at the Marjan Medical City's Oncology Centre, Iraq from 1st April 2024 to 9th February 2025.

Methods: This cross-sectional descriptive study was conducted at Marjan Medical City's Oncology Centre, Iraq and 200 women with breast cancer were enrolled.

Results: 26.5% women between 50-59 years of age, 76% women were married, 29% have bachelors degree, 61.5% were belonged to urban areas 69% women were unemployed. The majority of applicants lived with family (93%). 27.5% had a first-degree comparative with breast cancer, whereas 54.5% had no chronic diseases and 51.5% were overweight. 42% were spotted within the earlier 1-2 years and 51% were at phase II diseases. 59.5% women treated with radical mastectomy and 53% received both radiotherapy and chemotherapy and moderate perceived social support. No association were founded among quality of life and sociodemographic factors. However, health-related issues showed solid correlations, time since diagnosis (p=0.000), surgery type (p=0.002) and type of treatment (p=0.003) were all significantly accomplished to quality of life.

Conclusion: The sociodemographic issues did not influence quality of life, health-related factors such as surgical intervention, diagnosis duration and type of treatment had a significant influence. Social support levels were moderate among participants.

Key Words: Beast-cancer, Quality of life, Sociodemographic factors, Health information, Social support

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INTRODUCTION

Health-related quality of life (QoL) is a multidimensional concept that captures the overall wellbeing of individuals in relation to their health status. It includes physical, mental, emotional and social functioning and reflects how a person's health affects their ability to live a fulfilling life. For breast cancer patients, health-related QoL is a crucial outcome as their QoL can be influenced by symptoms like pain, fatigue and psychological distress (e.g., anxiety, depression).[1,2]

Health-related QoL measures wellbeing related to or affect by the presence of a disease or treatments and it generally consist of a number of domains including physical functioning, psychological wellbeing such as levels of anxiety and depression, and social support. Ongoing symptoms, side effects of treatments, recurrence often results in a feeling of distress that affects physical and psychological functioning and impacts on lifestyle and social engagements of patients with breast cancer.[3]

Patients with breast cancer experience physical symptoms and psychosocial distress that adversely affect their health-related QoL. The World Health Organization defined health-related quality of life as involving a person's physical health, psychological state, degree of independent, social relationships, personal beliefs and environment.[4]

Health-related QoL refers to an individual's perceived physical, emotional and social wellbeing in relation to their health status, particularly in the context of chronic illnesses like cancer. It is a multidimensional idea that includes numerous key areas, including physical functioning, social relationships, emotional stability, and the capability to involve in regular daily activities. For breast cancer patients, health-related QoL mirrors not only the straight influence of the disease and its treatments, such as chemotherapy, radiation and surgery but also the wider consequences on their social interactions, mental health, and general life satisfaction.[5]

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Breast cancer is one of the most common cancers affecting women worldwide, with significant implications for both physical and emotional well-being. The journey from diagnosis through treatment and survivorship involves a range of challenges, including surgery, chemotherapy, radiation, and hormonal therapies, all of which can impact a patient's quality of life. The physical effects of breast cancer treatments such as fatigue, pain, and changes in body image are often compounded by psychological distress, including anxiety, depression, and fear of recurrence.[6]

METHODS

A descriptive cross-sectional study was conducted at Marjan Medical City, Oncology Cancer Centre, Babylon Province from 1st April 2024 to 9th February 2025 vide letter No. 4545/QM/Approval/3rvfDVDFG dated March 11, 2024 with non-probability (purposive sampling) 200 women with breast cancer with enrolled. The sociodemographic characteristics include age, occupation, education level, economic status, marital status and residence were noted. General information related to women health included having children, number of children lactation, women living with, age of menarche, first-degree relative have breast cancer, have chronic disease and which type, body mass index (kg/m^2), duration since diagnosis, stage of breast cancer, surgical intervention, type of surgery, undergoing other type of treatment. Health related quality of life scale/breast cancer patient: The quality of life-breast cancer (QOL-BC) instrument is composed of 28 items representing the two dimensions of HRQoL: physical wellbeing (seven items), psychological wellbeing (21 items). All items within each subscale are summed separately, and mean scores are calculated for each subscale. In addition, a total HRQoL mean score can be calculated. A higher overall mean score corresponds to better HRQoL. A face to face interview was conducted with women to complete the information. The data was analyzed by SPSS-23.

RESULTS

Mean age was 49.98±13.50, 76% of women were married, 29% of them had Bachelor's degree education, 61.5% urban resident, 69% unemployed, 54.5% had satisfied for something economic status. 81.5% of women had children, 25.5% of women had 1-3 children, 93% living with family, 27.5% had first degree relative breast cancer, 54.5% of women hadn't chronic disease, 51.5% overweight, 42% of women had 1-2 years of disease diagnosis, 51% of women at 2nd stage of cancer, 59.5% of women had radical mastectomy, 53% of had chemo and radiotherapy (Table 1).

The findings of table 2 related to women response to physical health domain shows that women had mean at high level of assessment in items (1,3,6, and 7) and moderate level of assessment in items (2,4, and 5).

Table No. 1: Distribution of demographic characteristics of women (n=200)

Variable	No.	%
Age (years): 20-29	21	10.5
30-39	44	22.0
40-49	28	14.0
50-59	53	26.5
60-69	35	17.5
>70	19	9.5
Marital status: Married	152	76.0
Single	23	11.5
Divorced	7	3.5
Separated	4	2.0
Widow	14	7.0
Educational level		
No read & write	36	18.0
Read & write	24	12.0
Primary school	33	16.5
High school	48	24.0
Bachelors	59	29.5
Residency: Rural	39	38.5
Urban	161	61.5
Occupation: Employee	42	21.0
Un-employee	140	70.0
Retired	18	9.0
Economic status: Satisfied	38	19.0
Satisfied for something	109	54.5
Non-satisfied	53	26.5
Have children: Yes	163	81.5
No	37	18.5
Number of children: No	119	59.5
1-3	51	25.5
4-6	23	11.5
>7	7	3.5
Women living: Alone	14	7.0
Family	186	93.0
First degree relative have breast cancer		
Yes	55	27.5
No	145	72.5
Age of menarche: 8-11	85	42.5
12-15	108	54.0
16 & above	7	3.5
Stage of Breast cancer		
1st stage		21
2nd stage		51
3rd stage		17
4th stage		11
Surgery		
Simple mastectomy		7.5
Radical mastectomy		59.5
Skin conservating surgery		3.5
No		14.5
Treatment type: Chemotherapy	41	20.5
Radiotherapy	5	2.5
Chemo & radiotherapy	106	53.0
Hormone therapy	48	24.0

Table 3 showed the physical health women had mean and standard deviation (2.990±0.656), in regard to psychological wellbeing women had mean and standard deviation (1.382±0.189). Finally, according to quality of life overall the mean and standard deviation (2.224±0.213).

There is highly significant difference between women health information (time of diagnosis, surgery and treatment type) and quality of life at p value 0.000, 0.002, and 0.003 respectively (Table 4). There is highly significant difference between women health information (time of diagnosis, surgery and treatment type) and quality of life at p value 0.000, 0.002, and 0.003 respectively (Table 5).

Table No. 2: Distribution of women response to quality of life (physical health domain)

Item	Severe		Moderate		Mild		Not present		Mean	Ass.
	No.	%	No.	%	No.	%	No.	%		
Fatigue	74	37.0	96	48.0	29	14.5	1	0.5	3.22	H
Appetite changes	61	30.5	84	42.0	42	21.0	13	6.5	2.97	M
Aches or pain	76	38.0	86	43.0	30	15.0	8	4.0	3.15	H
Sleep changes	68	34.0	73	35.5	44	22.0	15	7.5	2.87	M
Weight gain	32	16	51	25.5	42	21.0	75	34.5	2.20	M
Vaginal dryness/menopausal symptoms	129	64.5	23	11.5	12	6.0	36	18.0	3.23	H
Menstrual changes or fertility	139	69.5	9	4.5	6	3.0	46	23.0	3.21	H

Ass. = assessment, L=low (1-2), M=moderate (2.01-3), H=high (3.01-4)

Table No. 3: Distribution of women response to quality of life domains by mean and standard deviation

Scale	No.	Mean	Standard deviation	Minimum	Maximum
Physical health	200	2.990	.656	1.00	4.00
Psychological well being	200	1.382	.189	1.00	1.91
Quality of life	200	2.224	.213	1.63	2.65

Table No. 4: Difference between women demographic characteristic and quality of life

Variable		No.	Mean	Standard deviation	Significance
Age	20-29	10	2.22	.30	F=.579 P=.716 NS
	30-39	40	2.18	.20	
	40-49	48	2.25	.21	
	50-59	53	2.21	.19	
	60-69	30	2.24	.22	
	70 & more	19	2.21	.23	
Marital status	Married	152	2.22	.21	F=.324 P=862 NS
	Single	23	2.20	.22	
	Divorced	7	2.24	.16	
	Separated	4	2.31	.10	
	Widow	14	2.19	.22	
Education level	Illiterate	36	2.17	.21	F=.942 P=.441 NS
	Read & write	24	2.25	.22	
	Primary school	33	2.26	.22	
	High school	48	2.21	.19	
	Bachelor's degree	59	2.22	.21	
Residency	Rural	77	2.26	.22	T=.066, P=.948 NS
	Urban	123	2.23	.020	
Occupation	Employee	42	2.22	.16	F=.318 P=.331 NS
	Students	2	2.47	.11	
	Unemployed	138	2.21	.21	
	Retired	18	2.26	.22	
Economic status	Satisfied	38	2.20	.25	F=.159 P=.853 NS
	Satisfied or something	109	2.24	.23	
	Non satisfied	53	2.23	.21	

F=calculated value of ANOVA test, T= t test, P=p-value, NS= no significant, HS=highly significant

Table No. 5: Difference between women health information and quality of life

Variable		No.	Mean	Standard deviation	Significance
Have children	Yes	163	2.27	.21	T=.432, P=.666 NS
	No	37	2.21	.19	
No. of children	None	119	2.20	.22	F=.631 P=.596 NS
	1-3	51	2.23	.19	
	4-6	23	2.26	.24	
	7 & more	7	2.25	.17	
Women living with	Alone	14	2.22	.12	T=.022, P=.983 NS
	Family	186	2.28	.21	
1st degree relative have breast cancer	Yes	55	2.21	.20	T=.416, P=.678 NS
	No	145	2.28	.22	
You have chronic disease	Hypertension	43	2.25	.26	F=.664 P=.575 NS
	Diabetes mellitus	13	2.47	.21	
	Hypertension & DM	35	2.25	.22	
	No	109	2.21	.23	
Body mass index	Normal weight	42	2.22	.18	F=2.683 P=.071 NS
	Overweight	103	2.25	.24	
	Obese	55	2.17	.23	
Time diagnosis (years)	<1	82	2.11	.23	F=7.133 P=.000 HS
	1- <2	84	2.22	.17	
	2-5	30	2.29	.18	
	>5	4	2.14	.12	
Stage breast cancer	1st	42	2.27	.21	F=.91 P=.437 NS
	2nd	102	2.06	.23	
	3rd	34	2.14	.17	
	4th	22	2.16	.16	
Surgery	Simple mastectomy	15	2.14	.13	F=4.416 P=.002 HS
	Radical mastectomy	119	2.06	.21	
	Skin conserving	7	2.15	.13	
	Breast conserving	24	2.17	.22	
	No	35	2.16	.24	
Treatment type	Chemotherapy	41	2.19	.24	F=4.797 P=.003 HS
	Radiotherapy	5	2.10	.24	
	Chemo & Radiotherapy	106	2.26	.21	
	Hormone therapy	48	2.22	.15	

F=calculated value of ANOVA test, T= t test, P= p-value, NS= no significant, HS=highly significant

DISCUSSION

This study found no significant association between sociodemographics age, marital status, education, residency, occupation, and economic status and overall quality of life (QoL), with p-values ranging from 0.331 to 0.948. These results suggest that, during active treatment and early recovery, the cancer experience itself may overshadow demographic influences. This is supported by Abu-Helalah et al[7], who observed that once disease stage and treatment burden were accounted for, sociodemographic factors became statistically insignificant predictors of QoL.

Al-Naggar et al[8] reported that older age correlated with lower QoL among Jordanian breast cancer survivors, particularly in physical domains, attributing this to comorbidities and treatment side effects. Similarly, Hassan et al[9] found that unemployment was a strong predictor of poorer QoL in Egyptian patients due to financial strain and social role disruption. The divergence highlights the importance of context, timing, and other mediating factors such as social support and cultural expectations.

In relation to burden of physical symptoms, numerous treatment-related problems appeared as prominent alarms. Aches, fatigue and pain, menopausal symptoms and vaginal dryness, and menstrual fluctuations or fertility problems were the furthermost severe, with mean scores above 3.01. Fatigue was stated as severe by 37% of contributors, while 38% informed severe pain and aches. Menopausal symptoms and vaginal dryness were valued severe by 64.5% of women, and 69.5% stated severe fertility-related indications. Sleep and appetite changes were reported as moderate,

together with a mean score of 2.97, while weight increasing was a lesser amount of severe (M=2.20). These results repeat international literature. Schmidt et al[10] and Lee et al[11] highpoint pain and fatigue as prevalent across treatment types and closely linked to reduced physical and social functioning. Abu-Helalah et al (2024) highlighted how treatment-induced menopause intensely influences quality of life in premenopausal Arab women, mainly given cultural sensitivities around femininity and fertility.

The mean scores through quality of life fields presented that physical health had the uppermost mean score (M=2.990), whereas psychological wellbeing had the lower most cut (M=1.382). General QoL was moderately valued (M=2.224). The heavy emotional and physical toll, the low psychological wellbeing score probable influenced by opposite scoring of adverse emotional states proposes that these worries manifest as depression and anxiety, it is not sufficient to fully alleviate the psychological burden as supported by Kim et al.[12]

When discovering personal health features, no significant association found between QoL and having kids in the study, living arrangements, the number of children and family history of cormorbidities for example diabetes and hypertension, or breast cancer. Menarche age was also not related to QoL. These outcomes are consistent with Abu-Halalah et al[7], who stated that family construction did not directly influence QoL if referred by perceived social support. Likewise, whereas family history is a identified risk factor, it seems to have slight direct effect on QoL throughout active treatment, in line with the results of Hassan et al.[9] The absence of implication for chronic situations contrasts with Greenlee[13], who labeled long-term harmonious effects on fatigue and inflammation n survivorship. Although body mass index did not influence statistical significance (p-0.071), there was a tendency toward lesser QoL in obese contributos, which could reflect cultural alterations in body perception or a timing effect linked treatment stage.

Several clinical variables emerged as significant predictors of QoL. Time since diagnosis showed a strong relationship, with women diagnosed within the past year reporting the lowest QoL (M=2.149), while those diagnosed over five years ago had the highest (M = 2.386), F=7.133, p=.000. This aligns with the literature on adjustment phases, where acute distress, treatment side effects, and fear dominate the initial phase, as described by Lebel[14] and Hersch et al.[15] Over time, survivors develop coping mechanisms, regain function, and reintegrate into daily life. Nonetheless, long-term survivors continue to face issues such as lymphedema and "scanxiety," indicating the need for enduring support systems, as noted by Smith.[16]

Surgical type also significantly influenced QoL (F=4.416, p=0.002). Radical mastectomy was associated with lower QoL (M=2.272) compared to breast-conserving (M = 2.152) and skin- conserving procedures (M = 2.060). The psychological toll of radical mastectomy linked to altered body image, perceived loss of femininity, and potential sexual dysfunction has been well documented.[17] Although breast-conserving surgeries preserve physical appearance, they can also generate concerns over recurrence risk, especially in the absence of clear margins, as noted by Pinto.[18]

Treatment modality had the most significant impact on QoL (F=4.797, p=0.003). Participants who underwent combined chemotherapy and radiotherapy reported the lowest QoL (M=2.266), reflecting the cumulative burden of side effects such as fatigue, skin toxicity, and neuropathy. In contrast, those receiving hormone therapy (M=2.225) or radiotherapy alone (M=2.109) reported comparatively higher QoL. Chemotherapy's chronic effects, including "chemo brain" and peripheral neuropathy, are well documented and substantially interfere with daily life.[19,20] These results underscore the importance of balancing treatment intensity with post-treatment quality of life, especially in early-stage patients.

Healthcare-related variables clearly had a dominant influence on QoL outcomes, reinforcing the need for multidisciplinary, patient-centered care. While demographic variables were largely unrelated to QoL, factors such as time since diagnosis, type of surgery, and treatment modality proved critical. These findings emphasize the need for collaborative care models that incorporate oncologists, mental health professionals, dietitians, and physical therapists. For instance, dietary interventions are needed to address the high prevalence of overweight participants (51.5%), which is linked to inflammation and decreased physical function.[13] Mental health interventions such as cognitive-behavioral therapy and mindfulness are necessary to address the 32% of women who reported severe anxiety, consistent with the recommendations of Andersen.[21] Rehabilitative and supportive services should also be integrated to address fatigue, sleep disturbances, and long-term treatment effects.

CONCLUSION

During the active and immediate post-treatment phase, quality of life in breast cancer patients is shaped primarily by clinical and treatment-related variables rather than demographic or static personal health factors. This supports a shift in intervention strategies toward comprehensive, personalized, and holistic care frameworks that respond to the evolving and multifactorial nature of patient needs across the cancer care continuum.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Duaa Saeed Obaid, Wafaa Ahmed Ameen
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Final Approval of version:	All the above authors
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Systemic Review

Comparative Efficacy and Safety of Mesenchymal Stem Cell Therapy and Hyaluronic Acid in Knee Osteoarthritis: A Systematic Review and Meta-Analysis

MSc Therapy and Hyaluronic Acid in Knee Osteoarthritis

Mohammed Farhan A Alfarhan

ABSTRACT

Knee osteoarthritis (OA) is the most common d disease of degenerative nature leading to the pain, reduced mobility, and diminished quality of life. Currently, treatment options are conservative approaches and advanced therapies like mesenchymal stem cell (MSC) therapy and hyaluronic acid (HA) in the injectable form Current systematic review assesses the effectiveness, safety, and comparative outcomes of MSC therapy versus HA in managing knee OA.

Methods: This systematic review and meta-analysis was conducted following PRISMA guidelines (PROSPERO registration: CRD42023473958). Literature searches across multiple databases identified randomized clinical trials (RCTs) comparing MSC therapy with HA in knee OA patients. Risk of bias was assessed using RoB 2.0, and meta-analyses employed a random-effects model to aggregate data from eligible studies.

Results: Four RCTs involving 120 patients were encompassed. Studies showed that MSC therapy, especially at higher doses, improved pain, function (WOMAC and VAS scores), and cartilage quality compared to HA. MSC-treated groups exhibited sustained benefits, including improved joint mobility and reduced inflammation, over long-term follow-ups. Meta-analysis indicated significant heterogeneity ($I^2 > 80\%$), limiting definitive conclusions on pooled results. No severe adverse events were considered for MSC therapy, underscoring its safety profile. Risk of bias ranged from low to intermediate across studies.

Conclusion: MSC therapy shows promise in improving clinical outcomes for knee OA compared to HA. However, high heterogeneity in study outcomes and intermediate risk of bias warrant caution. Further large-scale, well-designed RCTs are necessary to verify these findings and optimize therapy protocols.

Key Words: Knee osteoarthritis, mesenchymal stem cells, hyaluronic acid, systematic review, meta-analysis, regenerative medicine

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INTRODUCTION

Knee osteoarthritis (OA) is a prevalent deteriorating joint disease primarily affecting the knee joint, constituting the most common form of arthritis [1,2]. This condition arises as the protective cartilage that cushions the ends of the bones in the knee gradually wears down over time. Cartilage degradation, essential for smooth joint movement, leads to signs and symptoms such as swelling, stiffness, pain and a diminished range of mobility in the affected knee[3].

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Symptoms often worsen with time, impacting daily activities like walking or climbing stairs[4]. Therapy options range from conventional modalities like lifestyle changes, physical therapy, and medications to major interventions such as surgery in severe cases[2]. Treatment of knee osteoarthritis involves a variety of medications tailored to address symptoms and enhance the overall quality of life for individuals with KA. Topical NSAIDs, such as diclofenac creams or patches, provide localized relief when applied directly to the affected joint. In cases of more severe symptoms, corticosteroid injections may be administered directly into the joint to provide temporary relief from inflammation and pain. Hyaluronic acid (HA) injections, known as visco-supplementation, aim to improve joint lubrication and reduce pain[6].

This therapy aims to stimulate local cells, encouraging their participation in the repair process. Even with ongoing research and clinical studies investigating the safety and efficacy of mesenchymal stem cell therapy for knee arthritis, there is a need of consensus on its overall efficacy and safety in comparison to HA[7]. Therefore, this review aims to appraise and summarize

existing evidence regarding the outcomes, safety, and potential benefits of MSC in comparison to hyaluronic acid, providing a comprehensive assessment of the current state of knowledge on these regenerative interventions for knee arthritis management.

METHODS

Focused question and protocol registration: Prior to commencement of the review, a focused question was constructed via the Participants, Intervention, Control, and Outcomes (PICO) principal as recommended in the Preferred Reporting Items for Systematic Reviews and Meta-analysis[8]. The focused question was: Does mesenchymal stem cell (MSC) therapy lead to higher improvement in clinical outcomes in comparison to hyaluronic acid in patients with KA?'. A protocol was registered on PROSPERO (registration number CRD42023473958).

Literature search: A comprehensive electronic search for studies published from inception to 15th November 2025 was carried out by a medical information specialist across multiple databases, including PubMed/MEDLINE, Cochrane Library, CENTRAL, EMBASE, Scopus, and ISI Web of Knowledge. The search strategy used a string of keywords and Medical Subject Headings (MeSH) related to stem cell–based therapies, hyaluronic acid, and degenerative knee osteoarthritis: [((stem cell therapy) OR (mesenchymal stem cells) OR (stem cells) OR (progenitor cells) OR (undifferentiated cells) OR (precursor cells) OR (pluripotent cells) OR (multipotent cells) OR (embryonic cells) OR (primary cells) OR (primitive cells) OR (germ cells) OR (differentiation-capable cells)) AND ((hyaluronan) OR (sodium hyaluronate) OR (hyaluronate) OR (HA) OR (glycosaminoglycan) OR (hyaluronic polymer) OR (hyaluronic gel) OR (hyaluronic fluid) OR (hyaluronic serum) OR (hyaluronic solution) OR (viscosupplementation)) AND ((knee degenerative joint disease) OR (knee osteoarthritis) OR (degenerative arthritis of the knee) OR (knee joint degeneration) OR (knee cartilage wear) OR (knee joint osteoarthrosis) OR (knee articular degeneration) OR (knee joint degenerative disorder) OR (osteoarthrosis of the knee) OR (knee OA))].

Eligible studies were randomized controlled trials (RCTs) that directly compared the clinical efficacy of mesenchymal stem cell (MSC) therapy with hyaluronic acid in patients with degenerative knee osteoarthritis. Studies were excluded if they were non-comparative cohort studies, case series, case reports, letters to the editor, or published in languages other than English or Arabic due to reviewer language constraints. Additionally, studies involving traumatic or non-degenerative forms of knee osteoarthritis were excluded.

Data extraction: Following piloting the data extraction forms two reviewers extracted the data independently. General data regarding the study author(s) and date, study groups, location details, and study setting was extracted. Furthermore, the following data was extracted associated with the patient population: ethnicity, sex, mean/median age, age, selection criteria, and duration of the disease. Mean differences in all outcomes, such as pain, quality of life, function, and other tests such as blood biomarker levels were extracted. The data was tabulated using Microsoft Excel, and any conflicts were resolved by discussion.

Risk of bias assessment: RoB assessment, utilizing RoB 2.0, covered areas like randomization, change from intended interventions, missing data, outcome measurement, and reported result selection. Criteria for bias judgment were explicitly defined. Data synthesis methods, statistical or narrative, and provisions for addressing heterogeneity and sensitivity were outlined.

Meta-analysis: The meta-analysis was performed on Review Manager (RevMan) software version 5.4, applying a random-effects model to account for any possible heterogeneity among the included studies.[9] A systematic search strategy was employed, and predefined inclusion criteria were applied to identify eligible studies. Data extraction was individually conducted by two reviewers (blinded to each other), with disagreements resolved through discussion or consultation with an additional reviewer.

RESULTS

Literature search: Initial search resulted in 1253 items. After exclusion of 1243 irrelevant articles on the basis of titles, 9 articles were selected for screening of full texts. After 5 articles were further excluded (reasons explained in Table 1), 4 randomized clinical trials that met our PICO criteria were included[10-11].

Patient characteristics of the studies

The table presents detailed information from four distinct studies focusing on osteoarthritis (OA) treatment in Spain and China. In the study by Vega et al. (2015)[10] conducted in Spain with 30 participants, the inclusion criteria involved Grade II-IV OA and chronic knee pain, with exclusion criteria such as infection and immunosuppression. The mean age was 57 years, with 17 females and 13 males. In the study by Lamo-Espinosa et al. (2016)[11], 30 patients with knee OA were randomly chosen to receive intraarticular HA alone (control) or hyaluronic acid along with either 10×10^6 or 100×10^6 cultured autologous BM-MSCs respectively, and included individuals aged 50-80 with knee OA. In their 2018 study[12], the authors then followed-up 26 of those 30 patients 4 years after the end of this study. Exclusion criteria included various conditions like polyarticular disease and recent arthroscopy. The studies compared high and low-dose mesenchymal stem cells (MSC) with hyaluronic acid (HA) treatment, reporting mean ages and gender distributions. In the Chinese study by Ho et al. (2022)

with 30 participants[13], eligibility criteria included age 50-65, primary knee OA, and a pain level ≥5 for at least 2 months. Exclusion criteria comprised conditions like alcoholism and recent steroid-based therapy. MSC and HA treatments were compared, reporting mean ages and gender distribution. These studies contribute diverse insights into OA treatment approaches, participant demographics, and outcomes.

Treatment regimens and overall outcomes

Vega et al 2015[10]: In this study, one group received intra-articular injections of allogeneic bone marrow MSCs, while the other was given intra-articular HA as a control. Over one year, the MSC-treated group showed significant enhancements in algofunctional indices (WOMAC and VAS), indicating enhanced pain relief and functional outcomes compared to the hyaluronic acid-treated control group. Quantitative magnetic resonance imaging T2 mapping revealed a notable reduction in areas of poor cartilage in the MSC-treated cohort, suggesting improvements in cartilage quality. The findings emphasize the potential efficacy of allogeneic MSC therapy, showcasing positive outcomes in pain alleviation, functional improvement, and cartilage quality enhancement for chronic knee osteoarthritis (Table 2).

Table No.1: General characteristics of the included study.

Study	Participants (N)	Country	Selected inclusion criteria	Selected exclusion criteria	Age (mean/median; years)	Sex (n)
Vega et al 2015	30	Spain	Grade II – IV osteoarthritis, chronic knee pain of mechanical origin, 18 – 75 years	Infection, co-morbidities, pregnancy, breast-feeding, neoplasia, immunosuppression	57 ± 9 years	F: 17; M: 13
Lamo-Espinosa et al 2016	30	Spain	Males and females aged 50–80, diagnosis of knee OA according to American College of Rheumatology criteria, visual analogue scale	Previous diagnosis of polyarticular disease, severe mechanical extra-articular deformation (>15° varus/15° valgus), systemic autoimmune rheumatic disease, arthroscopy	High-dose MSC: 57.8 (55.0 - 60.8) years Low-dose MSC: 65.9 (59.5 - 70.6) years HA: 60.3 (55.1 - 61.1) years	M: 19 F: 11
Lamo-Espinosa et al 2018	26	Spain	Males and females aged 50–80, diagnosis of knee OA according to American College of Rheumatology criteria, visual analogue scale	Previous diagnosis of polyarticular disease, severe mechanical extra-articular deformation (>15° varus/15° valgus), systemic autoimmune rheumatic disease, arthroscopy	High dose MSC: 65.9 (58.3 – 69.5) Low dose MSC: 57.8 (54.4 – 63.0) HA: 60.6 (58.9 - 61.1)	F: 8 M: 17
Ho et al 2022	30	China	Twenty eligible patients, aged between 50 and 65 years (58.00 4.51 years), affected by primary OA of the knee of Kellgren-Lawrence (K-L) grade 2–3 and with a pain level equal to or higher than 5 on a Visual Analogue Scale (VAS) scale of 10 for at least 2 months were recruited with informed consent.	Alcoholism or drug abuse; pregnancy and breast-feeding; serious pathologies such as carcinoma	MSC: 56.7 ± 4.83 HA: 59.1 ± 4.04	F: 12 M: 8

Lam Espinosa et al 2016[11]: In this phase I/II multicenter randomized clinical study with an active control (HA), 30 knee OA patients were randomly chosen to receive intra-articular HA alone or HA combined with either 10×10^6 or 100×10^6 cultured autologous bone marrow–derived mesenchymal stem cells (BM-MSCs). No treatment-related adverse events were reported following BM-MSC administration or throughout the follow-up period. Patients treated with BM-MSCs exhibited sustained improvements in pain and functional outcomes, as measured by the Visual Analog Scale (VAS) as well as Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Radiographic assessment revealed progressive narrowing of the knee joint space in the control group, which was not observed in the high-dose

BM-MSC group. Furthermore, magnetic resonance imaging (MRI) evaluated using the Whole-Organ Magnetic Resonance Imaging Score (WORMS) demonstrated modest reductions in joint structural damage, predominantly in the high dosage BM-MSC cohort. Overall, these findings suggest that intra-articular BM-MSC therapy, especially at higher doses, is associated with sustained improvements in pain, functional capacity, and joint integrity in patients with knee osteoarthritis (Table 2).

Lam Espinosa et al 2018[12]: This study investigated the long-term effects of mesenchymal stromal cell (MSC) treatment on knee osteoarthritis (OA) based on the evaluation of patients from the previous randomized clinical trial detailed above (Lam Espinosa et al 2016) (CMM-ART, NCT02123368). The study concludes that a single intraarticular injections of autologous BM-MSCs are a safe and viable treatment option for knee OA, resulting in long-term clinical and functional improvements for knee OA (Table 2).

Ho et al 2022 [13]

This study aimed to evaluate the effectiveness of autologous bone marrow–derived mesenchymal stem cells (BM-MSCs) in comparison to hyaluronic acid (HA) in patients with no prior surgery with knee osteoarthritis. In this single-blind, single-center randomized clinical study, 20 patients were enrolled and equally allocated to receive intra-articular injections of either cultured BM-MSCs or HA. Clinical outcomes were assessed at baseline and at 12 months, including pain intensity, functional status, and quality of life, along with radiographic and magnetic resonance imaging (MRI) evaluations to assess compositional changes in joint cartilage. Overall, the findings indicate that intra-articular administration of autologous BM-MSCs resulted in superior pain relief, functional improvement, and enhancement of quality-of-life measures compared with hyaluronic acid at one year (Table 2).

Table No.2: Summary of findings reported in the included studies.

Study	Source of MSCs	Doses and number of MSCs	Time points of outcomes assessed (months)	Study groups (n)	Duration of study	Post-treatment follow-up	Outcomes of interest reported	Serious/ severe adverse effects	Overall outcomes
Vega et al 2015	Allogenic	40 X 10^6 cells (1 dose)	BL, 3, 6, 12 months	MSC (15) HA (15)	12 months	NR	VAS, WOMAC	None	Higher improvement in MSC-treated patients.
Lamo-Espinosa et al 2016	Autologous	Low dose: 10 × 10^6 or High dose: 100 × 10^6 (1 dose)	BL, 3, 6, 12 months	Low dose MSC + HA (10) High dose MSC + HA (10) HA (10)	12 months	NR	WOMAC, VAS	None	MSC groups resulted in higher improvement up to 12 months.
Lamo-Espinosa et al 2018	Autologous	10 × 10^6 or 100 × 10^6 (1 dose)	BL, 3, 6, 12 months *	Low dose MSC + HA (8) High dose MSC + HA (8) HA (9)	5 years	4 years	WOMAC, VAS	None	MSC groups resulted in better improvements in WOMAC and VAS scores than HA alone.
Ho et al 2022	Autologous	6 X 10^6 cells (1 dose)	BL, 1, 3, 6, 9, 12 months	MSC (10) HA (10)	12 months	NR	WOMAC, VAS, SF36	None	MSC groups resulted in better improvements in WOMAC and VAS scores than HA.

* This study was a 4-year follow-up of Lamo-Espinosa et al 2016. Hence, only 4-year follow-up data is presented to avoid repetition.
BL = Base-line; WOMAC = Western Ontario and McMaster Universities Osteoarthritis index; VAS = Visual Analog Scale; SF36 = 36-Item Short Form Survey; MSC = Bone-Marrow Derived Mesenchymal Stem Cells; HA = Hyaluronic Acid; NR = Not Reported.

Table No.3. Risk of bias assessment of the included studies.

Study (author, year) (Name)	Domain					
	ROB arising from the randomization process	**ROB due to deviations from the intended outcomes**	**ROB due to missing outcome data**	**ROB in measurement of outcome**	**ROB in selection of the reported result**	**Overall ROB**
Vega et al 2015	Low	Some concerns	Low	Low	Low	**High**
Lamo-Espinosa et al. 2016, 2018	Low	Low	Low	Low	Low	**Low**
Ho et al 2022	NI	Some concerns	Low	Some concerns	Low	**Some concerns**

Results of the meta-analysis: The findings of the meta-analysis are presented in Figures 2 (WOMAC scores 12 months after administration) and 3 (VAS scores 12 months after administration). Due to the variability in the outcome measurement, only the scores at 12 months from two studies could be pooled. Dude to high heterogeneity of the outcomes (I^2 = 85% for WOMAC and 89% for VAS) it was difficult to ascertain the overall efficacy of MSC on improving both of these indices.

Risk of Bias Assessment: Table 3 summarizes the risk of bias (ROB) assessment for three studies: Vega et al. (2015)[10], Lamo-Espinosa et al. (2016, 2018)[11,12], and Ho et al. (2022) [14]. The evaluation includes different domains of potential bias. Vega et al. (2015)[10] is characterized by low ROB arising from the randomization process and missing outcome data but raises concerns in deviations from the intended outcomes, measurement of outcome, selection of the reported result, leading to an overall high ROB. In contrast, Lamo-Espinosa et al. (2016, 2018) exhibit low ROB across all domains[11,12], indicating a generally low risk of bias. Ho et al. (2022)[13] shows unclear risk in the randomization process, low risk in missing outcome data and selection of the reported result, but some concerns in deviations from the intended outcomes and measurement of outcome, resulting in an overall intermediate risk of bias.

DISCUSSION

Considering the diverse findings across these studies, several factors could contribute to the superior outcomes observed in BM-MSC-treated groups compared to HA alone. First, the regenerative properties of BM-MSCs may play a crucial role in promoting tissue repair and reducing inflammation, contributing to long-term improvements in pain and function[14]. Additionally, the ability of BM-MSCs to differentiate into various cell types, including chondrocytes, may facilitate the regeneration of damaged cartilage, a key aspect in OA management[15]. The studies also suggest that higher doses of BM-MSCs may lead to more significant and sustained improvements, as seen in Lamo-Espinosa et al. (2018) where the high-dose BM-MSC group exhibited notable decreases in WOMAC scores and joint damage over the long-term[11,12]. Assessing the impact of different medications on knee osteoarthritis involves a comprehensive evaluation of various outcomes. One crucial aspect is pain relief, measured through standardized scales like the Visual Analog Scale (VAS), as a reduction in pain intensity signifies the effectiveness of the medication[16]. The WOMAC is a questionnaire used to evaluate knee osteoarthritis severity. It assesses pain, stiffness, and physical function, with respondents rating the intensity and frequency of their symptoms. Scores range from 0 to 96, with higher scores indicating more severe symptoms and functional limitations. Healthcare providers use WOMAC scores to track changes over time and assess the effectiveness of treatments in managing knee osteoarthritis symptom Functionality and physical activity are assessed through tests or patient-reported outcomes, highlighting improvements in the ability to perform daily tasks and maintain an active lifestyle. Evaluating joint stiffness, particularly after periods of inactivity, provides insights into improved joint mobility. Radiographic assessments, such as measuring joint space width through X-rays, help gauge the medication's impact on osteoarthritis progression[17]. Quality of life is assessed using questionnaires that capture the psychological, social, and recreational aspects affected by knee OA[18].

The collective evidence from the studies described in this review supports the potential efficacy of BM-MSCs as a treatment for knee OA, showcasing improvements in pain relief, functional outcomes, and cartilage quality. The regenerative properties, differentiation capabilities, and autologous nature of BM-MSCs contribute to their promising therapeutic potential. Furthermore, no serious and severe adverse effects or failures of treatment were reported 4 years after a 12-month observation of the cohort treated by Lam Espinosa et al [17,18]. Future clinical trials required with large sample sizes and prolong follow-up durations are warranted to further validate the effectiveness and safety of BM-MSC therapy for knee OA. However, it's important to interpret the overall results of this systematic review with caution, as some aspects of the studies exhibit a risk of bias, and the meta-analysis was inconclusive in deducing the overall comparative efficacy and safety of stem-cell therapy relative to HA. Further well-designed studies with large sample sizes

and prolong follow-up durations are warranted to further validate the effectiveness and safety of BM-MSC therapy for knee OA. These studies should also aim to address potential confounding factors and biases to provide more robust evidence for the use of BM-MSCs in OA management.

CONCLUSION

In conclusion, while BM-MSC therapy holds promise as a potential treatment for knee OA, additional research is warranted to fully elucidate its therapeutic effects, optimal dosing regimens, and long-term safety profile. Nevertheless, the existing evidence suggests that BM-MSCs offer a feasible option for improving the management of knee OA and may represent a valuable addition to current treatment strategies.

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Case Report

A Case Report of Primary Amoebic Meningoencephalitis in Thi-Qar Province, Southern of Iraq

Shaymm Abd Al Hussein Khalaf, Bassad A. AL-Abood

ABSTRACT

Naegleria fowleri is one of the most important free living amoeba can caused usually fatal disease, such as "primary amoebic meningoencephalitis" is a rare. We diagnosed of amoebic meningoencephalitis by Naegleria fowleri. This case was the first study in the province of Thi Qar to be recorded. This case was identified in Nassaryia City, where the patient was an 11-year-old boy. Thi-Qar province Naegleria fowleri was diagnosed in direct cerebral spinal fluid sample and cerebral spinal fluid cultured on non-nutrient agar medium, the flagellated and amoeboid trophozoite stages were observed, as well as the cystic form. The molecular detection of Naegleria fowleri showed PCR product was 183bp for 18srRNA gene. The cerebral spinal fluid must be microscopically examined in order to identify the amoeba stages and rule out the bacterial cause. Accurate and rapid diagnosis is important to treat the condition as quickly as possible Genetic identification can also be used to diagnose amoeba. Amoebic meningoencephalitis treatment may benefit from a prompt diagnosis. Preventing amoebic meningoencephalitis infection can be achieved in part by refraining from swimming or utilizing freshwater. Finally, clinical and laboratory personnel must be trained on the importance of prompt diagnosis and proper patient care for individuals with suspected primary amebic meningoencephalitis.

Key W: Primary amebic meningoencephalitis, Naegleria fowleri

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INTRODUCTION

The highly infectious free-spirited amoeba Naegleria fowleri is predominantly harvested from freshwater and soil during the sun-drenched summer months, as the sweltering temperatures forge a perfect environment for its proliferation and dissemination.[1] Naegleria fowleri, a self-sustaining amoeba, thrives in thermal freshwater, flourishing best in temperatures ranging from 35 to 46 degrees Celsius, and multiplies through the process of binary fission. This parasite undergoes a tripartite life cycle comprising a dormant cyst phase, a mobile flagellate phase, and a vigorous amoeboid trophozoite phase; notably, the amoebic form is consistently identified in brain biopsies, and tragically, the disease proves fatal regardless of timely and accurate diagnosis due to the challenge of delivering effective drug concentrations to the infection site.[2]

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The first recorded human infection case by N. fowleri took place in South Australia, documented by Fowler and Carter.[3] This remarkable microbial organism often thrives alongside various other microbial counterparts. Within the natural world, microorganisms frequently engage in interactions with eukaryotes.[4] Such interactions occur between humans and free-living amoebas; Primary amebic meningoencephalitis (PAM) is an uncommon and swiftly progressing illness that generally culminates in death within a mere 3 to 6 days. The condition is instigated by the bacterium Naegleria fowleri and has a dire impact on the central nervous system. The emergence of symptoms follows shortly thereafter.[5] Dubbed the "brain-eating" thermophilic flagellate amoeba, primary rhizopodan meningoencephalitis stands as a lethal and debilitating waterborne affliction.[6] Naegleria fowleri makes its journey to the brain, traversing the cribriform plate and infiltrating the body through the nasal canal.[7] Alternatively, it may navigate via the olfactory nerve and cribriform plate to reach the olfactory bulbs. Thus, it causes the central nervous system (CNS) to be destroyed, which ultimately results in primary amebic meningoencephalitis.[8] Globally, N. fowleri has been reported, notably in Thailand, Taiwan, and Hong Kong. America and Australia.[9] There has only been one instance reported in to yet in the north of Iraq in Mousal City.[10] In another study, it was widely diagnosed N. fowleri in Iraq by AL-Aboody[11] in clinical and environmental samples and founded widespread in

contaminated water and soil. Our case, this is first case in Thi-Qar and the second in Iraq, and none of them survived because of a severe infection in Brain, very late cases and increase of cerebrospinal fluid (CSF).In our study and othere studies we observed increased accuracies and prevalence of N. fowleri and other species of Naegleria such as N. polary in water and soil in huge numbers in all studies presented in Iraq.

CASE REPORT

In this case, cerebrospinal fluid was obtained from Dr. Muhannad Al-Assadi's private laboratory, Iraq vide letter No. 4545/QM/Approval/SJKDH379 dated March 9, 2024, belongs to an 11-year-old boy who lives in the city of Nasiriyah. On September 2022, patient was sever from headache, fever, fatigue, also a facial rash and myalgia. After that, he was transferred to a hospital to receive the necessary treatment. but he died before any treatment was given. He was sever from his vital signs such as temperature, 39.1°C; respiratory rate, 32 breaths/min; blood pressure, 122/56 mmHg. The blood test showed the leukocytes count of 11cells/mm3 with 60% neutrophils and C-reactive protein (CRP) of 285 mg/L and a low glucose of 15 mg/dL,. Then, lumbar puncture was dispensed, the CSF was turbid. Within twenty-four hours of collection, one milliliter of CSF sample was grown on non-nutrient agar (NN-agar) medium in duplicates in the lab. The samples were then incubated in 26 C0, and amoebic growth was monitored every day using a light microscope on a slide. and examined weekly with addition page amoebic slain (PAS) solution .The cultured of CSF show clearly movements of amoeboid trophozoites ,flagellate stages and cyst during examined on light microscope .Also, Nelson's growth medium with fetal calf serum can be used to culture Naegleria fowleri (Nuha,2022). N. fowleri trophozoite was observed with oval shape which measured 10–20 µm in size (Fig. 1). Naegleria amoeboid trophozoite. Figure 2 showed (A) cyst and(B) cyst with flagellated trophozoites form. Figure 3 emergence trophozoites excyst when trophozoite have suitable environment such as increase food.

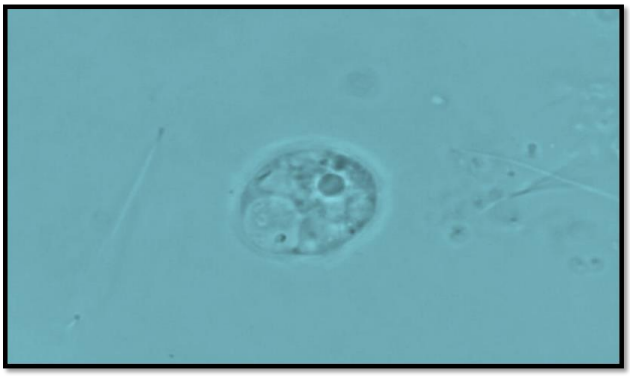
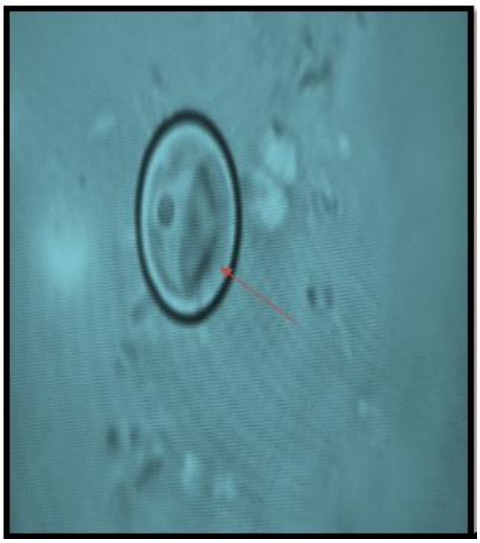


Figure No. 1: Naegleria fowleriamoeboid trophozoites (unstained)



A **B**

Figure No. 2: A Naegleria fowleri A-cyst stage. B} cyct and flagellated trophozoite and cyst from cultured CSF (unstained)

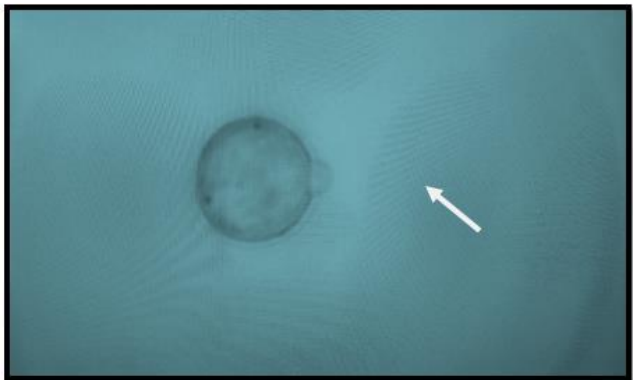


Fig. 3: Naegleria fowleri start emergence trophozoite from the cyst (unstained)

Analysis of Genetics: Genomic DNA was meticulously isolated from both direct CSF and CSF cultures, adhering to the company's guidelines using the innovative gSYNC TM Kit for DNA Extraction (Geneaid, Taiwan). Employing the 18S rRNA gene, PCR amplification was deftly utilized to detect the presence of N. fowleri. The PCR reaction was conducted within a total volume of 25 µl, encompassing 5 µl of each primer (10 mM), 12 µl of nuclease-free water, along with 5 µl of the Green Master Mix (Pre-Mix master mix Biolab/UK), and 5 µl of template DNA sourced from the CSF sample. The AccuPower® PCR PreMix Kit, which contains all essential components necessary for a successful PCR reaction, features tracking dye, dNTPs, KCl, MgCl2, stabilizer, and Tris-HCl at pH: 9.0.. The primers sequence applied (Bioneer/Korea) in the current study accord-ing to a previous study (Schild et al., 2007).Naegleria fowleri forward primer 3' -5' CAAACACCGTTATGACAGGG and Reveres primer 5' -3'

CTGGTTTCCCTCACCTACG (Schild et al., 2007). PCR step of thermo-cycling The conditions were: 1 minute of initial denaturation at 95 °C; 35 seconds of second denaturation at 95 °C for 35 cycles; 35 seconds of annealing at 58 °C for 35 cycles; and 40 seconds of extension at 72 °C for 35 cycles. However, the last extension was for ten minutes at 72°C. The PCR technique was performed for detection pathogenic Naegleria fowleri (FLA) based on subunit ribosomal rRNA gene (18SrDNA gene) from cell culture of N. fowleri, this study using using convential PCR. The PCR product was electrophoresed in 0.8% or 1.5% agarose gel powder, stained with Ethidium bromide, and visible under a UV lamp. The nucleotides from the DNA sequencing were sent to a Macrogene company in Korea, and the global isolates in Gen Bank were compared using BLAST.Ladder DNA (1517 bp) and the amplification band size of 183 bp were compared with primers to get the final results for PCR.

Figure No. 4: Image Agarose gel electrophoresis that show the PCR product (183bp) analysis of 18S r DNA gene from genomic DNA of Naegleria fowleri from CSF of patients with primary amebic meningoencephalitis. This image show (1,3 band direct from CSF and7,8 band from CSF cultured)

The sequencing and analysis of PCR products of Naegleria fowleri from CSF sample and CSF culture were showed 97. 96% homology identity to Naegleria fowleri. The 18S rRNA gene (accession numbers KT375442.1, OD958550.1, and MW033524.1) corresponds to Naegleria fowleri, which was found in a fatal case of primary amoebic meningoencephalitis in a Norwegian traveller who had just returned from Thailand.

DISCUSSION

The occurrence of Naegleria fowleri is surging among the youth and children in Iraq. This marks the second instance of reporting PAM in Iraq, with Thi-Qar being the location of the inaugural case. Typically linked to aquatic pursuits like diving and head immersion, the primary culprit behind this affliction is the inhalation of tainted water that finds its way into the nasal passages, potentially leading to the cribriform plate via the nasal cavity. Between 1962 and 2017, there were 143 documented cases of PAM in the USA.[12] Most of the patients in Chinese cases had prior encounters with fresh water.[13] The CSF chemistry of PAM mirrors that of bacterial meningitis, showcasing a decrease in glucose alongside an uptick in protein levels. N. fowleri fails to thrive on conventional culture media, as it necessitates bacteria-infused agar for growth. When "clearly motile" N. fowleri trophozoites make an appearance on the wet preparation of a fresh CSF sample, microscopy gains reliability.[14] Successful treatment of PAM cases hinges on swift and precise diagnosis.[15] A history of water exposure holds immense significance for prompt diagnosis. The clinical symptoms observed in this case study were remarkably similar to those in previously documented cases.[14] In other research efforts within Iraq, they validated the presence of highly contaminated samples with N. fowleri in both clinical and environmental contexts within Thi-Qar.[11] N. fowleri exhibits potent pathogenesis and produces regulatory surface proteins that shield the amoeba from the cytotoxic elements of the complement system, orchestrating lysis to deftly elude the host's immune defences.[7] Through the lens of molecular techniques, N. fowleri trophozoites, measuring 10–20 microns in diameter, can be seen to exhibit motility in freshly collected wet CSF samples under light microscopy. Nonetheless, the number of identifiable amoebae might be quite limited. These challenges can be effectively addressed via the molecular strategy of PCR testing.[16,17]

CONCLUSION

Primary amoebic meningoencephalitis is one of the most important and widespread diseases in Iraq, as evidenced by its spread in Iraqi water, frequent cases of meningitis after exposure to water swimming pools, rivers, as well as the water used for drinking that reaches homes, is also contaminated with this parasites, sterilizing drinking water in Iraq because it is also used to wash the body and accidentally inhaled by children and adults. CSF examined must be considered essential in identification PAM disease infection. Naegleria fowleri is considered one of the causes of meningitis throughout Iraq and Thi-Qar in particular. This is the first case recorded in Thi-Qar and the second case after case recorded in Mosul. Chlorinating heavily utilized swimming pools in Iraq - one part per million will halt the spread of N. fowleri. Local public health officials in high-risk areas ought to consider keeping an eye out for N. fowleri in recreational waterways, particularly in Iraq in the sweltering summer months.

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