Vol. 36, No. 9 September, 2025

ISSN 1029 - 385 X (Print) ISSN 2519 - 7134 (Online)

MEDICAL FORUM MONTHLY

RECOGNISED BY PMDC & HEC

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"Medical Forum" Monthly Recognised, Indexed and Abstracted by

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- EMBASE SCOPUS Database Since 2000
- Registered with International Standard Serial Number of France bearing ISSN 1029-385X (Print), ISSN 2519-7134 (Online) Since 1992
- Registered with Press Registrar Govt. of Pak bearing No.1221-B Copr. Since 2009
- ABC Certification Since 1992
- On Central Media List Since 1995
- Medical Forum Affiliated with Medical Academic Foundation (MAF)
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- EScience Press (CrossRef DOI)
- Email: med_forum@hotmail.com, medicalforum@gmail.cicalom
- website: www.medicalforummonthly.com, www.medforum.pk

MEDICAL FORUM MONTHLY

ISSN 1029 - 385 X (Print) ISSN 2519 - 7134 (Online)

APNS Member ✸ CPNE Member ✸ ABC Certified

Online Journal Published Since 1989

Online www.medicalforummonthly.com

on OJS Scopus Open Access Peer Reviewed

Affiliation With: Medial Academic Foundation (MAF) (Regd.)

Recognized, Indexed & Abstracted by

PMDC-IP-0048 (1998), HEC-Y-Category (2009), Excerpta Medica Netherlands (2000), EMBASE SCOPUS Database (2000), Index Medicus (IMEMR) WHO (1997), Cross Ref (DOI), SJR, HJRS, SCI Journal, Research Gate, Resurchify, Editage, Enago, Research Bib, Research Bite, Pastic and PSA, NLP, Pakmedinet & CPSP

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doi:10.60110/medforum

Business Manager: **Nayyar Zia Ch.**

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Published By: **Prof. Nasreen Azhar, Gohawa Road, Link Defence / New Airport Road, Opposite Toyota Motors, Lahore Cantt. Lahore.**
Mobile Nos. 0331-6361436, 0300-4879016, 0345-4221303, 0345-4221323.
E-mail: med_forum@hotmail.com, medicalforum@gmail.com
Website: www.medicalforummonthly.com

Printed By: **Naqvi Brothers Printing Press, Darbar Market, Lahore.**

Affiliation With: **Medial Academic Foundation (MAF) (Regd.)**

Ombudsman: **Dr. Munawar Abbas, Assistant Professor / HOD Medical Sciences, Times University, Multan.**
Mobile No. 0312-9233333 Email: drmabbas786@gmail.com

Rate per Copy: **Rs.3000.00**

Subscription Rates : (annually) **Pakistan (Rs.30000.00), USA & Canada (US$ 500.00), China, Japan, UK &Middle East (US$ 450.00)**

Med. Forum, Vol. 36, No.9 September, 2025 ISSN 1029-385-X (Print) ISSN 2519-7134 (Online)

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Editorial

Body Tremors – A Public Health Issue

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

Introduction

Tremor is one of the most common movement disorders and can interfere with a person's ability to work or perform basic activities of daily living[1,2.] Tremor is a movement about a joint that is involuntary, rhythmic and oscillatory[3]. Tremor is a neurological condition that includes shaking or trembling movements in one or more parts of the body, most commonly affecting a person's hands. It can also occur in the arms, legs, head, vocal cords, and torso. The tremor may be constant, or only happen sometimes. Tremor can occur on its own or as a result of another disorder.

Symptoms of Tremor

Tremor can make daily life tasks such as writing, typing, eating, shaving, and dressing more difficult.

Common symptoms may include Rhythmic shaking of the hands, arms, head, legs, or torso, Shaky voice, Difficulty with writing or drawing, small handwriting, loss of smell, trouble sleeping, trouble moving or walking, constipation, soft or low voice, masked face, dizziness and fainting. Some tremor can be triggered by stress or strong emotion.

Causes of Tremor

Tremor is most common among middle-aged and older adults, although it can occur at any age. Generally, tremor occurs in men and women equally.

Tremor is usually caused by a problem in the parts of the brain that control movements. Most types have no known genetic cause, although there are some forms that appear to be inherited and run in families.

Tremor can occur on its own or be a symptom of other neurological disorders such as Parkinson's disease, multiple sclerosis and stroke. Tremor sometimes can be caused by other medical conditions, including but not limited to:

- **Medicines.** Several drugs can cause tremors, including certain asthma medications, corticosteroids, chemotherapy, and drugs used for certain psychiatric and neurological disorders.
- **Heavy metals and other neurotoxins**. Exposure to heavy metals (such as mercury, manganese, lead, arsenic, etc.), organic solvents, or pesticides may cause tremors.
- **Caffeine.** Excessive caffeine may cause temporary tremor or make an existing tremor worse.
- **Thyroid disorder.** An overactive thyroid can cause tremors.
- **Liver or kidney failure.** Liver and kidney failure may cause damage in certain brain areas that leads to tremors or jerky movements.
- **Diabetes**. High or low blood sugar (hyperglycemia or hypoglycemia, respectively) may cause tremors or other involuntary movements.
- **Stress, anxiety, or fatigue** may be associated with tremors.

Types of tremor

Tremor is categorized based on when and how the tremor is activated. These categories are rest and action tremor.

Rest tremor occurs when people are at rest. People with Parkinson's disease often experience rest tremor.

Action tremor occurs when a muscle is moved voluntarily. There are several sub-classifications of action tremor, many of which overlap.

- **Postural tremor** occurs when holding a position against gravity, such as holding the arms outstretched.
- **Kinetic tremor** is associated with any voluntary movement, such as moving the wrists up and down or closing and opening your eyes.
- **Intention tremor** starts when the person makes an intended movement toward a target, such as lifting a finger to touch their nose.
- **Task-specific** tremor only appears when performing goal-oriented tasks such as handwriting or speaking.
- **Isometric tremor** occurs during a voluntary muscle contraction that is not accompanied by any movement, such as when holding a heavy book in the same position.

Tremor syndromes are defined based on the pattern of the tremor. Some of the most common forms include:

Essential tremor

Essential tremor is one of the most common movement disorders. Its key feature is a tremor in both hands and arms during action without other neurological signs. It also may affect a person's head, voice, or lower limbs. Although the tremor can start at any age. The exact cause of essential tremor is unknown.

Dystonic tremor

Dystonic tremor occurs in people who are affected by dystonia—a movement disorder in which incorrect messages from the brain cause muscles to be overactive, resulting in abnormal postures or sustained, unwanted movements.

Cerebellar tremor

Cerebellar tremor is typically a slow, big (high amplitude) tremor of the arms, legs, hands, or feet that worsens at the end of a purposeful movement such as pressing a button. It is caused by damage to the cerebellum and its pathways to other brain areas, often from a stroke or tumor, injury from a disease or an

inherited disorder, or from chronic damage due to alcohol use disorder.

Functional tremor

Functional tremor (also called psychogenic tremor) can appear as any form of tremor. Its symptoms may vary but often start suddenly and fluctuate widely.

Enhanced physiologic tremor

Enhanced physiologic tremor typically involves a fine amplitude (small) action tremor in both the hands and the fingers. It is generally not caused by a neurological disease but by reaction to certain drugs, alcohol withdrawal, or medical conditions including an overactive thyroid and hypoglycemia. It is potentially reversible once the cause is corrected.

Parkinsonian tremor

Parkinsonian tremor is common and one of the first signs of Parkinson's disease, although not all people with Parkinson's disease have tremor. Its shaking is most noticeable when the hands are at rest and may look as if someone's trying to roll a pill between the thumb and a finger. Parkinson's tremor may also affect the chin, lips, face, and legs. The tremor may initially appear in only one limb or on just one side of the body but may spread to both sides as the disease progresses. The tremor is often made worse by stress or strong emotions.

Orthostatic tremor

Orthostatic tremor is a rare disorder characterized by rapid muscle contractions in the legs that occur when a person stands up. The tremor usually stops when the person sits down or walks.

Epidemiology

In 2021 online survey of people with essential tremor found that nearly one-third of respondents were not seeing a physician[4]. These low rates of engagement with health systems are due to the mild nature of many ET cases, but other factors like access to care or health literacy may also play a role[5]. Epidemiological studies have established that ET is a common condition, especially among people aged 65 years and older, and that many ET cases in the general population are undiagnosed.

Genetics

A positive family history has been reported in 30–70% of patients with ET[6]. There is increased risk (4.7 fold) of developing ET for people with a first-degree relative with ET[7].

Pathophysiology

The cerebello-thalamo-cortical circuit is the main network involved in ET. This has been supported by postmortem, functional, and structural neuroimaging and electrophysiological studies[8].

Diagnostic criteria

Essential tremor is clinically diagnosed by a review of medical history, family history, and a physical examination. The examination evaluates for the presence of postural or kinetic tremor of the arms, legs, head, and voice. Criteria that help make this diagnosis include a family history of tremor, response to alcohol, and a long history of tremor, in addition to the absence of other neurological signs such as dystonia, ataxia, or parkinsonism[9].

Treatment of Tremors

There is no cure for most forms of tremor, treatments are available to help manage symptoms. In some cases, symptoms may be mild enough that they do not need treatment. Treating any underlying health condition can sometimes cure or reduce a person's tremor.

Medications

Some medications can slow tremor. Some medications commonly used to treat tremor include Beta-blocking drugs can treat essential tremor and other types, Tranquilizers (also known as benzodiazepines), Dopaminergic medications, Anticholinergic medications and Botulinum toxin (commonly known as Botox) injections can be useful for dystonic head tremor and hand tremor.

Surgery

Surgical procedures may be performed when tremor does not respond to medications or severely impacts daily life.

Deep brain stimulation (DBS) is the most common form of surgical treatment of tremor. It uses surgically implanted electrodes to send high-frequency electrical signals to the thalamus, the deep structure of the brain that coordinates and controls some involuntary movements. A small pulse-generating device placed under the skin in the person's upper chest (similar to a pacemaker) sends electrical stimuli to the brain to temporarily stop tremor. DBS is currently used to treat parkinsonian tremor, essential tremor, and dystonia.

Lifestyle changes for treating tremor

Certain lifestyle changes and techniques may provide some relief for mild to moderate tremor.

- Physical, speech, and occupational therapy may help control tremor and adapt to daily challenges caused by the tremor.
- Eliminating or reducing caffeine.
- Assistive tools, such as special plates, spoons, or heavier utensils can lessen tremor and make it easier to eat.
- Take medications on time. Talk with a doctor about stopping any medications that may be contributing to the tremor.
- Reduce stress or stressful situations that can aggravate the tremor.
- Wear clothes that make it easier to dress, such as those that use Velcro instead of buttons. Consider slip-on or no-tie shoes.
- Get enough sleep. Some tremors worsen when a person is tired. Physical activity and exercise can help prevent fatigue and improve sleep.

Vibration therapy

A recently published study evaluated the efficacy of a handheld vibratory device for the treatment of ET

called the Vilim Ball. The device vibrates between 8 and 18 Hz and 0–2 mm in amplitude. Seventeen participants with ET were treated with the device for 5 min minutes and tremor power was measured by accelerometer before and after treatment[10].

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Original Article

Effectiveness of Early Versus Delayed Surgical Intervention in Bile Duct Injury Post Cholecystectomy

Rumman Khan, Musarrat Hussain, Yousaf Jan, Aqib Ali Khan, Gohar Ali and Ammar Asadullah Khan

ABSTRACT

Objective: To compare the clinical outcomes of early versus delayed surgical repair in patients with post-cholecystectomy bile duct injury (BDI).

Study Design: Prospective observational study

Place and Duration of Study: This study was conducted at the General Surgery Department of Hayatabad Medical Complex, Peshawar from June 2023 to December 2024.

Methods: A total of 45 patients with BDI were divided into an early intervention group (n=22; definitive repair within 6 weeks) and a delayed intervention group (n=23; repair after ≥6 weeks following conservative management). Patients diagnosed with BDI were included in the study. All patients were managed with Roux-en-Y hepaticojejunostomy performed by hepatobiliary surgeons. We evaluated intraoperative parameters, postoperative complications, hospital stay, and long-term outcomes. Statistical analysis was performed using SPSS version 25, with $p<0.05$ considered significant.

Results: The results show mean age is 47.3 ± 12.1 years, with a male-to-female ratio of 1:1.5. Most BDIs occur after laparoscopic cholecystectomy were included in study. The early intervention group experience significantly higher postoperative complication rates compared to the delayed group (40.9% vs. 17.4%, p=0.03), including bile leaks, surgical site infections, and sepsis. Hospital stay is longer in the early group (12.5 ± 3.8 vs. 9.2 ± 2.6 days, p=0.01). There are no significant differences in operative time, blood loss, intraoperative complications, or long-term outcomes such as anastomotic stricture and recurrent cholangitis. The delay in intervention group has a higher, though not statistically significant, overall success rate (91.3% vs. 81.8%, p=0.43).

Conclusion: Delayed surgical repair of post-cholecystectomy BDIs is associated with fewer postoperative complications and shorter hospital stays without compromising long-term outcomes. These findings support a delayed repair approach after initial resuscitation and inflammation resolution.

Key Words: Bile duct injury, cholecystectomy, hepaticojejunostomy, early repair, delayed repair, surgical outcomes

Citation of article: Khan R, Hussain M, Jan Y, Khan AA, Ali G, Khan AA. Effectiveness of Early Versus Delayed Surgical Intervention in Bile Duct Injury Post Cholecystectomy. Med Forum 2025;36(9):4-8. doi:10.60110/medforum.360901.

INTRODUCTION

Laparoscopic surgery has been regarded as the standard treatment for symptomatic gallstone disease because it reduces perioperative pain and the need for analgesics, shortens hospital stays, and allows patients to return to normal activities more quickly than open surgery[1]. Cholecystectomy is one of the most common abdominal operations performed worldwide. Bile duct injury (BDI), which happens in roughly 0.3–1.5% of procedures, is a rare but dangerous complication even though it is usually safe[2]. These are devastating injuries with sequelae extending beyond the early postoperative period[3].

BDI comprises a spectrum of lesions extending from partial laceration to complete transection or ischemic destruction of the ducts, frequently complicated by associated vascular injury[4]. In severe cases, reconstruction of biliary continuity often requires complex operations such as hepaticojejunostomy[5]. Even if successful, it is associated with long-term complications such as secondary biliary cirrhosis, recurrent cholangitis, anastomotic strictures and poor quality of life.[6]

One of the most contentious issues in the treatment of BDI is when definitive surgical repair should be performed. There are basically two main ways to handle this issue: delay repair, which is typically done after six weeks[7], and early repair, which is typically thought of as those cases done two weeks (≤14 days)

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Received: April, 2025
Reviewed: May-June, 2025
Accepted: July, 2025

following injury. Proponents of early repair emphasize the ability to reduce psychological anxiety in patients, prevent subsequent biliary injury, and manage chronic inflammation[8]. Decompression of acute inflammation and oedema, according to proponents of delayed repair, provides a better plane for dissection with a lower risk of postoperative morbidity[9].

Owing to significant inflammation, maximal tissue softening, and the lowest threshold for technical failure, the intermediate phase—which falls between these two categories of treatments and usually takes place two to six weeks following injury—is normally the least preferred of all[10]. The clinical state of the patient, the degree of anatomical injury, the availability of skilled hepatobiliary surgeons, and institutional knowledge all play a role in the scheduling decision.

In addition to having severe financial and quality-of-life ramifications for healthcare systems, bile duct injury is a rare complication of cholecystectomy that can have catastrophic effects on patients. Both early and late surgical repairs are viable therapeutic alternatives; but, in this particular case as well, the best time to proceed will rely on consensus. While delayed repair may provide a potentially safer reconstruction, early repair may stop the advancement of biliary injury. However, the intermediate window is likewise thought to have unsolvable technical issues. Therefore, it is essential to evaluate the advantages and effectiveness of early versus late repair in this subgroup in order to guide clinical practice and enhance patient-centered outcomes for patients undergoing cholecystectomy with BDI.

METHODS

This prospective observational study was conducted at the department of general surgery, Hayatabad Medical Complex in Peshawar over a period of 18 months (June 2023 to December 2024). Patients that sustained a BDI following cholecystectomy were included in the study. A total of 45 patients with post-cholecystectomy BDI were enrolled. Patients were divided into two groups:

Early intervention group (n=22): Assuming local sepsis and patient optimization, participants in the early intervention group had definitive surgical repair within 6 weeks of injury.

Delayed intervention group (n=23): Patients in the delayed intervention group were treated conservatively for six weeks with endoscopic or percutaneous drainage, while the inflammation decreased before thrombin-based repair.

The diagnosis of bile duct injury was made using radiographic evidence, biochemical markers (elevated bilirubin/liver enzymes), and clinical suspicion (jaundice, bile leak, abdominal pain, or fever). Ultrasound, MRCP (Magnetic Resonance Cholangiopancreatography), and contrast-enhanced CT were often used imaging modalities as needed. In some cases, ERCP was performed for ductal mapping and, if practical, temporary drainage or stenting.

Consultant liver and hepatobiliary surgeons carried out all procedures. Roux-en-Y hepaticojejunostomy was the conventional surgical treatment for large BDIs. To guarantee the best plane for accurate anastomosis, all patients underwent intraoperative cholangiography and a meticulous dissection of the hepatic stump. The amount of blood loss, operative time, intraoperative complications, and the operative findings were recorded.

Immediate postoperative complications such bile leak, sepsis, surgical site infection, and reoperation were among the measured postoperative outcomes. Anastomotic stricture, recurrent cholangitis, and the requirement for further intervention through imaging and clinical and laboratory follow-up were assessed as long-term outcomes. Every patient had a follow-up from six months to one year.

A standardized proforma was used to gather the data, and SPSS version 25 was used for analysis. Age, length of surgery, and length of hospital stay were examples of continuous variables that were presented as mean with standard deviation. Categorical variables (such as reconstruction success and complication rates) were analyzed using the chi-square test. At $p < 0.05$, the significance level was set. The Hayatabad Medical Complex Ethical Review Committee gave their approval to the study. Informed consent papers were signed by each participant.

RESULTS

The mean age of patients was 47.3 ± 12.1 years, with a male-to-female ratio of 1:1.5. The majority of BDIs occurred following laparoscopic cholecystectomy (82.2%), while the remaining were post-open cholecystectomy (17.8%).

Table No.1: Baseline Characteristics of Study Participants

Variable	Early Intervention	Delayed Intervention	p-value
Age (years), mean ± SD	46.5 ± 11.8	48.1 ± 12.4	0.65
Gender (male: female)	9:13	8:15	0.78
Type of Cholecystectomy			
- Laparoscopic (%)	18 (81.8%)	19 (82.6%)	0.59
- Open (%)	4 (18.2%)	4 (17.4%)	
Injury Type (Strasberg Classification)			
- Type E1 (%)	6 (27.3%)	5 (21.7%)	0.42
- Type E2 (%)	9 (40.9%)	11 (47.8%)	
- Type E3 (%)	5 (22.7%)	4 (17.4%)	
- Type E4 (%)	2 (9.1%)	3 (13.1%)	

All patients underwent Roux-en-Y hepaticojejunostomy (HJ) as the definitive surgical procedure. The early intervention group had a significantly higher rate of postoperative complications (40.9% vs. 17.4%, p=0.03), including bile leaks, surgical site infections, and sepsis. The delayed intervention group had fewer complications and no reoperations required. Patients in the early intervention group had a longer hospital stay (12.5 vs. 9.2 days, p=0.01), likely due to higher complication rates.

Table No.2: Intraoperative and Postoperative Outcomes

Outcome	Early Intervention	Delayed Intervention	p-value
Operative Time (min), mean ± SD	185.4 ± 32.6	172.8 ± 28.9	0.18
Blood Loss (mL), mean ± SD	320 ± 85	290 ± 78	0.21
Intraoperative Complications	2 (9.1%)	1 (4.3%)	0.61
Postoperative Complications			
- Bile Leak (%)	3 (13.6%)	1 (4.3%)	**0.03**
- Surgical Site Infection (%)	4 (18.2%)	2 (8.7%)	
- Sepsis (%)	2 (9.1%)	1 (4.3%)	
- Reoperation (%)	2 (9.1%)	0 (0%)	
Hospital Stay (days), mean ± SD	12.5 ± 3.8	9.2 ± 2.6	**0.01**

Patients were followed for 6–12 months postoperatively to assess long-term complications, particularly anastomotic strictures and recurrent cholangitis. There was no statistically significant difference in long-term stricture rates or recurrent cholangitis between the two groups. However, the delayed intervention group had a numerically higher overall success rate (91.3% vs. 81.8%), though this was not statistically significant.

Table No.3: Long-Term Outcomes

Outcome	Early Intervention (n=22)	Delayed Intervention (n=23)	p-value
Anastomotic Stricture (%)	4 (18.2%)	2 (8.7%)	0.42
Recurrent Cholangitis (%)	3 (13.6%)	1 (4.3%)	0.34
Need for Reintervention (%)	3 (13.6%)	1 (4.3%)	0.34
Overall Success Rate (%)	18 (81.8%)	21 (91.3%)	0.43

DISCUSSION

The optimal timing for repair of bile duct injuries following cholecystectomy is a debate in hepatobiliary surgery. This is a valuable study that addresses the existing and controversial concepts of BDI management through comparison early Vs delayed surgical intervention. This extensive discussion is then followed by the assessment of our results in contrast with recent literature to critically consider consistent and inconsistent findings divergent from those reported here, and suggest possible explanations for between-study discrepancy.

Postoperative complication rates were a great way to distinguish between different intervention timings in our study. The study by Guerra et al[11], where the early vs. delayed was 35% to 18%, further supports this comparison. As a result, these parallels across many trials support the idea that the best outcomes from HBP surgery are achieved when inflammation following biliary trauma is managed prior to beginning restoration of a complex bile duct injury. Some explanation for this is provided by the classic work of Kambakamba et al[12] which found that early repairs are unsuccessful mainly because of continued periductal inflammation with eventual anastomotic breakdown. Additionally, our data on individual complications and the nearly three-fold higher incidence of bile leaks following early surgery (13.6 vs. 4.3%) are consistent with De Reuver et al.'s findings in the Dutch Bile Duct Injury Registry, which point to a suspect anastomosis due to acute edema and friability[13].

However, the advantages of delayed repair in our study extend beyond immediate postoperative morbidity; patients who underwent surgery after 48 hours also appeared to have a noticeably lower length of hospital stay (9.2 vs. 12.5 days). The cost-effectiveness analysis cited in Yang et al[14], whose model likewise indicated significant cost reductions if practice delayed because patients incur lower expenditures from complications, is consistent with this finding. However, this somewhat contradicts the findings of the Giuliante et al[15] study, which found no difference in the duration of hospitalization between the early and delayed timing groups.

During our cohort's follow-up, we observed a trend favoring the delayed group in terms of anastomotic stricture development, but no significant difference between the early and delayed groups (18.2% vs. 8.7%). This result lies somewhere between what is reported in the literature, Iannelli et al[16] demonstrated a significantly higher risk of strictures in early repair (23% vs 9%), whereas long-term patency rates were equal with or without early repair in Sreepathi et al[17]. These contradictory findings could be the result of a change in the biliary reconstruction technique. The conclusive early repair (ER) technique applied by our surgical team employed meticulous mucosa-to-mucosa approximation under magnification as opposed to a high-vs undiscriminating anastomosis that might still be possible even if mild inflammation is present.

The absence of an associated reduction in long-term stricture rates in response to the higher complication rate associated with early treatment was of special interest. Recently, two theories have been put out in the literature to explain this seeming paradox. Many early complications, including transient bile leakage, can be effectively handled without compromising long-term anastomotic function, as demonstrated by Kapoor et al[18]. According to the study by Torreta et al[19], timing may not be as important as the surgeon's experience and hospital volume. This could help to explain why, at our high-volume center, both groups have comparatively good long-term outcomes despite having different early morbidity profiles.

A few key points need to be taken into consideration when interpreting the safety and effectiveness of delayed repair in light of our findings. At first, the prospective method had a significant advantage in elective cases, but we agreed with a study of another researcher that certain clinical circumstances called for early management. Full biliary obstruction, which cannot be overcome with stenting, or unremittent sepsis despite adequate drainage are examples of situations in which one should think about deviating from the recommended delayed approach, according to the clinical decision tree in algorithms of the Americas Hepato-Pancreato-Biliary Association (AHPBA)[20]. Given that this only occurred under specific circumstances, this qualified viewpoint enables us to assess our overall results in favor of a delayed repair with minimal concern about an occasional need for the earlier intervention.

This study has limitations, including a single-center study design and a relatively small sample size (n = 45), which reduces the probability of generalizability compared to a larger multi-center trial; selective non-randomized allocation introduces the possibility of selection bias based on timing decisions that reflect clinical factors rather than protocol; strictures may develop after 6–12 months, leaving late strictures beyond a year in the follow-up interval. Further future research in larger RCTs with longer follow-up periods, improved patient selection using biomarkers like Claassen's cytokine profiling, and the inclusion of quality-of-life parameters to help more fully define surgical success over postoperative outcomes are all necessary to support recommendations for integrating these tools as aids to support individualized, timely decision-making.

CONCLUSION

We conclude that delayed (> 6 weeks) repair post-cholecystectomy bile duct injuries result in less morbidity and shorter postoperative hospital stays with similar long-term outcomes. This adds to mounting evidence for delayed repair when clinically feasible as it allows inflammation to settle and probably minimizes comorbidities. Conversely, our findings also show that in the hands of an experienced surgeon early repair may still result in good outcomes indicating that some portion of the risks associated with early intervention are mitigated by surgical expertise.

Recommendations: Further randomized, multi-center studies with extended follow-up are recommended to validate and generalize these findings.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Rumman Khan, Musarrat Hussain, Yousaf Jan
Drafting or Revising Critically:	Aqib Ali Khan, Gohar Ali, Ammar Asadullah Khan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.1759 Dated 25.05.2023

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Original Article

The Prognostic Value of C-Reactive Protein Levels in Patients with Community Acquired Pneumonia

Arshia Ijaz, Afsheen Batool Raza and Madiha Tahir

ABSTRACT

Objective: To determine the predictive accuracy of C-reactive protein levels in patients with community-acquired pneumonia in predicting the complications of pneumonia.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Pediatric medical unit of The Children's Hospital & UCHS Lahore from 05 December 2024 to 05 May 2025.

Methods: Non-Probability Consecutive Sampling technique used. The calculated with 95% confidence level with 7% margin of error and 80% magnitude 115 cases. A total of 115 children fulfilling the selection criteria were enrolled after taking written informed consent from parents. Clinical proforma was used to record data of the patients, e.g. demographic profile, C-reactive protein value at admission and on day 4, fever, cough, tachypnea, wheezing etc. The outcomes of interest were assessed as: 7-day mortality; need for mechanical ventilation and/or inotropic support; development of complicated pneumonia.

Results: Of these 115 study cases, 78(67.8%) were male patients, while 37(32.2%) were female patients. Mean age of our study cases was 27.65±14.68 months. Of these 115 study cases, 95(82.6%) were vaccinated and 20(17.4%) were un-vaccinated. Mean CRP level in our study was 8.23±6.12 mg/L and it was raised (>10mg/L) in 53(46.1%). Complications were noted in 37(32.2%), while sensitivity, specificity, PPV, NPV and diagnostic accuracy was 83.8%, 71.8%, 58.5%, 90.3% and 75.7%, respectively.

Conclusion: Our study results support use of serum CRP levels in children presenting with severe pneumonia as it was found to be highly sensitive, specific, high positive predictive value & negative predictive value and high diagnostic accuracy. All clinicians treating such patients can employ serum CRP levels for early diagnosis followed by timely management achieve desired outcomes.

Key Words: Pneumonia, Complications, Predictive Accuracy.

Citation of article: Ijaz A, Raza AB, Tahir M. The Prognostic Value of C-Reactive Protein Levels in Patients with Community Acquired Pneumonia. Med Forum 2025;36(9):9-13. doi:10.60110/medforum.360902.

INTRODUCTION

Pediatric community-acquired pneumonia in children can be identified by specific respiratory symptoms. These include cough, sputum production, rapid breathing, high fever, abnormal sounds during breathing, elevated or decreased white blood cell count, and findings on chest X-ray.

This type of pneumonia occurs outside hospitals and triggers a strong inflammatory response. Interleukins associated with CAP prompt the liver to generate the acute phase protein CRP, linked to disease severity prediction and clinical decisions as per current research.[1-3] CAP is a leading cause of death globally, particularly affecting children. Pneumonia contributes significantly to child mortality, with 14.9% of deaths under age five attributed to it. Around 0.9 million children die from pneumonia annually, making it a major cause of pediatric fatalities. Research indicates a range of infectious agents, including viruses and bacteria, causing pneumonia, with viral infections being predominant among patients.[4,5] No proven prognostic methods exist for determining the best site of care for CAP, a crucial decision. Around 48.2% had moderate to severe disease. Despite being a common cause of global deaths, CAP incidence decreased between 2000 and 2013 due to improved healthcare access, nutrition, vaccinations, and lifestyle changes, impacting its etiology, epidemiology, and mortality.[4,6] In a study of 1,222 community-acquired pneumonia patients, CRP levels were analyzed in 268 patients with specific diagnoses. Mean CRP levels were different in patients with various types of pneumonia. The sensitivity, specificity, and predictive values at a 25 mg/dL cut-off were 0.6, 0.83, 0.3, and 0.94.[7]According to UK research by Dr. Robin P. Smith, CRP is a sensitive marker for

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Received: June, 2025
Reviewed: July, 2025
Accepted: August, 2025

pneumonia. A persistently high CRP level suggests antibiotic failure or new infection. The study suggests CRP, not TNF or IL-6, as a pneumonia marker. In a 2008 study, a CRP level below 100 mg/L at admission indicated lower 30-day death risk. Failure to reduce CRP by 50% after day 4 signals poor prognosis in community-acquired pneumonia, making CRP an independent severity indicator.[8,9]Unfortunately, there is very limited data available on severity assessment tools for CAP in Asia particularly Pakistan. Owing to different demographic, environmental, socioeconomic and regional status of Pakistan especially central and southern Punjab regions, this study is proposed to study prognostic value of CRP in children of these regions which mostly come to Lahore for the treatment. Proper assessment is very important for better management of CAP and to make the policies for its prevention in the region in order to meet the sustainable development in the country.

METHODS

The prospective cross-sectional study was conducted at Children's Hospital and Institute of Child Health Lahore from 05 December 2024 to 05 May 2025. An informed consent of guardian was taken. Patients mentioned in the inclusion criteria who have diagnosed community acquired pneumonia were selected by non-probability consecutive sampling. A sample size of 115 was calculated with 95% confidence level, with 13% margin of error, expected sensitivity and specificity of CRP as 60% and 83% with expected prevalence of complications as 48.2%.[6,7]

Patients who signed consent form, in the age range 6 months to 56 months and those who were recently diagnosed as severe community acquired pneumonia according to operational definition were included in this study. Patients not willing for study, age below 6 months or above 56 months, patients with other respiratory ailments and patients being managed on OPD basis were excluded from this study. Clinical proforma was used to record data of the patients e.g. demographic profile, C-reactive protein value at admission, fever, cough, tachypnea, wheezing etc. The outcomes of interest were assessed as: 7-day mortality; need for mechanical ventilation and/or inotropic support; development of complicated pneumonia (lung abscess, empyema, or complicated para pneumonic effusion) etc. Data was analyzed using SPSS version 23.0. Characteristics of the population under study were noted in terms of frequencies. Qualitative variables e.g. gender and complications on both techniques were expressed as percentage or number while numerical variables e.g. age and CRP levels were expressed as mean and standard deviation. Predictive accuracy of CRP was calculated. Data was stratified with regards to gender and age. Post-stratification, predictive accuracy was calculated.

Operational definitions

Community Acquired Pneumonia

Cough, sputum production, tachypnea for age, a core body temperature > 38.0, rhonchi or cretps on auscultation, TLC > 10 or < $4*10^9$ cells L-1, and infiltrates on chest X-ray.

C Reactive protein

The acute phase protein C-reactive protein (CRP), produced by liver in response to IL-6, is used to either identify inflammation brought on by acute diseases or to track the progression of disease in cases of chronic conditions. CRP levels over 10 mg/L was regarded as positive in this investigation. CRP was measured at admission**.**

True Positive CRP

CRP > 10mg/L with any of the above mentioned complications.

True Negative CRP

CRP < 10mg/L and absence of any above complications.

False Positive CRP

CRP > 10mg/L and absence of any above complications.

False Negative CRP

CRP < 10mg/L and presence of any of above complications.

Sensitivity

TP/TP+FNx100

Specificity

TN/TN+FPx100

Positive Predictive Value (PPV)

TP/TP+FPx100

Negative Predictive Value (NPV)

TN/TN+FNx100

RESULTS

Our research comprised a total of 115 individuals who met the specific criteria set for selection in our study. Among these 115 cases under investigation, 78 individuals, constituting 67.8% of the total, were male, whereas the remaining 37 individuals, making up 32.2%, were female. The average age of the participants in our study was calculated to be 27.65±14.68 months, with the youngest individual being 6 months old and the oldest being 56 months old. Our findings have revealed that a significant portion of our study population, specifically 77 individuals, which accounts for 67.0%, were aged ≤30 months. Within the cohort of 115 subjects, 43 individuals, representing 37.4%, resided in rural areas, while 72 individuals, constituting 62.6%, lived in urban settings. An assessment of the socio-economic status disclosed that 38 individuals, amounting to 33.0%, were identified as having a poor socio-economic background, whereas 77 individuals, making up 67.0%, belonged to the middle-income category. The average duration of the disease among the participants was determined to be 8.43±4.32

days, with 69 individuals, accounting for 60.0%, reporting an illness duration exceeding one week. Out of the 115 individuals included in the study, 95 individuals, representing 82.6%, had received vaccinations, while the remaining 20 individuals, making up 17.4%, were not vaccinated. The mean level of C-reactive protein (CRP) in our study was documented as 8.23±6.12 mg/L, with levels exceeding 10mg/L observed in 53 individuals, constituting 46.1%. (Table 1). Complications associated with the condition were observed in 37 individuals, representing 32.2% of the study population(Fig:1) while the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated to be 83.8%, 71.8%, 58.5%, 90.3%, and 75.7%, respectively. Regarding distribution of CRP level with regards to complications, Of the 53 people who tested positive for CRP, 31 (58.49%) experienced complications, and 22 did not. In contrast, out of 62 people with a CRP of negative, only 6 (9.68%) experienced complications, whereas 56 did not. This suggests a substantial correlation between elevated CRP levels and a higher chance of complications. (Fig 2).

Table No.1: Frequency distribution of different variables

Gender	**Frequency**	**Percent**
Male	78	67.8
Female	37	32.2
Total	115	100.0
Age groups		
≤30 months	77	67.0
>30 months	38	33.0
Total	115	100.0
Duration of disease		
≤1 week	46	40.0
>1 week	69	60.0
Total	115	100.0
Vaccination		
Yes	95	82.6
No	20	17.4
Total	115	100.0
CRP		
Positive	53	46.1
Negative	62	53.9
Total	115	100.0

The ability of C-reactive protein (CRP) to predict complications can be elucidated by stratifying diagnostic accuracy by age. Whereas CRP-negative data indicate 6 and 35 instances of complications, CRP-positive results in newborns ≤30 weeks correspond with 25 occurrences of complications and 11 without. While CRP-negative findings indicate 0 and 21, CRP-positive results in newborns older than 30 weeks are associated with 6 occurrences of complications and 11 without. Clinical professionals can better manage baby health by customizing CRP tests based on age with the use of this data. (Fig 3)

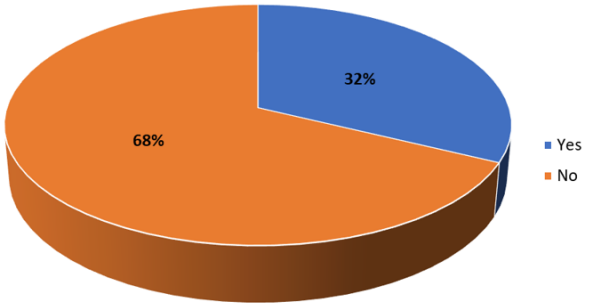

Figure No.1: Distribution of complications

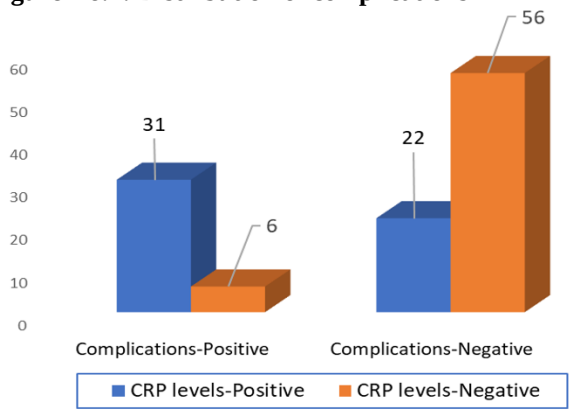

Figure No.2: Distribution of CRP levels with regards to complications

P value is 0.001 which is significant
Sensitivity = 83.78 % Specificity= 71.79 % PPV = 58.49 % NPV = 90.32 %
Diagnostic Accuracy = 75.65 %

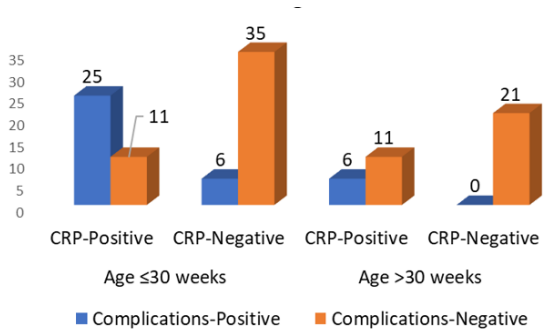

Figure No.3: Stratification of diagnostic accuracy with respect to age

P value is 0.001 which is significant
Age ≤ 30 weeks: Sn=80.6%, Sp=76.1%, PPV=69.4%, NPV=85.3%, DA=77.9%
Age > 30 weeks: Sn=100.0%, Sp=65.6%, PPV=35.3%, NPV=100.0%, DA=71.1%

DISCUSSION

Childhood pneumonia, a leading cause of death under age 5, sees higher prevalence in developing (0.29 cases per child-year) than developed countries (0.05). Globally, 156 million new cases occur annually, mostly

in developing countries like India, China, and Pakistan. Risk factors include lack of breastfeeding, under-nutrition, indoor pollution, low birth weight, crowding, and no measles immunization. Pneumonia contributes to 19% of under-5 deaths, with most in sub-Saharan Africa and south-east Asia. Studies connect Streptococcus pneumoniae, Haemophilus influenzae, and respiratory syncytial virus to childhood pneumonia.[10]Pneumonia is an important cause of illness and leading cause of death in young children in developing countries. More than 99% of pneumonia deaths occur in low- and middle-income countries (LMICs). The recent estimate is a median incidence of 0.22 episodes per child year with severe pneumonia contributing to 11.5% in LMICs.[11-12]The World Health Organization estimated 156 million new pneumonia cases globally annually, with the majority in developing countries. Pneumococcus is the main bacterial cause in these regions, along with H. influenzae type b, S. aureus, and K. pneumoniae. Treatment for childhood CAP should consider age and likely pathogens due to limited early diagnosis, often leading to empirical treatment.[13]Several attempts to understand global child pneumonia mortality over 30 years have faced challenges in estimating due to varying pneumonia definitions, low verbal autopsy specificity, symptom overlap with malaria, difficulty in distinguishing pneumonia from sepsis in neonates, and multiple disorders contributing to a single death. Pneumonia remains consistently identified as the main cause of childhood mortality. Our study included 115 patients meeting inclusion criteria, with 67.8% males and 32.2% females. Similar trends were seen in other studies by Tagarro et al, Mandal et al, and Yadav et al, with Fritz et al reporting different results.[14-17] The mean age of our study cases was 27.65±14.68 months. Most cases, 77(67.0%), were under 2.5 years. This was due to our inclusion criteria of patients aged 6-56 months. Fritz et al. reported a higher mean age of 4.7±5 years, and Tagarro et al. reported 4.67±0.39 years, attributing the differences to similar exclusions.[14,17] Mean disease duration was 8.43±4.32 days and 69(60.0%) had duration of illness more than 1 week. Fritz et al[17] from Iran reported 10.8 days mean disease duration which is close to our study results. Tagarro et al[14] reported similar results. In our study, the mean CRP level was 8.23±6.12 mg/L, with 46.1% having elevated levels (>10mg/L). Complications were seen in 32.2%. Our study found sensitivity of 83.78%, specificity of 71.79%, PPV of 58.49%, NPV of 90.32%, and diagnostic accuracy of 75.65%. Similar results were reported by Omran et al (90% sensitivity, 40% specificity, 62% PPV, and 79% NPV) and Alcoba et al (83.7% sensitivity, 50% specificity, 50% PPV, and 84.3% NPV) in children with pneumonia.[18-19]

CONCLUSION

Our study results support use of serum CRP levels in pediatric population presenting with severe pneumonia as it was found to be highly sensitive, specific, high positive predictive value & negative predictive value and high diagnostic accuracy. All clinicians treating such patients can employ serum CRP levels for early diagnosis followed by timely management achieve desired outcome.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Arshia Ijaz, Afsheen Batool Raza
Drafting or Revising Critically:	Arshia Ijaz, Madiha Tahir
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.1011/CH-UCHS Dated 30.11.2024

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Original Article

Influence of Jigsaw and Traditional Teaching Methods on Psychological Well-Being and Academic Performance of Undergraduate Nursing Students

Sabiha Tariq, Sarfraz Masih and Madiha Mukhtar

ABSTRACT

Objective: (i) To examine the influence of the Jigsaw teaching method on nurse educators' knowledge and practices, and ii) to compare the impact of Jigsaw versus traditional teaching on the psychological well-being and academic performance of undergraduate nursing students.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the public sector nursing college from 1st January 2025 to 30th June 2025.

Methods: About 4 nurse educators and 160 second-year bachelor of sciences in nursing students were included in study. Pre-intervention and post intervention data were collected from nurses' educators using knowledge, and practices check list. While pre and post intervention data were collected from nursing students on psychological well-being and academic performance.

Results: Nurse educators in the experimental group were younger between 25-30 years (50%) and had 6–10 years' experience (100%), while those in the control group were older (31–45 yrs: 100%) with 11–15 years' experience (100%). Knowledge scores improved in both groups post-intervention, from 100% poor knowledge to 50% fair and 50% good in the control group; however, statistical significance was not reached (p=0.102). Practice competency increased from 0-100% in the experimental group, compared with 0-50% in the control group (p=0.683). Among nursing students, psychological well-being significantly increased in the jigsaw group from 129.96±26.29 to 296.82±26.8 (p<0.001), and in the traditional group from 145.71±32.92 to 185.56±38.73 (p<0.001), with larger gains across all well-being subdomains in the experimental group. Academic performance in the experimental group shifted from 100% pass to 62.7% very good and 37.3% excellent post-intervention, whereas in the traditional group 96.5% remained in "pass" category.

Conclusion: The Jigsaw teaching method significantly improved psychological well-being, academic performance, skills, and attendance among nursing students compared to traditional teaching.

Key Words: Jigsaw teaching method, Traditional teaching, Psychological well-being, Academic performance, Nursing education

Citation of article: Tariq S, Masih S, Mukhtar M. Influence of Jigsaw and Traditional Teaching Methods on Psychological Well-Being and Academic Performance of Undergraduate Nursing Students. Med Forum 2025;36(9):14-18. doi:10.60110/medforum.360903.

INTRODUCTION

Teaching methods play a pivotal role in shaping nursing professionals by determining how effectively students acquire the knowledge, skills, and attitudes necessary for their profession.[1] Educators employ varied strategies to accommodate different learning styles and educational needs.[2]

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Received: July, 2025
Reviewed: July, 2025
Accepted: August, 2025

Traditional lectures provide organized delivery but often limit student participation and engagement, which can compromise retention and critical thinking in skill-based disciplines like nursing.[3] In contrast, the Jigsaw method is a cooperative, student-centred strategy that transforms learners from passive recipients to active participants through expert-group study and peer teaching.[4]

This approach fosters teamwork, communication, leadership, and accountability - competencies central to nursing practice.[5] Evidence suggests that Jigsaw enhances learning confidence, self-regulated learning, and satisfaction when implemented with clear facilitation and role structure.[6] Comparative studies indicate that Jigsaw and other active approaches can outperform lecture on knowledge, performance, and satisfaction outcomes.[7] Network and systematic reviews further support active and cooperative models for

improving educational effectiveness in nursing and allied health.[8,9] Given these advantages, structured educator preparation and facilitation are essential to ensure balanced participation and mastery of segment content during Jigsaw sessions.[10]

METHODS

This quasi-experimental design to assess the effects of the Jigsaw teaching method compared to traditional teaching on the psychological well-being and academic performance of nursing students was conducted at King Edward Medical University, Mayo Hospital, Lahore from 1st January 2025 to 30th June 2025 vide letter No. REC-UOL/497/08/24 dated 27-12-2024. The study involved 160 second-year BSN students divided into control (n=85) and experimental (n=75) groups, alongside four nurse educators (2 per group). Purposive sampling was used. Pre-intervention data were collected using validated tools: Ryff and Keyes' Psychological Well-Being questionnaire (Cronbach's α = 0.87), an academic performance sheet based on quizzes, skills assessments, and attendance, and knowledge/practice tools for nurse educators. The intervention phase involved educating nurse educators on the Jigsaw method, followed by its application in nine structured sessions for the experimental group. Control group students received traditional lectures. Post-intervention assessments were conducted for both students and educators. Data were analyzed using SPSS V27, applying descriptive statistics, Wilcoxon Signed-Rank test with a significance threshold of $p<0.05$

RESULTS

Most nursing students in both groups were from urban areas and had two or more siblings, indicating similar family and social backgrounds. All nurse educators in both groups were married and highly qualified, though those in the control group were slightly older and more experienced. Overall, the two groups were demographically well-matched, suggesting that any observed differences in learning outcomes are likely attributable to the teaching methods rather than demographic variations. The demographic profile of the nurse educators in the experimental and control groups was fairly diverse across age, experience, and qualifications. In terms of age, the experimental group included younger participants, whereas the control group comprised slightly older individuals. All participants in both groups were married. Regarding professional experience, the experimental group consisted of individuals with moderate experience, while the control group included more experienced educators. In terms of academic qualifications, the experimental group had a mix of individuals with a basic nursing degree and a post-registered nurse bachelor's degree, while all participants in the control group held post-registered nurse bachelor's degrees. No participants in either group held a master's degree in nursing (Tables 1-2)

The comparison includes the overall psychological well-being score and its six subdomains: Autonomy, Environmental Mastery, Personal Growth, Positive Relations with Others, Purpose in Life, and Self-Acceptance. The results indicate that both teaching methods led to statistically significant improvements in the psychological well-being of students. However, the jigsaw teaching group demonstrated a more pronounced enhancement across all measured domains compared to the traditional group (Table 3).

Initially, both groups showed similar performance levels. Following the intervention, students taught using the jigsaw method demonstrated a marked shift towards higher academic categories such as "very good" and "excellent," while those in the traditional group largely remained in the lower performance category.

Table No.1: Comparison of demographics of control and experimental group of nurse educators and student nurses

Variables	Category	Jigsaw Teaching Group	Traditional Teaching Group	Total
Resident Area (Student nurses)	Rural	19 (25.3%)	28 (32.9%)	47 (29.4%)
	Urban	56 (74.7%)	57 (67.1%)	113 (70.6%)
Number of Siblings(Student nurses)	≤ 1	6 (8.0%)	8 (9.4%)	14 (8.7%)
	≥ 2	69 (92.0)	77 (90.6%)	146 (91.3%)
Age (Educators only) [years]	25–30	1 (50.0%)	-	1 (25.0%)
	31–40	1 (50.0%)	1 (50.0%)	2 (50.0%)
	41–45	-	1 (50.0%)	1 (25.0%)
Marital status (educators only)	Married	2 (100%)	2 (100%)	4 (100%)
	Unmarried	-	-	-
Experience (Educators only) [years]	1–5	-	-	-
	6–10	2 (100%)	-	2 (50.0%)
	11–15	-	2 (100%)	2 (50.0%)
Qualification (Educators only)	BSN	1 (50.0%)	-	1 (25.0%)
	Post RN BSN	1 (50.0%)	2 (100%)	3 (75.0%)
	MSN	0	-	-

Table No.2: Comparison of pre and post intervention knowledge and practice of experimental group and control group of nurse educators

Variables	Category	Experimental Group	Control Group	P value
Knowledge (Pre-Intervention)	Poor knowledge	2 (100%)	2 (100%)	0.102
	Fair knowledge	-	-	
	Good knowledge	-	-	
Knowledge (Post-Intervention)	Poor knowledge	-	-	0.683
	Fair knowledge	-	1 (50%)	
	Good knowledge	-	1 (50%)	
Practices (Pre-Intervention)	Incompetent practices	2 (100%)	2 (100%)	1.000
	Competent practices	-	-	
Practices (Post-Intervention)	Incompetent practices	-	1 (50%)	0.683
	Competent practices	2 (100%)	1 (50%)	

Table 3: Comparison of psychological well-being of jigsaw and traditional teaching of nursing students before and after intervention

Variable	Jigsaw Teaching				Traditional Teaching			
	Pre	Post	Z-value	p-value	Pre	Post	Z-value	p-value
PWB	129.96±26.29	296.82±26.8	-7.52	<.001	145.71±32.92	185.56±38.73	-6.22	<0.001
Auto	21.58±5.54	49.45±5.12	-7.527	0.000	24.08±6.91	30.36±7.07	-5.562	0.000
Env	21.64±4.62	49.26±5.39	-7.528	0.000	24.48±5.80	29.45±7.30	-4.454	0.000
PG	21.08±5.34	49.72±4.12	-7.528	0.000	23.74±6.72	32.97±6.40	-6.858	0.000
PR	22.09±6.65	49.36±4.65	-7.527	0.000	24.10±6.98	28.92±6.70	-4.247	0.000
PL	21.84±6.08	49.38±5.70	-7.527	0.000	24.62±7.61	29.78±8.12	-4.115	0.000
SA	21.72±4.77	49.64±5.38	-7.528	0.000	24.68±6.10	34.04±8.66	-6.168	0.000

PWB= Psychological Well-Being, Auto= Autonomy, Env= Environment Master, PG= Personal Growth, PR=Positive Relations with Others, PL=Purpose in Life, SA= Self-Acceptance

Table No.4: Comparison of academic performance of jigsaw and traditional teaching of nursing students before and after intervention

Academic Performance	Experimental Group		Control Group	
Excellent	-	28 (37.3%)	-	-
Very Good	-	47(62.7%)	-	-
Good	-	-	-	3(3.5%)
Pass	75(100%)	-	85(100%)	82(96.5%)
Poor	-	-	-	-

This highlights the positive impact of the jigsaw teaching strategy on students' academic outcomes compared to traditional teaching (Table 4)

DISCUSSION

The findings of study indicate that both groups improved their knowledge scores post-iIn this study, both educator groups improved knowledge post-intervention, with the Jigsaw group showing a modest advantage; however, differences were not statistically significant, aligning with reports that cooperative strategies yield gains that may be modest in small cohorts.[11] Educator practices improved notably in the Jigsaw arm, echoing quasi-experimental findings that Jigsaw and related methods enhance skill performance, retention, and satisfaction when properly structured.[12] Among students, the Jigsaw approach produced significant improvements across psychological well-being domains - autonomy, environmental mastery, personal growth, positive relations, purpose in life, and

self-acceptance - consistent with evidence that cooperative learning promotes confidence, motivation, and social integration in health-science education.[13] These psychosocial gains are attributed to peer interdependence, shared accountability, and opportunities for feedback and reflection embedded in Jigsaw cycles.[14]

By contrast, traditional lecture can be associated with lower engagement and weaker affective outcomes, reinforcing the value of interactive learning for contemporary nursing cohorts.[15] Academic performance improvements in our Jigsaw group parallel meta-analytic and experimental results showing superior achievement, retention, and satisfaction under cooperative models versus lecture.[16] Importantly, Jigsaw's structured interdependence maps onto clinical teamwork, supporting transfer of collaborative competencies to practice settings.[17] Overall, the present findings add to a growing literature base positioning Jigsaw as an integrative pedagogy that advances cognitive, affective, and behavioural learning outcomes in nursing education.[18] With appropriate educator training, session design, and assessment alignment, Jigsaw can be scaled to large cohorts while preserving equity of participation and depth of learning.[19] Future studies should extend to multi-site trials with longer follow-up to evaluate durability of psychosocial and academic benefits and explore implementation frameworks for sustained curriculum integration.[20]

CONCLUSION

Before the intervention, both groups were similar in quiz scores, skill performance, psychological well-being, and attendance, reflecting equivalent baseline characteristics. After the intervention, significant progress was observed in the group instructed through the Jigsaw method. These students demonstrated improvements in multiple aspects of psychological well-being, such as autonomy, environmental mastery, positive relationships, and self-acceptance. Academically, a greater number of students in the Jigsaw group attained higher quiz and skill assessment scores, and their attendance rates also increased considerably compared to the control group.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.REC-UOL/497/08/24 Dated 27.12.2024

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Original Article

Use of Dipeptidyl Peptidase 4 (DPP4) Inhibitors in Diabetic Nephropathy: A Prospective Cohort Study

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ABSTRACT

Objective: To evaluate the glucose-lowering and reno protective properties of DPP-4 inhibitors in type 2 diabetes mellitus with nephropathy.

Study Design: Prospective Cohort Study.

Place and Duration of Study: This study was conducted at the Department of Medicine, Dr. Ruth KM Pfau Civil Hospital Karachi & Dow University Hospital, Ojha Campus from February 2022 to December 2023.

Methods: This prospective cohort study used systematic sampling to enroll 300 adults with type 2 diabetes. Baseline and follow-up assessments at 12 and 24 weeks included blood glucose, BMI, UMA, ACR, lipid profile, and HbA1c. Following blinded administration of either Vildagliptin (50–200 mg) or Sitagliptin (25–100 mg), the results were examined using SPSS v25 and the relevant statistical tests.

Results: Glycaemic and renal indices significantly improved in this 24-week study of 300 T2DM patients with renal dysfunction (Sitagliptin n = 164, Vildagliptin n = 136). FBS decreased from 154 to 135 mg/dl and the mean HbA1c decreased from 8.65% to 7.95% ($p<0.05$). Effectiveness with renal safety was demonstrated by the significant decreases in UMA and ACR, the stability of serum creatinine, and the slight decrease in BMI.

Conclusion: DPP4 inhibitors can be used safely in type 2 DM with renal dysfunction to have fairly good glycemic control. In addition, we found it renal friendly, showing improvement in urine microalbuminuria and ACR and maintaining serum creatinine. So it can be used safely in type 2 DM patients with mild to moderate renal dysfunction who are reluctant to take insulin.

Key Words: DPP4 inhibitors; glycemic control; UMA; renal dysfunction; ACR; type 2 diabetes

Citation of article: Kumar D, Bhatty S, Kashif SM, Kumar P, Luqman M. Use of Dipeptidyl Peptidase 4 (DPP4) Inhibitors in Diabetic Nephropathy: A Prospective Cohort Study. Med Forum 2025;36(9):19-24. doi:10.60110/medforum.360904.

INTRODUCTION

Diabetes affects 33M Pakistani adults who represent 26.3% of the population with greater numbers found in urban areas because of lifestyle changes and unhealthy eating habits and inactivity and rising obesity rates[1,2]. The microvascular and macrovascular diseases caused by DM increase the risk of cardiovascular, cerebrovascular, nephropathy, and retinopathy-related mortality and death.[3,4]

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Received: February, 2025
Reviewed: March, 2025
Accepted: April, 2025

The treatment of diabetes relies on a patient-based methodology. Type 1 DM requires insulin therapy while Type 2 DM patients need oral hypoglycemic along with the potential use of insulin for control. Among the oral hypoglycemic are metformin and gliptins and SGLT2 inhibitors with sulfonylureas and meglitinides and thiazolidines and alpha-glucosidase inhibitors.[5] Dipeptidyl peptidase 4 inhibitors (DPP4i) began treating type 2 diabetes patients after their introduction in 2006. Selective DPP4 action antagonism happens through orally absorbable peptides without affecting other biological functions and produces elevated levels of among others the incretin GLP-1. The method of action enables glitazone drugs to maintain low hypoglycemic reactions and demonstrate excellent safety characteristics.

In general, DPP-4 inhibitors have beneficial effects on glycemic control but are associated with a small increase in acute pancreatitis and occasionally hospitalization for heart failure. Collectively, DPP-4i is well tolerated with manageable side effects for many patients with Type 2 diabetes.[6] Gliptins/Dipeptidyl peptidase-4 inhibitors (DPP4) are preferred in patients with mild to moderate renal dysfunction, and may be used in patients with nephropathy who are reluctant to

use insulin when prescribed in such complications. Diabetic nephropathy is a leading diabetes complication and affects around one in three patients with DM. Albuminuria and GFR are the two main substituted indices of diabetes-related renal impairment. Compared with patients without CKD, DPP4 inhibitors seem to be equally effective in improving glucose levels in patients with chronic kidney disease. They appear safe to use in CKD, and consequently DPP4 inhibitors may decrease albuminuria and boost GFR. Chronic kidney disease of diabetes depends upon the time duration of diabetes, and proper control of risk factors and glycemic load can slow down disease progression[7].

METHODS

This prospective cohort research took place at the Dr Ruth K.M Pfau Civil hospital along with the Dow University hospital Ojha Campus Karachi during successive months from February 2022 to December 2023 following ethical approval with IRB-2366/DUHS/approval/2022/794. Using the WHO estimator (95% CI, 80% power, 5% error), a sample of 300 patients was determined and gathered through systematic sampling. Type 1 diabetes, gestational diabetes, chronic liver disease, and patients taking other oral antidiabetic medications were excluded, while adults with type 2 diabetes mellitus (HbA1c >7%, duration ≤10 years) between the ages of 18 and 70 were included . In addition to demographic and clinical data gathered through electronic questionnaires, baseline and follow-up data (12 and 24 weeks) included HbA1c, lipid profile, FBS, RBS, BMI, urine microalbumin, serum creatinine, and ACR. Depending on clinical necessity, patients were blindly and randomly assigned to receive either Vildagliptin (50–200 mg) or Sitagliptin (25–100 mg). SPSS v25 was used for data analysis, and nonparametric tests were used for variables that were not normally distributed.

RESULTS

Out of the 300 patients who were enrolled, 34.2% were men and 65.8% were women. 24.3% were between the ages of 50 and 60, 14% were over 60, 12.6% were between the ages of 20 and 40, and nearly half (49.2%) were between the ages of 40 and 50. 35.2% of participants had diabetes for more than ten years, and 37.2% had it for five to ten years. For glycaemic control, 164 of these were given Sitagliptin (25–100 mg once daily) and 136 were given Vildagliptin (50–100 mg daily).

Over the course of 24 weeks, DPP-4 inhibitor therapy markedly improved renal and glycaemic parameters in patients with type 2 diabetes who also had renal dysfunction. The mean HbA1c dropped from 8.65% to 7.95%, the mean FBS dropped from 154 to 135 mg/dl, and the mean RBS dropped from 210 to 187 mg/dl (all $p<0.05$). While BMI and serum creatinine decreased marginally and both reached statistical significance, UMA and ACR showed modest but steady declines (Table 1).

Table No.1: Pre and post therapy effect of DPP4 inhibitors on BMI; glycemic control and renal parameters

Parameters	mean± SD	DPP4 inhibitors (sitagliptin and vildagliptin)	
	Baseline	Post-therapy (12 weeks)	Post-therapy (24 weeks)
BMI (kg/m²)	27.88 ± 3.13	27.77± 2.99	27.58± 2.98
FBS (mg/dl)	154.15± 38.47	145.10± 37.53	134.98± 34.84
HbA1C (G%)	8.65± 1.43	8.01 ± 1.34	7.95± 1.33
UMA (mg/dl)	97.91± 110.22	90.96± 106.17	89.15± 104.05
	Baseline	Post-therapy (12 weeks)	Post-therapy (24 weeks)
BMI (kg/m²)	27.88 ± 3.13	27.77± 2.99	27.58± 2.98

Table No.2: Gender difference pre and post therapy of DPP4 inhibitors on BMI; glycemic control and renal parameters

	Mean	Male	Female
HbA1C			
Baseline	8.65 ± 1.43%	8.89 ± 1.708%	8.53 ± 1.25%
24 weeks	8.29% ± 1.33%	8.21±1.57%	7.81± 1.16%
Reduction		$p < 0.001$	$p < 0.001$
FBS and RBS			
Baseline	156 ± 38.21 mg/dl	155.04 mg/dl	157.92 mg/dl
24 weeks	134.85±34.93 mg/dl	132.11 mg/dl	136.29 mg/dl
Reduction	22 mg/dl ($p < 0.05$) both genders		
Baseline	210.31 ± 48.11 mg/d	209.76 mg/dl	210.74 mg/dl
24 weeks	186.98±44.35mg/dl	185.11 mg/dl	188.34 mg/dl
Reduction	23 mg/dl ($p < 0.05$) both genders		
Urine for micro albumin			

Baseline	79.76 ± 110.13 mg/dl	77.89 mg/dl	81.22 mg/dl
24 weeks	74.34 ± 105.78 mg/dl	72.65 mg/dl	75.89 mg/dl
Reduction	5.42 mg/dl ($p < 0.05$) both genders		
Albumin and creatinine ratio (ACR)			
Baseline	87.55±95.32 mg/g	85.67 mg/g	88.91 mg/g
24 weeks	82.45±90.74 mg/g	80.12 mg/g	83.78 mg/g
Reduction	5.10 mg/g ($p < 0.05$) both genders		
Serum creatinine			
Baseline	1.18 ± 0.23 mg/dl	1.20 mg/dl	1.16 mg/dl
24 weeks	1.16 ± 0.21 mg/dl	1.18 mg/dl	1.14 mg/dl
Reduction	0.02 mg/dl ($p > 0.05$)		

Table No.3: Glycaemic, Renal, and Lipid Parameters of sitagliptin and Vildagliptin

Variable	Timepoint	sitagliptin	vildagliptin	Estimated P value
FBS	Baseline	154.15mg/dl	153.62mg/dl	0.82
FBS	12 weeks	141.22 mg/dl	140.58mg/dl	0.77
FBS	24 weeks	135.22mg/dl	134.68mg/dl	0.74
HbA1C	Baseline	8.65%	8.61%	0.70
HbA1C	24 weeks	8.01%	7.87%	0.04 Significant difference; better in vildagliptin
UMA	Baseline	95.45 mg/dl	97.38 mg/dl	0.68
UMA	24 weeks	87.45 mg/dl	91.31 mg/dl	0.03 Significant difference; better in vildagliptin
Cholesterol	Baseline	188.21 mg/dl	187.56 mg/dl	0.85
Cholesterol	24 weeks	159.06 mg/dl	163.23 mg/dl	0.02 Significant difference; better in vildagliptin
TGs	Baseline	210.93 mg/dl	212.48 mg/dl	0.81
TGs	24 weeks	184.45 mg/dl	181.40 mg/dl	0.11
HDL	Baseline	33.55 mg/dl	34.61 mg/dl	0.39
HDL	24 weeks	37.16 mg/l	36.94 mg/dl	0.52

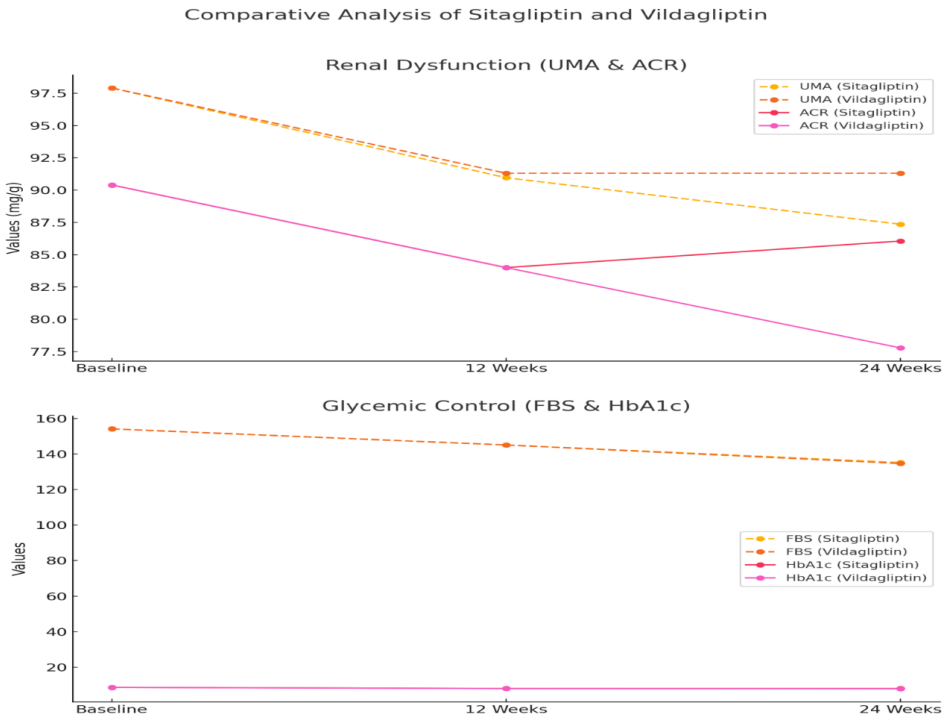

Figure No.1: comparative analysis of sitagliptin and vildagliptin

Gender difference of all these parameters are described in Table 2.

At baseline, there were no discernible differences between the Vildagliptin (n=136) and Sitagliptin (n=164) groups (p>0.05). Triglycerides sharply declined by week 12, and by week 24, there had been no change. LDL showed a slight but significant decrease from baseline to week 24, whereas HDL increased steadily (p<0.001). Generally, DPP-4 inhibitor treatment improved lipid profile and glycaemic control over the course of 24 weeks, with early metabolic benefits occurring within 12 weeks (Table 3).

Males reported three hypoglycemic episodes with sitagliptin and four with vildagliptin during the 24-week follow-up, whereas females reported four and five episodes, respectively. There were no gender-based differences that were statistically significant (p>0.05), and all 16 episodes were categorised as level 1 hypoglycemia. Figure 1.

DISCUSSION

Numerous therapeutic benefits of DPP-4 inhibitors are highlighted by research on diabetic patients with renal dysfunction. These drugs help stabilise renal function, improve glycaemic control, and positively alter lipid profiles. Both sitagliptin and vildagliptin reduce the risk of hypoglycemia, even in patients with renal impairment, by increasing incretin levels, improving insulin secretion, and inhibiting glucagon. In accordance with American Diabetes Association guidelines, their good safety profile supports their long-term use in type 2 diabetes with renal complications (2020)[8].

Our results on Vildagliptin and Sitagliptin in diabetic patients with renal impairment are consistent with the REAL trial, which demonstrated that low-dose Sitagliptin (12.5–25 mg/day) was safe and effective over a six-month period in terms of eGFR and HbA1c. In a similar vein, our study's two agents both preserved stable renal function and glycaemic control, demonstrating their safety in this population. Our study did not evaluate the cost-effectiveness of low-dose sitagliptin, despite the REAL trial's emphasis on this point. Interestingly, Vildagliptin resulted in a marginally higher decrease in HbA1c and ACR, indicating that patient-specific objectives for glycaemic rather than renal outcomes may influence treatment selection[9]. In our cohort of 300 patients, 16 experienced level 1 hypoglycemia (7 with Sitagliptin, 9 with Vildagliptin), giving an overall incidence of 5.3%. This closely parallels the 5.1% rate reported by Lukashevich et al. (2014) for Vildagliptin. Both drugs appear safe, though the slight variation in events underscores the importance of close monitoring in patients with renal impairment.[10] Due to glucose-dependent insulin secretion, DPP-4 inhibitors in combination with metformin or thiazolidinediones are linked to a low risk of hypoglycemia (Nauck et al., 2009). 5.3% of patients in our study experienced hypoglycemia, which is somewhat higher than anticipated. However, individuals taking sulfonylureas or insulin are at significantly higher risk (Goossen & Graber, 2012), necessitating close observation[11,12].

Our results are consistent with earlier research that supports DPP-4 inhibitors for renal and glycaemic outcomes. In line with reported decreases of 0.5–1% as monotherapy and 0.6–1.1% with metformin, HbA1c decreased from 8.65% at baseline to 7.95% at 24 weeks (American Diabetes Association, 2020). Similar HbA1c reductions (~0.5–0.6%) were observed in clinical trials comparing saxagliptin and sitagliptin, and meta-analyses verified that sitagliptin and vildagliptin were equally effective when compared to a placebo. Additionally, there is evidence that vildagliptin may offer marginally superior circadian glycaemic stability in comparison to sitagliptin[13,14].

Given that diabetic nephropathy is a frequent consequence of poorly managed type 2 diabetes, the observed decreases in urinary microalbumin and ACR point to possible renal protective effects of DPP-4 inhibitors (Zhang et al., 2019). These medications are safe for patients with impaired renal function, as evidenced by stable serum creatinine and the lack of renal decline.[15]

According to Yong Gong et al.'s systematic review and meta-analysis, DPP-4 inhibitors help maintain kidney function in patients with type 2 diabetes, which is in line with our findings. The meta-analysis revealed a similar decrease in ACR (WMD −2.76 mg/g; 95% CI −5.23 to −0.29) without any change in eGFR, whereas our study showed a significant ACR decline of 5.10 mg/g (p<0.05) without affecting serum creatinine. Thus, there is proof that DPP-4 inhibitors can lower albuminuria and enhance renal outcomes in diabetics.[16]

Since there are more female participants (60.5%) in the study data, there is uncertainty regarding gender-based differences in diabetes treatment responses and refractory diabetes, so caution should be exercised when extrapolating these findings. The studies mentioned show that female patients have different cardiovascular outcomes than male patients and respond differently to diabetes treatment (Hoffmann et al., 2018). More research on gender disparities is necessary since it will result in better treatment strategies that can improve the outcomes of diabetes care[17]. This study validated the effectiveness of Vildagliptin and Sitagliptin in glycaemic control in patients with type 2 diabetes. With mean decreases of 22 mg/dl and 0.68%, respectively, HbA1c dropped from ~8.6% to ~8.0% and baseline FBS values (~154 mg/dl) decreased to ~135 mg/dl after 24 weeks (p<0.05). A Malaysian study found that DPP-4 inhibitors decreased HbA1c by 0.9% and FBS by 19.8

mg/dl. However, sitagliptin demonstrated a higher reduction in HbA1c and vildagliptin slightly improved control of FBS. Study variations could be related to treatment adherence and sample size. Although more study is required to elucidate outcome variations, both medications demonstrated overall effectiveness[18].

Similar to our 0.68% decrease over 24 weeks with sitagliptin and vildagliptin, a meta-analysis on predictive factors for DPP-4 inhibitor efficacy in T2DM found a 0.6% HbA1c reduction after a year. Both studies found a correlation between effectiveness and baseline HbA1c. Additionally, the meta-analysis identified early HbA1c response, low BMI, and the lack of CAD as indicators of improved long-term results. Our cohort's BMI dropped only marginally (from 27.88 to 27.58 kg/m2), indicating that weight had little effect. These results underline how crucial it is to customise treatment regimens based on unique patient characteristics in order to maximise DPP-4 inhibitor response[19]. In T2DM patients with renal impairment, DPP-4 inhibitors enhanced glycaemic control and renal outcomes while maintaining good safety. Vildagliptin and sitagliptin demonstrated low hypoglycemia, little effect on BMI, and comparable effectiveness.

CONCLUSION

The effectiveness and tolerability of sitagliptin and vildagliptin in T2DM patients with renal complications were evaluated in this study. Both medications enhanced FBS, HbA1c, UMA, and ACR over a 24-week period, suggesting improved renal function and glycaemic control. Similar results were observed in both sexes, and a slight decrease in BMI also suggested possible weight benefits. These results demonstrate how DPP-4 inhibitors help control blood sugar levels and maintain renal function. Confirming long-term benefits, elucidating renal mechanisms, and defining population-specific considerations all require more research.

Abbreviations:

- BMI = Body Mass Index
- UMA = Urine Microalbumin
- ACR = Albumin-to-Creatinine Ratio
- HbA1c = Hemoglobin A1c
- FBS = Fasting Blood Sugar
- RBS = Random Blood Sugar
- LDL = Low-Density Lipoprotein
- HDL = High-Density Lipoprotein
- TGs = Triglycerides

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.IRB-2366/DUHS/Approval/ 2022/794 Dated 16.02.2022

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Original Article

Impact of Central Obesity on the Clinical and Biochemical Profile of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)

Central Obesity Associated Steatotic Liver Disease

Ahmed M Shaker and Bilal Natiq Nuaman

ABSTRACT

Objective: To assess the association between central obesity (waist-to-height ratio >0.5) and liver fibrosis, steatosis, and inflammatory biomarkers in metabolic dysfunction-associated steatotic liver disease patients.

Study Design: A case-control study

Place and Duration of Study: This study was conducted at the Department of Medicine, College of Medicine, AL-Iraqia of University, Iraq from 15th March 2024 to 30th May 2025.

Methods: A case-control study was conducted on 120 adults diagnosed with metabolic dysfunction-associated steatotic liver disease in Baghdad. Participants were divided into centrally obese (n=60) and non-obese (n=60) groups. Clinical and biochemical parameters including liver enzymes (alanine aminotransferase, aspartate aminotransferase), C-reactive protein, homeostatic model assessment for insulin resistance and Fibro scan-based fibrosis staging were compared.

Results: Centrally obese patients showed significantly elevated alanine aminotransferase (49.7 vs. 33.1 U/L, $p<0.001$), C-reactive protein (19.9 vs. 9.7 mg/L, $p<0.001$), and homeostatic model assessment for insulin resistance (5.93 vs. 3.39, $p<0.001$). Fibrosis grades ≥F2 and steatosis stages ≥ S2 were significantly more frequent among the obese group ($p<0.01$).

Conclusion: Central obesity is significantly associated with worsened liver fibrosis and metabolic derangement in metabolic dysfunction-associated steatotic liver disease patients. These findings support the utility of waist to hip ratio as a clinical screening tool in metabolic dysfunction-associated steatotic liver disease management.

Key Words: Central obesity, Metabolic dysfunction-associated steatotic liver disease, Liver fibrosis, C-reactive protein, Homeostatic model assessment for insulin resistance, Fibro Scan

Citation of article: Shaker AM, Nuaman BN. Impact of Central Obesity on the Clinical and Biochemical Profile of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). Med Forum 2025;36(9):25-30. doi:10.60110/medforum.360905.

INTRODUCTION

Metabolic dysfunction–associated steatotic liver disease (MASLD) is a redefined clinical entity that replaces the historical term non-alcoholic fatty liver disease (NAFLD), aiming for semantic clarity and pathophysiologic precision. NAFLD, once the most prevalent chronic liver disease worldwide, affects over one-third of adults and is closely linked to metabolic syndrome, central obesity, insulin resistance, and type 2 diabetes mellitus.[1-3]

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Received: June, 2025
Reviewed: July, 2025
Accepted: August, 2025

However, the term NAFLD has been criticized for its exclusionary definition based on alcohol intake and for failing to highlight metabolic risk factors, especially in lean individuals.[2,3]

In 2023, the Liver Disease Nomenclature Consensus Group introduced MASLD to more accurately describe hepatic steatosis associated with metabolic dysfunction.[2,4] The diagnosis requires hepatic steatosis plus at least one cardiometabolic risk factor such as obesity, dyslipidemia, hypertension, or type 2 diabetes.[5,6] Although MASLD overlaps with NAFLD in up to 99% of cases, it provides a clearer, mechanism-based framework emphasizing metabolic risk and cardiovascular relevance.[7-9]

The global prevalence of MASLD is rapidly increasing due to aging, sedentary lifestyle, poor diet, and obesity.[10] Among individuals with type 2 diabetes, prevalence exceeds 65%, peaking in the Middle East (71.2%) and lowest in Africa (53.1%)..[11] In the United States, MASLD cases rose by 138% between 1988 and 2018, surpassing obesity growth rates[12], reflecting a growing clinical and economic burden.[13]

MASLD is associated with elevated risks of all-cause, cardiovascular, and liver-related mortality.[14] Mortality is further aggravated in those with metabolic alcohol-associated liver disease (Meted).[15] In acute settings, such as myocardial infarction, MASLD correlates with obesity, diabetes, chronic kidney disease, and higher mortality.[16]

Given central obesity's pivotal role, early identification using simple anthropometric measures is crucial. Waist-to-height ratio (WtHR) has emerged as a practical, reliable index for central adiposity and metabolic risk, outperforming BMI and waist circumference [21, 22]. This study investigates the impact of central obesity, assessed via WtHR, on the clinical and biochemical profiles of MASLD patients, and evaluates its predictive value for disease severity and progression.

METHODS

This multicenter, hospital-based case–control study was conducted at in five tertiary hospitals in Baghdad, Iraq (Baghdad Teaching Hospital, Medical City, Gastroenterology & Hepatology Hospital, Al-Yarmouk, and Al-Numen) from 15th March 2024 to 30th May 2025. All centers followed unified diagnostic and laboratory standards. Adults ≥18 years with ultrasonographic evidence of hepatic steatosis were included. Central obesity was classified by waist-to-height ratio (WtHR): cases: WtHR >0.50 and controls WtHR <0.50. Participants with other liver diseases, pregnancy, or excessive alcohol intake (>140–350 g/week for females; >210–420 g/week for males) were excluded. Convenience sampling was used. Power analysis (α = 0.05, 80% power) estimated ≥60 participants per group; 120 subjects were enrolled equally (60/60).

Data were obtained via a standardized form including: Demographics: age, sex, marital status, education, income. Lifestyle: smoking, physical activity, diet quality. Medical History: diabetes, hypertension, malignancy. Anthropometry: waist circumference, height, WtHR. Clinical Sign: neck acanthosis nigricans (insulin resistance marker). Biochemistry: ALT, AST, glucose, triglycerides, HDL-C, CRP, insulin (HOMA-IR=insulin×glucose/405). Fibrosis Indices: FIB-4=(age×AST)/(platelets×√ALT) and FibroScan® stiffness (kPa). Imaging: Ultrasound grading (normal to cirrhosis).

MASLD was defined per 2023 Liver Disease Nomenclature Consensus criteria: hepatic steatosis plus ≥1 metabolic risk factor (obesity, hypertension, dyslipidemia, or diabetes) excluding secondary causes. Cases with excess alcohol intake were classified as MetALD and excluded. Analyses were performed using SPSS-27. Independent *t*-tests and χ^2 or likelihood-ratio tests assessed between-group differences, with $p<0.05$ considered significant.

RESULTS

The mean age 43.3±12.35 years; cases were significantly older (46.65±11.34 vs. 39.95±12.50 years, $p = 0.003$). Age distribution also differed ($\chi^2 = 9.956$, $p = 0.041$). Females predominated (54.2%), with no significant sex difference between groups ($p> 0.05$). Marital status varied significantly (p=0.030): married participants were more frequent in cases (68.3% vs. 46.7%), while single and divorced were higher in controls. Educational level differed (p=0.003); illiterate and primary education were more common among cases, while secondary education predominated in controls. Income adequacy showed no significant difference ($p> 0.05$) [Table 1).

Notable lifestyle differences were evident between groups (Fig. 1). Half of the centrally obese participants reported no physical activity, and 31.7% engaged only in light exercise, whereas 56.7% of controls practiced moderate to vigorous activity, highlighting the protective role of regular exercise. Unhealthy dietary patterns were more prevalent among centrally obese individuals (41.7% vs. 6.7%), while healthy or moderately healthy diets predominated in controls, emphasizing diet quality as a determinant of central adiposity. Smoking rates showed no significant variation between groups, indicating limited influence on central obesity. Clinically, neck hyperpigmentation - a visible marker of insulin resistance was significantly more frequent in centrally obese participants, reflecting higher metabolic burden and its utility as a simple screening indicator.

Clinical signs of insulin resistance particularly neck hyperpigmentation were significantly more prevalent in the central obesity group (61.7%) than in controls (11.7%) (χ^2=32.297, df=1, p<0.001). Conversely, absence of such signs was more common among controls (88.3% vs. 38.3%). Biochemically, ALT levels were significantly elevated in centrally obese participants compared to controls (49.70±28.97 vs. 33.12±17.84; t= –3.776, df=118, p<0.001), indicating greater hepatic involvement in central obesity. The mean level of fasting blood sugar was also significantly higher among central obesity group than that of central obesity free group (110.27±40.569 vs. 87.75±21.248) respectively with significant mean difference of -22.517 (t= -3.808, df:118, P=0.000). The mean level of lipids of triglycerides found to been significantly higher among cases group of study's sample in comparison to that in the controls group (235.63±66.350 vs. 161.07±23.989) respectively with significant difference of -74.567 (t= -8.187, df:118, P= 0.000). However, the mean level of lipids of high HDL was significantly lower among cases group of study's sample in comparison to that in the controls group (35.08±4.073 vs. 41.95±3.039) respectively with significant difference of 6.867 (t= 10.467, df:118, P= 0.000). The mean total cholesterol level was significantly higher in the central obesity group compared to controls (253.78±60.84 vs. 183.42±33.56; t = –7.844, df = 118,

p<0.001). Similarly, the inflammatory marker C-reactive protein (CRP) showed a marked elevation among centrally obese participants (19.90±25.36 vs. 9.70±5.38; t = –3.047, df = 118, p<0.001), reflecting enhanced systemic inflammation associated with central obesity. The mean Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) value was significantly higher in the central obesity group compared to controls (5.93±1.54 vs. 3.39±1.33; t = –9.666, df = 118, p<0.001), confirming a markedly greater degree of insulin resistance among centrally obese participants. The Fibrosis-4 (FIB-4) index calculated from age, AST, ALT, and platelet count was significantly higher in the central obesity group compared to controls (1.75±1.07 vs. 1.40±0.80; t = –2.021, df=118, p = 0.046). This indicates a greater likelihood of hepatic fibrosis among centrally obese participants (Table 2).

Table No.1: Baseline sociodemographic characteristics of the study's sample (n=120)

Characteristics	Cases (Yes, n=60)	Control (No, n=60)	Total	Significancy
Age (years)	46.65±11.342	39.95±12.497	43.30±12.350	*t*= -3.075, df: 118, *P*= 0.003[a]
< 30	4 (6.7%)	11 (18.3%)	15 (12.5%)	χ^2= 9.956, df: 4, P =0.041[b]
30-40	14 (23.3%)	23 (38.3%)	37 (30.85)	
41-50	18 (30%)	14 (23.3%)	32 (26.7%)	
51-60	16 (26.7%)	8 (13.3%)	24 (20%)	
> 60	8 (13.3%)	4 (6.7%)	12 (10%)	
Gender				
Female	34 (56.7%)	31 (51.7%)	65 (54.2%)	χ^2= 0.302, df: 1, P=0.583[b]
Male	26 (43.3%)	29 (48.3%)	55 (45.85)	
Marital status				
Single	11 (18.3%)	22 (36.7%)	33 (27.5%)	Likelihood Ratio: 8.974, df: 3, P=0.030[c]
Married	41 (68.3%)	28 (46.7%)	69 (57.5%)	
Divorced	-	2 (3.3%)	2 (1.7%)	
Widowed	8 (13.3%)	8 (13.3%)	16 (13.3%)	
Education				
Illiterate	8 (13.3%)	2 (3.3%)	10 (8.3%)	Likelihood Ratio: 16.009, df: 3, P=0.003[cc]
Primary school	16 (26.7%)	5 (8.3%)	21 (17.5%)	
Secondary school	35 (58.3%)	53 (88.3%)	88 (73.3%)	
Bachelor	-	-	-	
Postgraduate	1 (1.7%)	-	1 (0.8%)	
Income				
Not adequate	18 (30%)	19 (31.7%)	37 (30.8%)	χ^2= 0.039, df: 1, P =0.843[b]
Adequate	42 (70%)	41 (68.3%)	83 (69.2%)	

[a]Unpaired T-Test, [b]Chi-Square Test, [c]Likelihood Ratio (Alternative Chi-Square Test)

Table No.2: Mean comparison of different biochemical parameters (n=120)

Biochemical Parameters	Study groups (central obesity)		Mean difference	Significance[a]
	Cases (Yes, n=60)	Control (Yes, n=60)		
Alanine Transaminase (U/L)	49.70±28.965	33.12±17.836	-16.583	t= -3.776, df:118, P= 0.000
Fasting blood sugar (mg/dL)	110.27±40.569	87.75±21.248	-22.517	t= -3.808, df:118,P= 0.000
Triglycerides (mg/dL)	235.63±66.350	161.07±23.989	-74.567	t= -8.187, df:118, P= 0.000
High-density lipoproteins (mg/dL)	35.08±4.073	41.95±3.039	6.867	t= 10.467, df:118, P= 0.000
C-reactive protein (mg/L)	19.90±25.363	9.70±5.378	-10.200	t= -3.047, df:118, P= 0.003
HOMA-IR (mg/dL)	5.930±1.5389	3.392±1.3303	-2.5383	t= -9.666, df:118, P= 0.000
Fibrosis-4 (FIB-4)	1.75033±1.067136	1.40183±0.803314	-0.348500	t= -2.021, df:118, P=0.046

[a]Unpaired T-Test

Table No.3: Distribution of study samples according to their level of fibrosis (n= 120)

Fibrosis' levels	Cases (n=60)		(Controls (n=60)		Total
	No.	%	No.	%	
F0 (No fibrosis)	4	6.7	-	-	
F (0-1)	33	55	53	88.3	86
F1 (Portal fibrosis without septa)	5	8.3	1	1.7	6
F2 (Portal fibrosis with few septa)	8	13.3	1	1.7	9
F3 (Portal fibrosis with numerous septa without cirrhosis)	4	6.7	3	5	7
F4 (Cirrhosis)	6	10	2	3.3	8

Likelihood Ratio: 21.584, df: 5, P = 0.001

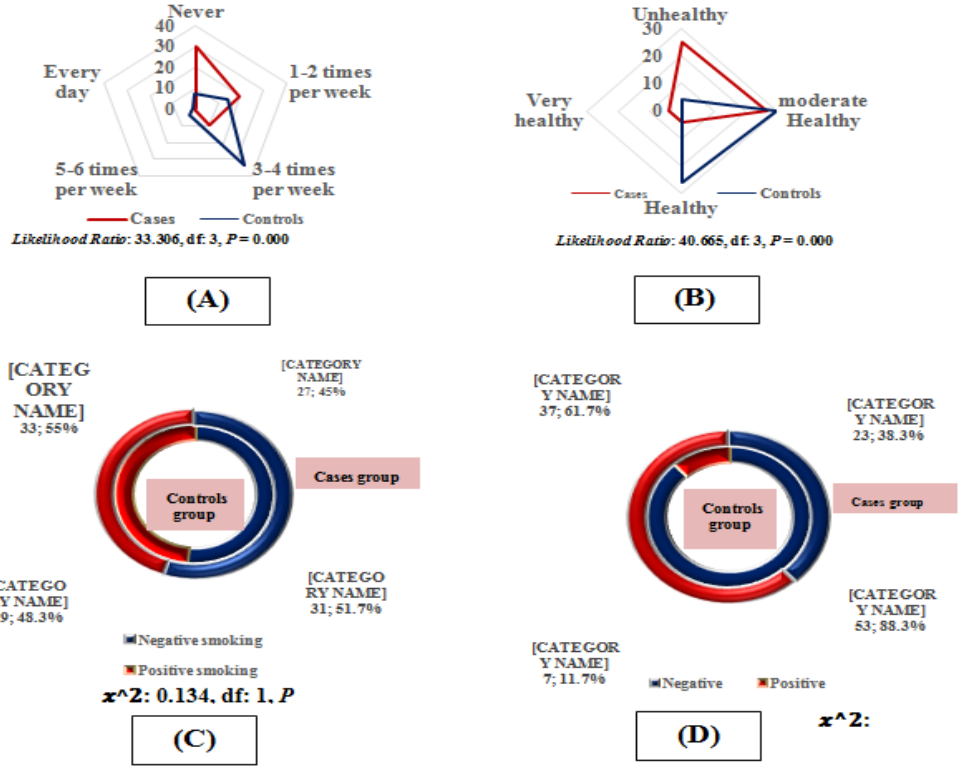

Figure No. 1: Comparison of lifestyle factors and insulin resistance marker between centrally obese and control groups

Table No.4: Distribution of study samples according to their level of steatosis (n= 120)

Fibrosis' levels	Cases (n=60)		(Controls (n=60)		Total
	No.	%	No.	%	
S0 (No steatosis)	31	51.7	50	83.3	81
S1 (5-33%)	9	15	4	6.7	13
S2 (34-66%)	7	11.7	3	5	10
S3 (> 66%)	13	21.7	3	5	16

χ^2 = 14.230, df: 3, P = 0.003

Table No.5: Mean comparison of anthropometric parameter of waist circumference among study's groups (n=120)

Anthropometric Parameter	Study groups (central obesity)		Mean difference	Significance[a]
	Cases (Yes, n=60)	Control (Yes, n=60)		
Waist circumference cm	107.65±12.107	78.63±5.434	-29.017	t= -16.938, df:118, P=0.000

[a]Unpaired T-Test

Table No.6: Mean comparison of anthropometric parameter of waist to height ratio among study's groups (n=120)

Anthropometric Parameter	Study groups (central obesity)		Mean difference	Significance[a]
	Cases (Yes, n=60)	Control (Yes, n=60)		
Waist to height ratio (WtHR)	0.6435±0.07897	0.4815±0.08205	-0.16200	t= -11.019, df:118, P= 0.000

[a]Unpaired T-Test

Other fibrosis and steatosis scores and grading were invesitigated using fibroscan and illustrated that, the higher grading of fibrosis were significantly more among cases groups than that at control group of study samples for F1, F2, F3, and F4 (8.3%, 13.3%, 6.7%, and 10%) vs. (1.7%, 1.7%, 5%, and 3.3%) respectively (Likelihood Ratio 21.584, df: 5, P=0.001) [Table 3].

Likewise, the higher steatosis grading was significantly more among cases groups than that of control group of study samples for S1, S2, and S3 (15%, 11.7%, and 21.7%) vs. (6.7%, 5%, and 5%) respectively ($\chi^{2:}$ 14.230, df: 3, P = 0.003) [Table 4].

The mean waist circumference was significantly higher in the central obesity group compared to controls (107.65±12.11 vs. 78.63±5.43; t = –16.938, df=118, p<0.001), indicating a markedly greater central fat accumulation among obese participants (Table 5).

The mean waist-to-height ratio was significantly higher in the central obesity group compared to controls (0.6435±0.07897 vs. 0.4815±0.08205; t = –11.019, df=118, p< 0.001), confirming a strong association between increased central adiposity and metabolic risk (Table 6).

DISCUSSION

In the present study, strong associations between central obesity and adverse metabolic, inflammatory, and hepatic outcomes, including insulin resistance, elevated liver enzymes, and advanced fibrosis and steatosis. Lifestyle differences were evident: physical inactivity was significantly more common among centrally obese patients (50% vs. 11.7%), aligning with epidemiological evidence linking sedentary behavior to obesity and MASLD.[17] Similarly, unhealthy dietary habits were more frequent in cases (41.7% vs. 6.7%), supporting the established link between poor diet quality and metabolic disorders.[18,19] These findings emphasize lifestyle modification as a key component in MASLD prevention and control.

Anthropometric results confirmed the superiority of central obesity indices (waist circumference and WtHR) over general obesity measures in predicting MASLD risk, reinforcing prior validations of WtHR as a simple and reliable screening tool.[20]

Clinically, insulin resistance was reflected by a significantly higher prevalence of acanthosis Nigricans in centrally obese individuals (61.7% vs. 11.7%), supporting its role as a non-invasive indicator of metabolic dysfunction and hepatic steatosis severity.[21,22]

Metabolic biomarkers demonstrated significantly higher fasting glucose, HOMA-IR, and CRP levels among cases, indicating marked insulin resistance and systemic inflammation - key drivers of MASLD pathogenesis.[21-23] Liver enzymes (ALT, AST) were also elevated, reflecting hepatocellular injury and inflammatory activity associated with MASLD.[23]

Lipid abnormalities, including increased triglycerides and total cholesterol with reduced HDL, were consistent with atherogenic dyslipidemia patterns reported in metabolic and hepatic disorders.[24]

Fibrosis assessment showed significantly higher FIB-4 scores and FibroScan stages among centrally obese participants, confirming central obesity's contribution to fibrosis progression. These findings support FIB-4 as a reliable non-invasive fibrosis index, though accuracy may decline in morbid obesity.[24,25] The combined use of elastography and fibrosis scoring enhances MASLD staging and risk stratification.[26]

CONCLUSION

Central obesity is a key determinant of MASLD severity, promoting insulin resistance, metabolic derangement, systemic inflammation, and fibrosis. Integrating WtHR screening, encouraging lifestyle modification, and using non-invasive fibrosis assessment are essential to improving MASLD management and outcomes.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ahmed M Shaker, Bilal Natiq Nuaman
Drafting or Revising Critically:	Ahmed M Shaker, Bilal Natiq Nuaman
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 4795 Dated 04-02-2025

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Original Article

The Association of Telomerase Reverse Transcriptase (TERT) Promoter Single Nucleotide Polymorphism (SNP) rs2853669 with Aggressive Features in Papillary Thyroid Cancer

Wafaa Khalel Ibrahim[1] and Khitam Razzak AL-Khafaji[2]

ABSTRACT

Objective: To detect the single nucleotide polymorphism rs2853669 in the telomerase reverse transcriptase promoter among patients' samples of papillary thyroid carcinoma and control samples, and then explore its correlation with specific clinicopathological parameters related to aggressive characteristics of papillary thyroid carcinoma.

Study Design: A retrospective study

Place and Duration of Study: This study was conducted at the Department of Pathology & Forensic Medicine, Faculty of Medicine, AL Anbar University, Ramadi Iraq and University of Baghdad, Baghdad, Iraq. from 1st June 2022 to 30th June 2023.

Methods: The samples collected were formalin-fixed paraffin-embedded tissue blocks from registered and stored cases of histologically proven papillary type thyroid carcinoma collected from the National Center for Education Laboratories, Medical City Campus - Baghdad and AL-Kimma private hospital. These tissue samples include 60 cases of papillary thyroid cancer and 15 cases of multinodular goiter. The telomerase reverse transcriptase promoter single nucleotide polymorphism rs2853669 was examined in 75 samples of papillary thyroid carcinoma and multinodular goiter, by real-time polymerase chain reaction subsequently correlating the findings with the clinicopathological features of papillary thyroid carcinoma, like age, sex, papillary thyroid carcinoma variants, size of the tumors, presence of capsules, multifocality, extrathyroid extension and lymph node metastasis.

Results: The rs2853669 A>G polymorphism was detected as AG genotype 40 (67%) the odd ratio was 1.111, p=0.0852 and confidence interval was (0.3658 to 3.3748), while GG genotype was evident in 14 of patients group (23%), the odd ratio was 0.687, P=0.567 and confidence interval was (0.1901 to 2.4864). The AA genotype was evident in 6/60 (10%), the odd ratio was 0.22, p=0.022, and the confidence interval was (0.0503 to 0.7956.), Allele study, the most frequent allele in the patients' group was G allele 68 (57%), with a p-value of 0.0852. Regarding the correlation with clinicopathological parameters, multifocality is present in 52% of cases, with a p-value =0.05

Conclusion: The rs2853669 polymorphism is present in high rate(67% of AG genotype, and 23% in GG genotype) in patients with papillary thyroid carcinoma , and correlates significantly with multifocality.

Key Words: Papillary thyroid carcinoma, Single nucleotide polymorphism, rs2853669, TERT promoter

Citation of article: Ibrahim WK, AL-Khafaji KR. The Association of Telomerase Reverse Transcriptase (TERT) Promoter Single Nucleotide Polymorphism (SNP) rs2853669 with Aggressive Features in Papillary Thyroid Cancer. Med Forum 2025;36(9):31-36. doi:10.60110/medforum.360906.

INTRODUCTION

Papillary thyroid carcinoma is the predominant form of thyroid carcinoma, constituting about 80% of instances.[1,2]

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Received: February, 2025
Reviewed: March-April, 2025
Accepted: May, 2025

The prevalence of papillary thyroid carcinoma (PTC), especially microcarcinoma, has risen due to the extensive use of ultrasonic examination.[3-5] The reported incidence of papillary carcinoma has more than quadrupled in several nations during the past fifty years.[6-8] Thyroid carcinoma in Iraq constitutes 1.7% Among recently identified neoplasms and 2.75% of female cancers, ranking second among the ten most prevalent tumors in females in the last Iraqi Cancer Registry.[9,10] Subtyping depends upon a mix of architectural characteristics, the existence or nonexistence of cytological features, and the magnitude

of the growth.[11] PTC typically exhibits an indolent nature and a favourable prognosis post-surgery, with 10-year survival rates for adults ranging from 92% to 98%.[12] However, a subset of PTC patients (5–10%) may progress to extremely severe metastatic malignancy.[13] Several risk factors indicate a poor prognosis, including male gender, advanced age, increased tumor size (≥4 cm), and gross extrathyroidal extension (ETE)[14], the existence of lymph node metastases measuring ≥3 cm and distant metastases.[15]

The TERT SNP, rs2853669 A>G, is situated in the promoter region of TERT. It impedes an ETS2 binding point situated adjacent to an Ebox. Research on rs2853669 indicated a significant association with decreased survival and elevated cancer risk in patients with hepatocellular carcinoma.[16]Numerous studies suggest that rs2853669 may influence cancer development in conjunction with certain TERT mutations.[17] It was reported that it could influence telomere length and telomerase activity.[18] A research by Rachakonda et al[19] indicated that, in patients with urothelial bladder cancer, TERT rs2853669 may be associated with survival, prognosis, and tumor recurrence. There is controversy about the role of TERT SNP role in different malignancies, our aim is to detect the frequency of TERT polymorphism among PTC samples and detect the correlation with aggressive features of PTC.

METHODS

This study represents a retrospective study, all samples collected were formalin-fixed paraffin-embedded tissue blocks that represent registered and stored cases of histologically proven papillary type thyroid carcinoma that were collected from the National Center for Education Laboratories, Medical City Campus - Baghdad and AL-Kimma private hospital-Baghdad/Iraq, from 1stJune2022 to 30thJune 2023.These tissue samples consist of 60 samples with a final diagnosis of papillary thyroid carcinoma (12males and 48 females), and 15 control tissue samples(1 male and 14 females) that represent samples with the final diagnosis of multinodular goiter. Inclusion criteria encompassed all cases with a final diagnosis of PTC, regardless of patient age or sex. Exclusion criteria involved papillary microcarcinoma variants, due to their typically low-risk clinical behaviour, as well as any samples that were damaged, inadequately preserved, or lacking complete clinical or pathological data were excluded. Several clinicopathological parameters that including sex, age (all ages included in the study), histopathologic types, size of tumor (maximum diameter), multifocal lesions, capsulation, lymph nodes metastasis and existence of extrathyroidal extension. Paraffin blocks were prepared for all cases, and slices were stained with haematoxylin and eosin (H and E). Subsequently, all slides were re-evaluated, and findings were recorded.

DNA extraction: By using Quick-DNA™ FFPE Kit [Catalog No. D3067-E (50 Preps.), Zymo Research Corporation D3067/ USA]. Deparaffinization was done by removing a trim (≤20µm thick) of a tissue sample of paraffin block that was transferred toa 1.5 ml microcentrifuge tube, then added400µl of deparaffinization solution to the sample. incubated at55ºc for 1minute, vortexed then the deparaffinization solution. Then tissue digestion through adding a mixture of (H2O45µl, 2X digestion Buffer45µl and Proteinase K10µL) then incubated at 55ºC overnight.DNA purification by adding 350µl of Genomic lysis buffer and mixed then added 135µl of isopropanol then mixed and centrifuged, transferred the supernatant to a zymo-spin II column in collection tube and centrifuged. added 400 µl of genomic DNA wash 1,700µl of genomic DNA wash 2 and 200µl of genomic DNA wash 2 subsequently and centrifuged after each step then transferred the content to a clean microcentrifuge tub and added ≥50µl of DNA elution buffer and incubation then centrifuged to elute the DNA

Molecular detection of TERT SNP rs2853669 A/G by real-time PCR: For TERT SNP rs2853669 A/G (ID:C-8773290-20,20X Assay Working, Thermo-Fisher Scientific / USA), Diluted the 40X Custom SNP Genotyping Assay to a 20X working stock solution. Vortexed and centrifuged the 20X Assay Working Stock. Completely homogenize TaqMan® Genotyping Master Mix by spinning the container. Resuspended the thawed frozen samples by vertexing, and then centrifuged the tubes briefly and calculatedthe overall number of reactions to be conducted for each assay. Calculated the total volume of each component required for each assay. We added (2X TaqMan® Master,20X Assay Working, DNA Sample Volume, and nuclease-free water) to a sterile, tube then transferred the mixture to the thermal cycler (Table 1).

The data was entered and analyzed through SPSS-29. The significance of the disparity between various means (quantitative data) was assessed utilizing the Student's t-test for the difference between two independent means or the ANOVA test for differences among more than two independent means. The importance of the variance across various percentages (qualitative data) was assessed using the Pearson Chi-square test(χ^2-test), using Yate's adjustment or the Fisher Exact test where appropriate. Statistical significance was deemed present when the P value was less than or equal to 0.05.

RESULTS

According to Hardy Weinberg equilibrium, the patient's group was 10.15, P-value=0.005. Genotyping study: the majority were AG genotyping 40 out of 60 of the patient's group (67%) and in 6 samples out of 15 (33%)

in the control group, the odd ratio was 1.111, p-value =0.0852 and confidence interval was (0.3658 to 3.3748), while GG genotype was evident in 14 samples out of 60 of patient group, (23%), the odd ratio was 0.687, P value = 0.567 and confidence interval was (0.1901 to 2.4864). The AA genotype was evident in 6 samples out of 60 (10%), the odd ratio was 0.22, p-value = 0.022, and confidence interval was (0.0503 to 0.7956). The major allele in the patients' group was G allele 68 (57%) and in the control group 14 (47%), the minor allele in the study was A 52 (43%), and in the control group16 (53%). Odd ratio 0.6691 p-value = 0.326 and confidence interval 0.2997 to 1.4937 (Table2, Fig. 1).

Regarding clinicopathological parameters the mean age for the patient group was 46.6±14.0(23-85) years, regarding gender there were 47(78.3%) females and 13(21.7%) males (Table 3), classical variant was evident in 43/60 (71.7%) while follicular variant was evident in 16(26.7%)(PTC histological variant in Figure 2), infiltrative tumors was evident in 42(70%), regarding the size of tumors, the medium and large size in which the tumors more than 2cm were 35(58.3%) while tumors ≥4cm is only 6(10%), multifocal tumors were present in 30(50%), extra thyroid extension was established in only 3(5%), while LN metastasis was evident in 17(28.3%) [Table 4].

Among the correlation of TERT polymorphism with the clinicopathological parameter classical variant was more evident in AG and GG genotypes than the follicular variant 72%, and 64% respectively, regarding gender female gender was more evident in all genotypes, the infiltrative tumors was more evident in AG and GG genotype 67% and 86% respectively, multifocal tumors evident in AG and GG genotype in 52% and 50% while LN metastasis was evident in AG and GG in 27% and 36% (Table 5). Tumor size more than 2cm was in AG and GG 26/40(65%),7/14(50%) respectively (Tables 6-7).

The correlation between age and the presence of SNPis more evident with age groups 30-39 14/40 and 50-59 years 10/40 in AG genotype, 40-49 4/14 and 50-59-years 6/14, in GG genotype (Table 8, Fig. 3).

Table No.1: cycles of real-time PCR for detection of TERT polymorphism

Predesigned SNP		
Temperature	**Duration**	**Cycles**
95°C	10 minutes	HOLD
95°C	15 seconds	40
60°C	1 minute (scanning)	

Table 2: Detection of TERT polymorphism in patient and control groups

Genotype	Control	Patient	ODD	P-Value	C.I.
AA	5 (33%)	6 (10%)	0.22	0.022	0.0503 to 0.7956
AG	6 (40%)	40 (67%)	1.111	0.0852	0.3658 to 3.3748
GG	4 (27%)	14 (23%)	0.687	0.567	0.1901 to 2.4864
A	16 (53)	52 (43)	0.6691	0.326	0.2997 to 1.4937
G	14 (47)	68 (57)			

Table No.3: Age and genderparameters rates inPTC and control group

Parameter		Patients (n=60)		Controls (MNG) [n=15]		P value
		No.	%	No.	%	
Age (years)	<30	3	5.0	-	-	0.273
	30-39	19	31.7	4	26.7	
	40-49	12	20.0	7	46.7	
	50-59	18	30.0	3	20.0	
	60-69	3	5.0	1	6.7	
	≥70	5	8.3	-	-	
Gender	Male	13	21.7	2	13.3	0.535
	Female	47	78.3	13	86.7	

Table No.4: Clinicopathological parameters in 60 PTC samples

Parameter		No.	%
Type of PTC	Classical	43	71.7
	Follicular	16	26.7
	Sclerosing + Hobnail	1	1.7
Presence of capsule	Infiltrative	42	70.0
	Encapsulated	18	30.0
Size (cm)	Small	25	41.7
	Medium	18	30.0
	Large	17	28.3
Size (cm)	1.0---	11	18.3
	1.5	14	23.3
	2.0	13	21.7
	2.5	5	8.3
	3.0	6	10.0
	3.5	5	8.3
	4.0	6	10.0
Multiplicity	Unifocal	30	50.0
	Multifocal	30	50.0
Extra thyroid extension	Positive	3	5.0
	Negative	52	86.7
	Not identified	5	8.3
LN metastasis	Positive	17	28.3
	Negative	34	56.7
	Not identified	9	15.0

Table No.5: The correlation between clinicopathological parameters and TERT polymorphism

Clinicopathological Parameter	AA	AG	GG	P-value
Type of PTC				
Classical	3 (50%)	29(72.5%)	9(64.3%)	0.724
Follicular	3 (50%)	9(22.5%)	4(28.6%)	
Classical with poorly differentiated	-	2 (5%)	-	
Sclerosing + Hobnail PTC	-	-	1 (7.1%)	
Gender				

Female	5(83.3%)	30 (75%)	12(85.7%)	0.673
Male	1(16.7%)	10 (25%)	2 (14.3%)	
Presence of capsule				
Encapsulated	3 (50%)	13(32.5%)	2 (14.3%)	0.376
Infiltrative	3 (50%)	27(67.5%)	12 (85.7)	
Extra thyroid extension				
Positive	-	3 (7.5%)	-	0.454
Negative	6 (100%)	37(92.5%)	14 (100%)	
Multiplicity				
Multifocal	2(33.3%)	21(52.5%)	7 (50%)	0.05
Unifocal	4(66.7%)	19(47.5%)	7 (50%)	

Lymph Node metastasis				
Positive	1(16.6%)	11(27.5%)	5 (35.7%)	0.945
Negative	5(83.4%)	29(72.5%)	9 (64.3%)	

Table No.6: Correlation between the size of tumors (2 cm) and SNP presence

SNP	Size <2	Size ≥2	Total	P-value
AA	4 (66.7 %)	2(33.3 %)	6 (100%)	0.263
AG	14 (35%)	26 (65%)	40(100 %)	
GG	7 (50%)	7 (50.%)	14(100 %)	
Total	25(41.7 %)	35(58.3 %)	60100 %	

Table No.7: Correlation of size of tumor groups and TERTSNP

SNP	Size in cm							Total
	1-<1.5	1.5-<2	2-<2.5	2.5-<3	3-<3.5	3.5-<4	≥4	
AA	1 (16.7%)	3 (49.9%)	1 (16.7%)	-	-	-	1 (16.7%)	6 (100%)
AG	7 (17.5%)	7 (17.5%)	9 (22.5%)	4 (10%)	5 (12.5%)	4 (10%)	4 (10%)	40 (100%)
GG	3 (21.5%)	4 (28.6%)	3 (21.5%)	1 (7.1%)	1 (7.1%)	1 (7.1%)	1 (7.1%)	14 (100%)
Total	11 (18.3%)	14 (23.3%)	13 (21.7%)	5 (8.3%)	6 (10%)	5 (8.3%)	6 (10%)	60 (100%)

P value = 0.46

Table No. 8: Correlation between the size of tumors and genotypes ofTERT SNP in PTC and control samples

Age (years)	AA		AG		GG	
	Patients	Controls	Patients	Controls	Patients	Controls
30-39	3 (13%)	2 (8.7%)	14 (60.87%)	2 (8.7%)	2 (8.7%)	-
40-49	1 (5.26%)	2 (10.53%)	7 (36.84%)	4 (21.05%)	4 (21.05%)	1 (5.26)
50-59	2 (9.52%)	1 (4.76%)	10 (47.62%)	-	6 (28.57%)	2 (9.25)
60-69	-	-	2 (50%)	-	1 (25%)	1 (25)
<30	-	-	2 (66.67%)	-	1(33.33%)	-
>70	-	-	5 (100%)	-	-	-

P value = 0.228

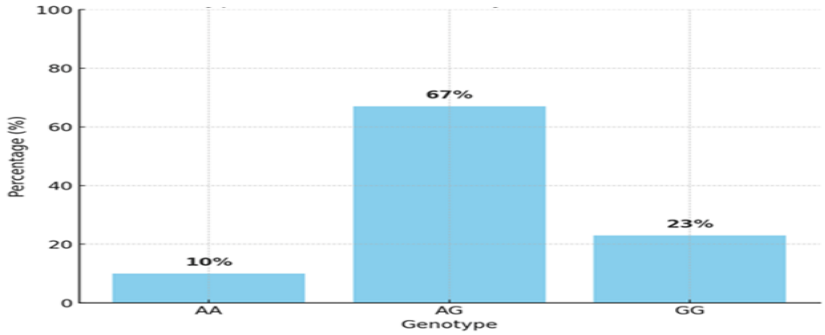

Figure No. 1: Percentage and distribution of SNP genotypes in PTC samples

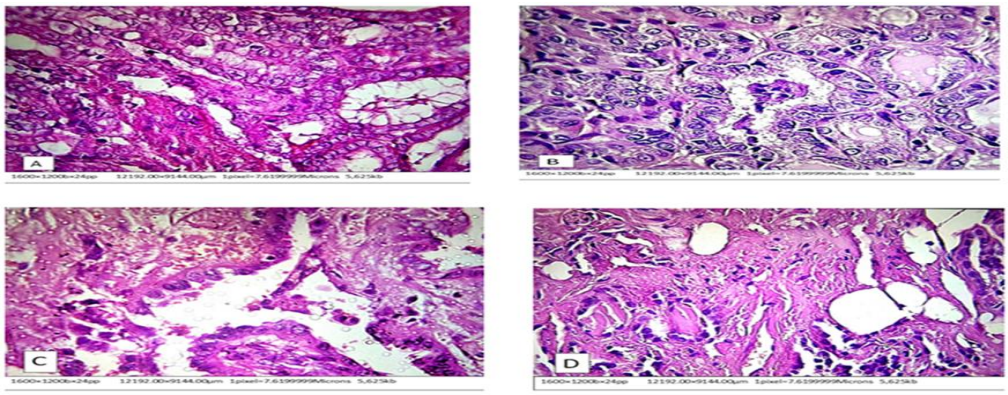

Figure No. 2: Variants of PTC. A: classical variant X400 B: follicular variant X400 C: hobnail variant X400, and D: sclerosing variant X100.(H &E stain)

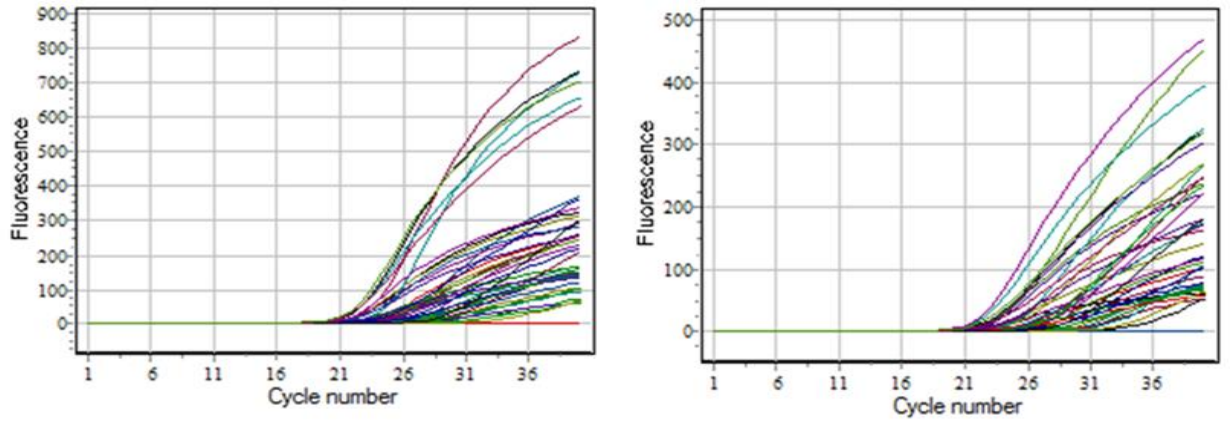

Figure No. 3: RT-PCR curves of the First allele (FAM channel), RT-PCR curves of the second allele (HEX channel)

DISCUSSION

Papillary thyroid carcinoma represents the most prevalent form of thyroid cancer. While most cases exhibit a favourable prognosis, some may be associated with aggressive behaviour. Numerous studies have identified a correlation between aggressive behaviour and various genetic alterations, particularly involving the TERT and BRAF genes. TERT polymorphism refers to variations in the TERT gene, while single nucleotide polymorphisms (SNPs) are variations occurring at single nucleotide locations within the genome. SNPs are categorized into two forms: coding SNPs and noncoding SNPs. Noncoding SNPs encompass regulatory, untranslated, intron, silent, and genomic SNPs. Regulatory SNPs (rSNPs) are located in noncoding regions, including promoters, enhancers, and the 3′ ends of genes(20).Some rSNPs may influence the expression of adjacent genes and the tumorigenesis or proliferation of certain carcinomas.[21]rs2853669 has been identified in some malignant neoplasms, including hepatocellular carcinomas[22], other neoplasms such as lung carcinomas and glioblastoma. A study revealed the conjunction of rs2853669 and C228T is substantially associated with increased tumor development in patients with PTC.[23]

Investigated in TERT rs2853669 SNP in Iraqi patients with Papillary thyroid carcinoma was 40 (67%)for heterozygous genotype AG and homozygous GG genotype 14(23%). The result showed that AG genotype may be a risk factor since odd ratio 1.111 but it is statistically not significant (CI=0.3658 to 3.3748, p-value=0.0852) While AA genotype odd ratio=0.22 that is mean it maybe protective factor that is confirmed by (CI=0.0503 to 0.7956, p vale=0.022) that mean is statistically significant. The G allele was found in 68 (57%) while the A allele was 52 (43%). A comparable result was observed in K. Vidinov et.al[24]in Bulgarian population they found that G allele frequency was 52.49%, homozygous GG genotype 5/18(27.7%)and heterozygousgenotype 13/18(72.3%), although the study done by Sanger sequencing, while in Tatsuya et al[23]the rate of genetic frequencies of rs3853669 was 58.6%. High variation in the percentage of genotypeslike AA in Tatsuya etal[25] 87.5%, thesevariations may related to differences in samples size, exclusion criteria, method of DNAextraction, detection method of polymorphism, and possible geographical difference in the pattern TERT promotor polymorphism. Regarding the clinicopathological parameters including gender, variant of PTC, size of tumors, presence of capsule, extra thyroid extension and LN metastasis, although some parameters shown an increasing rate with the TERT SNP presence but failed to establish statistically significant correlation except for multifocal tumors. While the finding that reported by Vidinov etal[24], all clinical parameters were statistically not significant.

CONCLUSION

The rs2853669 polymorphism is present at an increasing rate in PTC samples, in 67% in AG genotype, 23% in GG genotype, and correlates significantly with multifocality with P-value=0.05 while other parameters of tumor aggressiveness like large tumor size, lymph node metastasis and extra-thyroid extension shown no significant correlation with TERT SNP.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Wafaa Khalel Ibrahim, Khitam Razzak AL-Khafaji
Drafting or Revising Critically:	Wafaa Khalel Ibrahim, Khitam Razzak AL-Khafaji
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 3982/QM/Approval/287863J Dated 01.05.2022

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Original Article

Effectiveness of the Environmental Safety Measures Program on Nurses' Knowledge and Practices in Hemodialysis Units

Shady Shafeek Anees Dowarah and Sahar Adham Ali

ABSTRACT

Objective: To evaluate the effectiveness of an environmental safety measures educational program on nurses' knowledge and practices in hemodialysis units.

Study Design: A quantitative, quasi-experimental study

Place and Duration of Study: This study was conducted at the Adult Health Nursing, University of Babylon, College of Nursing, Iraq from 8th January 2024 to 18th June 2025.

Methods: A quantitative, quasi-experimental study was conducted at Adult Health Nursing, University of Babylon, College of Nursing, Iraq. A total of 100 nurses from hemodialysis units was selected and divided equally into control and interventional groups.

Results: Unsatisfied levels of knowledge and practices in both groups during the pre-test. However, the interventional group showed significant improvement in knowledge and practices in both post-tests.

Conclusion: The educational program proved its effectiveness to improve nurses' knowledge and practices toward environmental safety measures in hemodialysis units. Ongoing training and mentorship at the unit levels were recommended.

Key Words: Effectiveness, Safety measures, Hemodialysis, Knowledge, Practices.

Citation of article: Dowarah SSA, Ali SA. Effectiveness of the Environmental Safety Measures Program on Nurses' Knowledge and Practices in Hemodialysis Units. Med Forum 2025;36(9):37-42. doi:10.60110/medforum.360907.

INTRODUCTION

Environmental safety encompasses the physical layout, waste disposal, and emergency preparedness. Proper spacing, adequate ventilation, and ergonomic designs reduce cross-contamination and occupational hazards. Effective waste management systems protect staff and patients from infectious agents.[1]

Maintaining humidity at 55-60% prevents the growth of microorganisms, reducing infection risk.[2] The dialysis area should be 70°F-72°F (21.11°C–22.22°C). Adequate temperature control insure patient comfort environment and minimize risks like hypothermia or overheating.[3] Proper lighting is crucial for visibility and preventing errors. Sufficient lighting in treatment areas and corridorsis essential.[4] Ventilation systems must ensure sufficient air exchange to remove airborne contaminants.[3]

The World Health Organization (WHO) recommends hospital noise levels not exceed 35 dB during the day and 30 dB at night. though actual levels are around 72 dB during the day and 60 dB at night.[5] Thenoise range in HD unit ranges from 48.40 to 72 dB.[6] Therefore, controlling noise sources is vital, raising awareness and education of nurses, doctors, and other staff is the most effective way to change behavior.[7]

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Received: June, 2025
Reviewed: July, 2025
Accepted: August, 2025

METHODS

This quantitative quasi-experimental was conducted at in hemodialysis centers at Imam Al-Hussein Medical City and Imam Al-Hassan Al-Mujtaba Teaching Hospital in Karbala from 8th January 2024 to 18th June 2025 vide letter No. 57 dated 17th December 2024. Nurses who met inclusion criteria provided written informed consent after the study's purpose and data confidentiality were explained. A non-probability purposive sample of 100 nurses was selected and randomly divided into two equal groups (control and interventional) using the lotto method. All hemodialysis nurses with one year or more of experience, scoring less than 60% in the pre-test, and with patient's direct contact (care provider) were included. Needs

assessment was conducted using a 14-question questionnaire for 30–45-minute educational program was then developed based on WHO guidelines. A special questionnaire and checklist were used, the questionnaire adapted from Wadi RJ 2022 after obtaining permission via email[8], included four parts: Demographical characteristic (6 items), clinical workplace data (3 items), and nurses' knowledge about environmental safety measures (9 items).

Facility availability and policies, nurses practices were evaluated using an Observational Checklist adopted from the CDC[9], it was observed three times and included dialysis station routine disinfection checklist includes: before beginning routine disinfection (6 items), and after patient has left station (7 items) and sharp disposable checklist (6 items).

For the questionnaire, a correct answer scored (2) and an incorrect (1). Levels were defined as: Poor (>1.5), Fair (=1.5), Good (<1.5). For the practice checklist, observations were scored: Always (3), Sometimes (2), Never (1). Levels were: Poor if the mean (1-1.6), Fair (1.7-2.39), Good (2.4-3). Content validity of the instruments was assessed by a panel of 9 faculty members with at least 15 years of experience. A pre-test was given to all participants. The control group took post-tests after two and four weeks. The interventional group received the educational program in sessions of 4-6 nurses, followed by post-tests at the same intervals.

Data were analyzed using SPSS-26. T-test and Pearson Chi-square were used to assess homogeneity at baseline. Significance levels were set at $P<0.05$.

RESULTS

There was no significant difference in age (p=0.162) or sex (p=0.134). Both groups had identical gender ratios. There was no significant difference in marital status (p=0.160) or residency (p=0.069). A significant difference was found in educational qualifications (p=0.036), with the control group having a higher percentage of bachelor's degree holders (68% vs. 52%) and the interventional group having more diploma holders (44% vs. 22%) [Table 1].

Table 2 showed a statistically significant difference in years of experience (p=0.023), though the majority in both groups had 1–5 years of experience (88% interventional, 78% control). No significant difference was found in working shifts (p=0.690). A near-significant difference was noted in prior participation in safety courses (p=0.028), with more in the control group (78%) having attended courses than the interventional group (64%). The number of courses attended did not differ significantly (p=0.639).

The significant positive change in the knowledge scores of the interventional group over time. For example, knowledge on distinguishing surface disinfection from physical cleaning improved from a mean of 1.30 in the pre-test to 1.68 in post-test 2. Similarly, knowledge on proper lighting, temperature, and humidity increased, with post-test 2 means ranging from 1.70 to 1.80. The control group, however, showed minimal or no improvement. This emphasizes the educational program's effectiveness (Table 3).

The interventional group showed a continuous increase in mean scores across all items related to pre-disinfection procedures, indicating improved compliance. Significant gains were seen in disposal practices, contamination checks, glove removal, and hand hygiene. In contrast, the control group had slight and mostly insignificant changes. The educational intervention positively influenced the promotion of adequate disinfection practices in the interventional group (Table 4). The checklist for disinfection after a patient has left was not applicable, as this task was handled by a services company, not nurses.

Table No. 1: Demographical Characteristics (n=50)

Characteristics		Control group		Interventional group		P value
		No.	%	No.	%	
Age (years)	22-26	26	52.0	14	28.0	0.162
	27-31	8	16.0	21	42.0	
	32-36	7	14.0	7	14.0	
	37-41	5	10.0	6	12.0	
	42-46	4	8.0	3	6.0	
Gender	Female	17	34.0	17	34.0	0.134
	Male	33	66.0	33	66.0	
Marital status	Single	20	40.0	31	62.0	0.160
	Married	30	60.0	19	38.0	
Educational Qualifications	Secondary Nursing School	4	8.0	2	4.0	0.036
	Diploma	11	22.0	22	44.0	
	Bachelor	34	68.0	26	52.0	
	Postgraduate	1	2.0		100.0	
Residence	Rural	12	24.0	13	26.0	0.69
	Urban	38	76.0	37	74.0	

Table No. 2: Employment Characteristics (n=50)

Clinical workplace		Control group		Interventional group		P value
		No.	%	No.	%	
Period of working in hemodialysis unit (years)	1-5	39	78.0	44	88.0	0.023
	6-10	7	14.0	6	12.0	
	11-15	4	8.0			
Working Shift	Morning	23	54.0	22	44.0	0.693
	Evening	27	46.0	28	56.0	
A tendency of special courses related to safety measure	Yes	11	22.0	18	36.0	0.028
	No	39	78.0	32	64.0	
The number of courses	No there	11	22.0	18	36.0	0.639
	1-3	30	60.0	25	50.0	
	4-6	9	18.0	7	14.0	

Table No. 3: Nurses' knowledge toward environmental safety

Control Group				Interventional Group			
Pre-test	Post-test 1	Post-test 2	P value	Pre-test	Post-test 1	Post-test 2	P value
1.40±0.49	1.42±0.49	1.38±0.49	0.243	1.30±0.46	1.58±0.49	1.68±0.47	0.095
1.58±0.49	1.42±0.49	1.42±0.49		1.34±0.47	1.52±0.50	1.80±0.40	
1.00±.0.0	1.00±0.0	1.08±0.27		1.34±0.47	1.38±0.49	1.74±0.44	
1.54±0.50	1.66±0.47	1.62±0.48		1.44±0.51	1.70±0.46	1.80±0.43	
1.28±0.54	1.16±0.37	1.18±0.38		1.40±0.49	1.58±0.49	1.70±0.46	
1.38±0.49	1.36±0.45	1.32±0.47		1.56±0.50	1.44±0.50	1.74±0.44	
1.38±0.49	1.30±0.46	1.26±0.44		1.54±0.53	1.62±0.49	1.94±0.24	
1.42±0.49	1.74±0.44	1.64±0.48		1.58±0.49	1.72±0.45	1.80±0.40	
1.02±0.14	1.04±0.19	1.04±0.19		1.54±0.53	1.84±0.37	1.94±0.24	

Table No. 4: Nurses' practices: before routine disinfection

Control Group				Interventional Group			
Pre-test	Post-test 1	Post-test 2	P value	Pre-test	Post-test 1	Post-test 2	P value
2.20±0.99	2.92±0.39	2.28±0.97	0.028	1.84±0.99	2.60±0.80	2.60±0.80	0.116
2.18±0.98	2.76±0.55	2.42±0.88		1.84±0.99	2.60±0.80	2.60±0.80	
1.12±0.35	1.12±0.38	1.14±0.35		1.08±0.39	2.36±0.85	2.36±0.85	
2.02±0.97	2.84±0.37	2.22±0.92		1.82±0.98	2.60±0.80	2.60±0.80	
1.54±0.70	1.52±0.61	1.84±0.79		1.36±0.72	2.48±0.81	2.48±0.81	
1.28±0.45	1.30±0.46	1.28±0.45		1.10±0.36	2.32±0.79	2.32±0.79	

Table No. 5: Nurses' practices: sharps disposal

Control Group				Interventional Group			
Pre-test	Post-test 1	Post-test 2	P value	Pre-test	Post-test 1	Post-test 2	P value
1.58±0.64	2.92±0.27	2.16±0.97	0.242	2.06±0.99	2.32±0.95	2.80±0.60	0.038
1.00±0.0	1.00±0.0	1.00±0.0		1.00±0.0	1.98±0.82	2.34±0.65	
1.58±0.64	2.98±0.14	2.14±0.99		2.02±1.0	2.32±0.97	2.96±0.28	
1.92±0.75	1.92±0.72	1.70±0.81		1.58±0.64	2.32±0.95	2.80±060.	
1.56±0.73	1.46±0.67	1.56±0.78		1.24±0.47	2.24±0.91	2.62±0.66	
1.20±0.57	1.20±0.60	1.08±0.39		1.42±0.70	2.32±0.95	2.80±0.60	

Table No. 6: Comparison of Post-Test Knowledge and Practices

Responses of sample	Control Group		Level	Interventional Group		Level	P value	Significance
	Post-test 1	Post-test 2		Post-test 1	Post-test 2			
Nurses' knowledge toward environmental safety at hemodialysis units	1.34±.381	1.32±.415	Poor	1.59±.474	1.79±.390	Good	0.013	Significance

Knowledge: Poor level <1.5 Fair level = 1.5 Good level >1.5

Table No.7: Comparison of Post-Test Results Between the Control and Interventional Groups Regarding Nurses' practices

Responses of sample	Control Group		Level	Interventional Group		Level	P value	Significance
	Post-test 1	Post-test 2		Post-test 1	Post-test 2			
Nurses' practices toward before beginning routine disinfection of the dialysis station: at hemodialysis units	2.07±.463	1.86±.730	Fair	2.49±.813	2.79±.409	Good	0.032	Significance
Nurses' practices toward sharp disposable at hemodialysis units	1.91±.403	1.60±.660	Poor	2.25±.927	2.72±.571	Good		

Knowledge: Poor level <1.5 Fair level = 1.5 Good level >1.5

Table 5 shows the interventional group's improvement in sharp material disposal. Mean scores for immediately placing sharps in containers increased from 2.06 (pre-test) to 2.80 (post-test 2). The control group's scores remained lower and inconsistent. A significant deficit was noted in both groups regarding the placement of sharps containers close to the point of use, with the control group showing no change (mean = 1.00). Overall, the interventional group exhibited improved compliance compared to the control group.

Table 6 reveals a significant improvement in the interventional group's knowledge compared to the control group (P=0.013). The control group consistently demonstrated "poor" knowledge (mean ≈ 1.32-1.34), while the interventional group achieved "good" knowledge levels (mean ≈ 1.59-1.79).

Table 7 clear improvement in the interventional group's practices, the control group remained at "poor" to "fair" levels for disinfection and sharps disposal, while the interventional group reached "good" practice levels in both post-tests, with a significant difference noted for pre-disinfection practices (P = 0.032).

DISCUSSION

The demographic profile of the participants is similar to previous studies in similar settings. The age distribution with most nurses being young, aligns with findings by Mahmood and Khudur.[10] Younger nurses may be more adaptable to new knowledge, which is a factor in evaluating educational programs.

The sex distribution (66% male) differs from some other in Egypt and Arab nations where nursing is female-dominated[11], suggesting educational materials should be gender-neutral.

A significant difference was found in educational levels (p= .036), with more bachelor's degree holders in the control group. This disparity is in line with regional and international research-based results. Such as study by Abdelsatir.[12] This suggests that training programs should consider participants' educational backgrounds. Most participants were from urban areas, which could influence access to professional development opportunities compared to nurses in rural areas.

There was a significant difference in work experience (p=0.023), with most nurses having 1-5 years of experience. Novice nurses often show greater improvement after structured training programs, as demonstrated by Singh et al.[13] Although not statistically significant, working shifts can influence training outcomes, as morning shifts may offer more learning opportunities.[14]

The fact that many nurses in both groups had previously attended safety courses suggests a desire for professional development, which impacts baseline knowledge. This result was guaranteed by the study of Aldawaha et al.[15]

In the interventional and control groups, 50% and 60% respectively completed one to three courses. Although statistically insignificant (p= .639). This can have an influence on the practice and knowledge retention. Similar to the current literature Eltib et al[16] which found that recurrent training supports safety standards and enhances long-term adherence.

An educational study significantly improved the interventional group's knowledge of environmental safety. Participants better understood surface disinfection, lighting, noise control, and patient station spatial needs. These results support that educational programs boost knowledge and compliance with safety protocols[17], and that environmental design awareness is vital for quality of care.[18]

The improvement in the environmental knowledge of the interventional group due to effective lighting and noise control corresponds to the findings by Koon.[19] In addition Himmelfarb et al[20] support the study findings, by emphasized that sufficient spatial planning of dialysis units is essential to avoid cross-contamination and provide safe care delivery.

The control group showed no improvement, supporting the need to reinforce safety education. This aligns with

existing research that emphasizes continuous professional learning to improve healthcare and patient safety, specifically in hemodialysis units.[21]

The findings also prove positive changes in routine disinfection and sharps disposal practices for the interventional group. These findings are supported by the recent literature that outlines the significant role of structured educational program to improve the infection control and safety competencies.[22] Additionally, the findings align with recent study results showing that trained nurses better follow safe sharp disposal in hemodialysis.[23]

The progressive improvement in mean scores for practices like hand hygiene and proper sharps handling demonstrates greater adherence to safety protocols post-intervention. These results align with recent literature showing that specific training enhances nurses' safety competencies in dialysis units.[24]

The greater post-test scores in the interventional group in all safety practices validate the success of the program in improving compliance, which are consistent with previous studies that note that well-structured training increases nurse safety compliance.[25]

The study recommended implementing regular, targeted safety training for novice nurses using interactive methods, and advanced workshops for experienced nurses.

CONCLUSION

Most participants were young, male, urban residents with less than ten years of experience. At pre-test, both groups showed unsatisfied levels of knowledge and practices. The educational program was effective, as the interventional group showed significant improvements in knowledge and practices in both post-tests.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Shady Shafeek Anees Dowarah, Sahar Adham Ali
Drafting or Revising Critically:	Shady Shafeek Anees Dowarah, Sahar Adham Ali
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.57 Dated 17.12.2024

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Original Article

Cytotoxic and Genotoxic Effects of the Cold Aqueous Extract of Glycyrrhiza Glabra Roots on MCF-7 Breast Cancer Cells and REF Normal Cells in Vitro

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ABSTRACT

Objective: To evaluate the cytotoxic and genotoxic effects of the cold aqueous extract of Glycyrrhiza glabra roots on MCF-7 breast cancer cells and REF normal cells in vitro.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the Al-Nahrain University Biotechnology Research Centre from 22nd December 2024 to 20th March 2025,
Methods: Glycyrrhiza glabra extract was administered to MCF-7 breast cancer cells at different concentrations (70, 140, 210, 280, and 350 µg/ml) for 24, 48, and 72 hours. The embryonic fibroblast cells were treated 72 hours. Cell viability, IC_{50} values, and deoxyribonucleic acid damage was assessed using the comet assay.
Results: This extracts reduced MCF-7 cell proliferation time- and dose-dependently at 350 µg/ml inhibition rates was significantly ($p<0.05$): 77.778% (24 h), 79.349% (48 h), and 87.262% (72 h). The IC_{50} of embryonic fibroblast cells was 5.59 mg/ml after 72 hours, demonstrating selective susceptibility. In the comet test, MCF-7 cells treated with increasing extract concentrations demonstrated moderate and severe DNA damage after 72 hours.
Conclusion: Gglycyrrhiza glabra root cold aqueous extract is dose and time-dependently cytotoxic and genotoxic to MCF-7 breast cancer cells but not normal fibroblast cells.

Key Words: Glycyrrhiza glabra, Cytotoxicity; Comet assay; MCF-7 cells

Citation of article: Kadhim MM, Mardan AR. Cytotoxic and Genotoxic Effects of the Cold Aqueous Extract of Glycyrrhiza glabra Roots on MCF-7 Breast Cancer Cells and REF Normal Cells in Vitro. Med Forum 2025;36(9):43-48. doi:10.60110/medforum.360908.

INTRODUCTION

Medical plant bioactive substances are investigated globally for therapeutic and pharmacological uses. To cure cancer, plants help. Traditional medicine prevents sickness using herbs. Modern medicine uses plants and drugs. Principal herbal drug is plant secondary metabolites, alkaloids, glycosides, chlorophyll, carotenoids, protein, minerals, vitamins.[1] Plant extracts treat diabetes, heart, and cancer, Glycyrrhiza glabra L., bushy perennial licorice with lateral blooms and semi-creeping branches. Licorice grows 50–125cm^2, depending on blossom color. Fabaceae has licorice. The bioactive components glycyrrhizin, 18-β glycyrrhetinic acid, and isoflavones have medicinal promise.[3]

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Received: April, 2025
Reviewed: May, 2025
Accepted: June, 2025

Immunomodulatory, anti-inflammatory, antiviral, antioxidant, and antidiabetic, this plant kills bacteria. Cancer is uncontrolled cell development that tumors. A tumor might be malignant or benign cancer may spread.[4] DNA alterations that disrupt cell cycle may cause cancer.[5] Bone, brain, lungs, blood cells, etc. may spread cancer. Tissue origin classification of malignant cancers diseases may spread from blood or lymphatic vessels, complicating therapy.[6]

METHODS

This experiment study was conduct at Al-Nahrain University Biotechnology Research Centre from 22nd December 2024 to 20th March 2025. Glycyrrhiza glabra roots were available in Baghdad. After washing off dust and dirt, the roots were air-dried at room temperature and crushed into powder. 30 g powder and 300 ml distilled water were put to a clean flask. The mixture rested 24 hours at room temp. The flask extracted surface-active components best when power-swirled. The extract was filtered through sterile gauze after 24 hours to separate liquid and solid residues. The filtered extract was lyophilized at 4°C before use.

Cell Lines and Culture Conditions: Al-Nahrain University's Biotechnology and Genetic Engineering Research Center offered MCF-7 breast cancer cells and

normal rat embryonic fibroblasts for investigation. The cell lines were grown in DMEM with 10% FBS and 1% penicillin-streptomycin, cells grown in a sterile CO_2 incubator (5% CO_2, 37°C).

Cytotoxicity Assay (MTT Assay): MTT was used to investigate Glycyrrhiza glabra root cold aqueous extract cytotoxicity. The first MTT experiment tested MCF-7 cells with cold aqueous extract at various concentrations (70, 140, 210, 280, 350 µg/ml) for 24 hours, 48 hours, and 72 hours. For 72 hours, REF cells received the same cold aqueous extract. MCF-7 and REF-exposed cells received MTT after 4, 24, 48, and 72 h. An ELISA reader measured 620 nm absorbance after dissolving formazan crystals in 0.04 ml DMSO.

Genotoxicity Assay (Comet Assay): The extract was administered to MCF-7 cells for 72 hours and tested for genotoxicity using the alkaline comet assay. Cells were prepared and stained with Green SYBR dye as normal. Image analysis program estimated genotoxicity from comet tail DNA after staining (a greater ratio indicates more genetic damage). The data was entered and analyzed through SPSS-25.

RESULTS

Cold aqueous Glycyrrhiza glabra root extract was evaluated for cytotoxicity on normal REF. The table demonstrates extract concentration lowered cell viability. Cytotoxicity was concentration-dependent with an IC_{50} of 5.59 mg/ml. Cytotoxicity was shown by REF cell density decrease, rounding, and detachment after 72 hours with the cold aqueous G. glabra extract (Table 1). These morphological changes were more obvious at increasing dosages, but untreated control cells did not change (Figs. 1-2)

After 24 hours, the cold aqueous extract showed considerable cytotoxicity ($p<0.05$) compared to the control group (Table 2). Cancer cell proliferation decreased by 4.483% at 70 µg/ml. After 24 hours, cytotoxic activity peaked at 77.778% at 350 µg/ml as concentration increased. After 48 hours, inhibition increased at all levels, with 70 µg/ml reaching 5.881% and 350 µg/ml reaching 79.349%. The percentage inhibition increased significantly after 72 hours, reaching 13.887% at 70 µg/ml and 87.262% at 350 µg/ml.

Table No.1: The impact of cold aqueous extract of Glycyrrhiza glabra root at different concentrations on the viability of normal cells (REF) after 72 hours of exposures

Concentrations (mg/ml)	Mean±SD
1	92.12±1.72
2	79.04±5.66
4	52.31±6.25
6	39.81±3.82
8	30.56±1.40
10	15.94±2.87
12	12.50±1.29

Table No.2: The effect of Cold Aqueous Extract of Glycyrrhiza glabra roots on MCF-7 Cells at different concentrations after 24, 48, and 72hrs

Time/Conc. (µg/ml)	IR (%) 24 hrs	IR (%) 48 hrs	IR (%) 72 hrs	LSD
0µg/ml	E, b 3.520±1.678	E, b 4.773±1.694	F, a 6.656±1.046	1.293615
70µg/ml	E, b 4.483±2.566	E, b 5.881±1.624	E, a 13.887±3.010	2.125452
140µg/ml	D, b 24.505±5.220	D, b 26.518±5.330	D, a 38.551±5.080	4.482866
210µg/ml	C, b 35.809±6.500	C, b 37.979±6.640	C, a 49.930±5.860	5.455869
280µg/ml	B, b 60.630±3.480	B, b 62.254±3.930	B, a 73.529±2.150	2.818712
350µg/ml	A, b 77.778±2.794	A, b 79.349±3.010	A, a 87.262±2.980	2.520545
P-value	0.00012	0.00046	0.00068	
LSD	3.416072	3.479814	3.145972	

Table No.3: The impact of cold aqueous extracts of Glycyrrhiza glabra roots on DNA damage and the Comet assay in MCF-7 breast cancer cells at various concentrations

Parameter/Conc. (µg/ml)	No Damage	Low Damage	Medium Damage	High Damage
0µg/ml	A: 42.563±1.756	AB: 42.218±1.707	D: 7.045±0.428	D: 8.174±0.755
70µg/ml	B: 39.123±4.030	A: 42.439±3.482	C: 8.867±0.393	D: 9.571±0.516
140µg/ml	BC: 37.468±2.130	C: 38.981±1.823	B: 11.471±1.751	C: 12.080±1.636
210µg/ml	CD: 35.762±3.176	BC: 39.820±1.885	B: 12.133±1.373	C: 12.285±1.406
280µg/ml	CD: 34.859±1.229	D: 35.335±0.999	A: 14.684±0.757	B: 15.122±0.921
350µg/ml	D: 32.981±0.879	D: 34.413±1.060	A: 15.649±0.818	A: 16.957±1.094
P-value	0.0014	0.0007	0.00033	0.00056
LSD	2.643509	2.153496	1.122144	1.205672

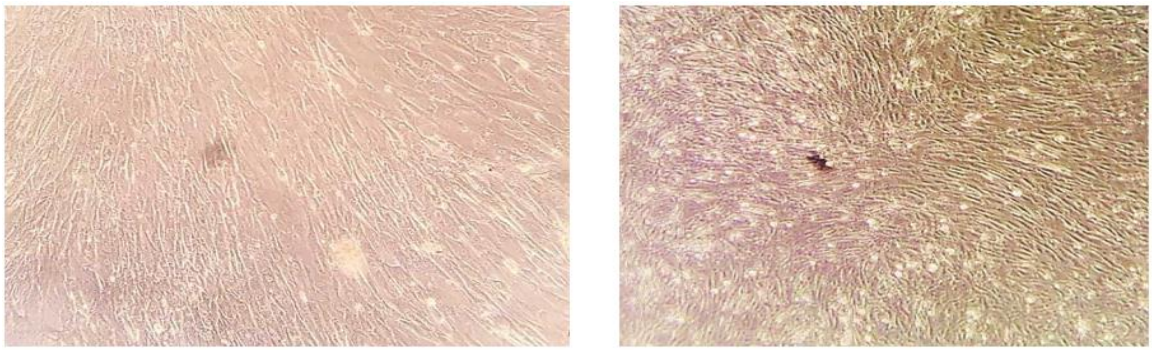

Figure No. 1: Glycyrrhiza glabra root old aqueous extract treatment alters normal cell morphology (REF). REF cells treated with extract for 72 hours, 40X magnification. Control (untreated REF cells)

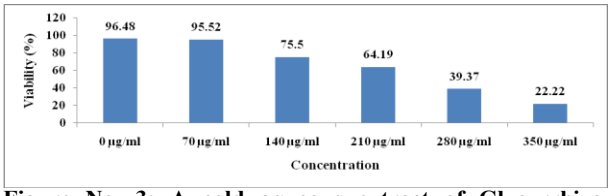

Figure No. 3: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 24 hours of exposure to different concentrations

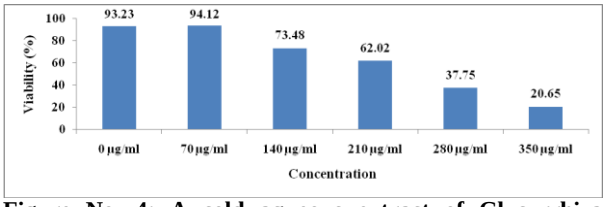

Figure No. 4: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 48 hours of exposure to different concentrations

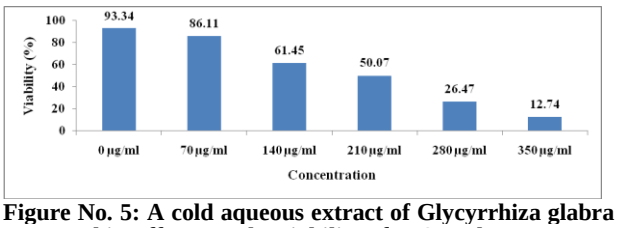

Figure No. 5: A cold aqueous extract of Glycyrrhiza glabra roots and its effects on the viability of MCF-7 breast cancer cells, after 72 hours of exposure to different concentrations

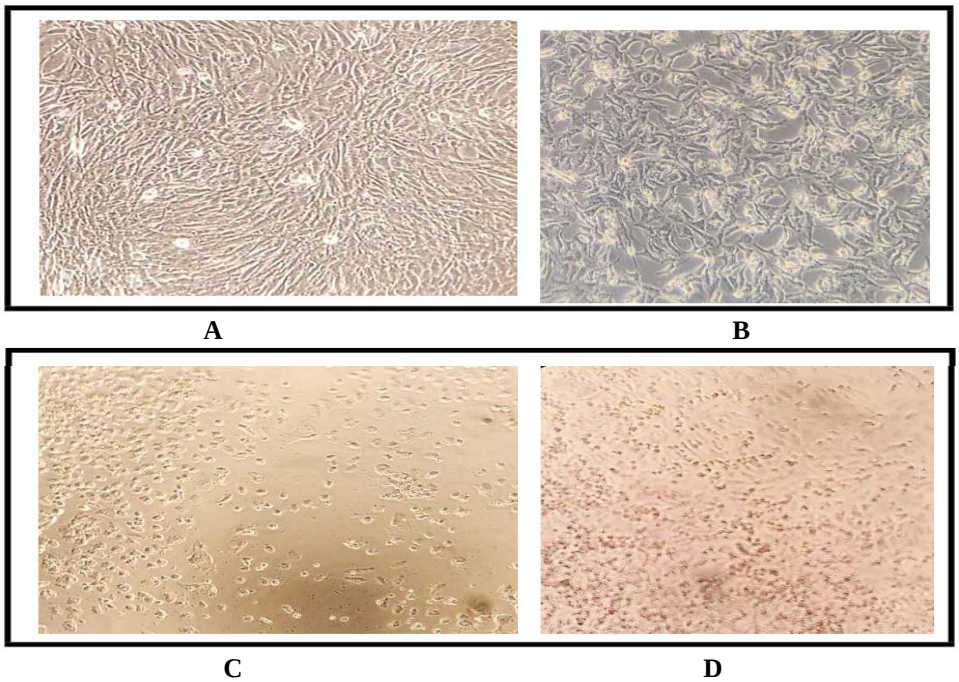

Figure No. 6: Morphological alterations in MCF-7 breast cancer cells treated with the aqueous extract of Glycyrrhiza glabra for different exposure periods

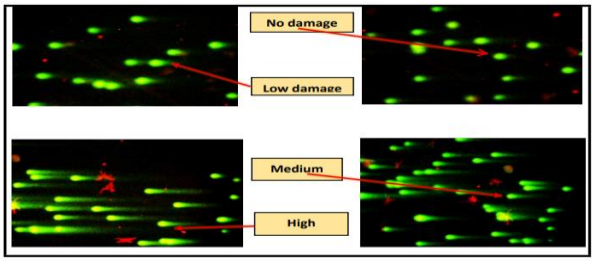

Figure No. 7: DNA damage levels in MCF=7 cells, evaluated using the Comet assay

After 24 hours, cancer cell viability ranged from 22.22% at 350 µg/ml to 95.52% at 70 µg/ml. Time reduced same-concentration cell survival. At 48 hours, viability was 20.65% at 350 µg/ml and 94.12% at 70 µg/ml. Cancer cell viability decreased to 12.74 percent at 350µg/ml after 72 hours. Figures 3–7 show the decay system's Cimabue oty cell cutoff from start to viability.

Table 3 showed the cold aqueous extract of Glycyrrhiza glabra roots substantially affected the comet test on MCF-7 breast cancer cells at low, medium, and high damage levels. At low damage levels, the comet rate was highest at 70 µg/ml (39.123) and decreased to 32.981 at 350 µg/ml ($p \leq 0.005$) after 72 hours. At medium and severe damage levels, the maximum comet rate was from 350 µg/ml concentration, while the lowest comet rate was from 70 µg/ml concentration. The medium damage value was 15.649 at 350 µg/ml, compared to 8.867 at 70 µg/ml while the high damage value was 16.957 at 350 µg/ml, compared to 9.571 at 70 µg/ml, during the same exposure duration and significance level.

DISCUSSION

Aqueous Glycyrrhiza glabra root extract very cytotoxic in embryonic fibroblast normal cells (IC_{50} = ~5.59 mg/ml). Low extract doses injure normal cells less. The extract concentration decreased cell viability from 92.12% at 1 mg/ml to 12.50% at 12 mg/ml. Toxin dose-dependent extraction 72-hour in vitro cytotoxic. Assess cell viability early and late after exposure. Ganguly and Breen[7] reported no oxidative damage or cellular signaling pathways from short dosages. Medical or environmental impacts of the extract may be best examined after 72 hours.

Some bioactive plant compounds may damage normal cells.[7] A significant indicator of toxicity and therapeutic safety is the IC_{50} value. Glycyrrhizin and liquiritigenin in the extract do this. Wang asserts Xuhui[8] reported that oxidative stress and ROS production may destroy these compounds at large concentrations. Yang and Zhang[9] demonstrated that magnesium isoglycyrrhizinate reduces ROS in normal fibroblasts via p38MAPK/Akt/Nox4 signaling and suggest these medicines may affect cell oxidative balance. According to Muhamad Plengsuriyakarn[10], certain phytochemicals attack cancer cells without affecting normal cells. Purity, extract chemical composition, and exposure time effect selectivity. High therapeutic extract concentrations restrict cell viability. Pérez-Soto et al[11] validates the cell growth and antioxidants may be compromised by overdosing.

Cold water Glycyrrhiza glabra root extract inhibits MCF-7 breast cancer cells. High extract concentration and exposure time increased inhibition. Extract bioactives may collectively inhibit cancer cell survival. Husain et al[12] reported that early exposure to lower dosages showed little inhibition (24 h), whereas 350 µg/ml showed considerable inhibition, indicating that dosage concentration considerably affects cytotoxicity. This validates Sohel et al[13] conclusion that active ingredient concentration boost plant extracts' early impacts.

Wang et al[14] found Glycyrrhiza glabra root extract suppresses MCF-7 breast cancer cells concentration-dependently. Ahmad et al[15] also found that MTT and ELISA doses and exposure times killed cells. Cytotoxic inhibition ratios rose considerably across all dosages after 48 hours, demonstrating that bioactive chemicals are cumulative and cellular growth pathway regulation persists. Plant extracts may slow cell development, claim Jain et al.[16] Flavonoids and terpenes may alter gene expression and cause apoptosis, according to Tuli et al.[17]

After 72 hours, cell death and cytotoxic inhibition peaked, allowing the extract to concentrate and activate complicated cancer cell-killing signaling pathways. Nizam et al[18] found that Glycyrrhiza glabra extract inhibited MCF-7 cell growth and promoted apoptosis via the PI3K/Akt and MAPK pathways. Its cytotoxicity and effect on cancer cells may make it an anticancer drug, especially in combination.

Cold aqueous glycyrrhiza glabra root extract damaged MCF-7 breast cancer cells' DNA at various dosages in the comet test. High DNA damage levels reached 70 µg/ml, whereas moderate damage was 350 µg/ml.[19] Too many active chemicals in the extract may protect or reduce cell responsiveness at higher doses. Hormesis occurs when a little amount of chemical boosts biodefenses yet poisons, cytotoxicizes, or inhibits function. Hormetic cellular toxicity is well-known. Al-Naqeb et al[20] used plant extract to preserve and destroy cell DNA. Like cytotoxicology's hormesis.

Rising extract concentration significantly correlates with medium-level DNA damage, from 8.867% at 70 µg/ml to 15.649% at 350 µg/ml. Bioactive chemicals in the extract damaged MCF-7 breast cancer cells' DNA dose-dependently, indicating genotoxicity. Glycyrrhizin and liquiritigenin enhance intracellular ROS, DNA damage, and oxidative stress, according to Sharifi-Rad et al[21], these components may induce apoptosis and double-strand DNA breaking. Zhang et al[22] found that isoliquiritigenin, another licorice component, may increase ROS-mediated DNA damage

in cancer cells and inhibit autophagy, which cleans damaged organelles and proteins and maintains homeostas Study's Comet test confirms this.

Significant DNA damage increased cell damage from 9.571% at 70 µg/ml to 16.957% at 350 µg/ml. Its high genotoxicity hinders DNA repair. DNA damage may damage cell cycle checkpoints, causing apoptosis or preventing it to maintain genomic integrity. Lagunas-Rangel and Bermúdez-Cruz[23] discovered natural chemicals that may hinder cancer cell DNA repair, causing genetic damage or enhancing antitumor effects. The data validate this study's Comet assay.

No harm percentages reduced from 42.563% in the control group to 32.981% at 40% extract. In prescriptive pharmacology and toxicology, the dose-dependent genotoxic theory states that higher dosages injure cells more aligns with Vrbovac-Madunić et al.[24] A statistical study found substantial damage levels at different doses ($P \leq 0.005$), supporting these conclusions. Cordelli et al[25] found that the comet assay, a sensitive DNA damage detection instrument, strongly suggests that the extract affects MCF-7 breast cancer cells' genetic material.

CONCLUSION

In a dose- and time-dependent study, Glycyrrhiza glabra root cold aqueous extract inhibited normal fibroblast proliferation and killed MCF-7 breast cancer cells. A comet experiment reveals cold water Glycyrrhiza glabra root extract destroys breast cancer DNA. The study studies Glycyrrhiza glabra root anticancer properties. In vivo and molecular research is required to understand Glycyrrhizin's breast cancer treatment properties.

Author's Contribution:

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Drafting or Revising Critically:	Muslim Mohammed Kadhim, Aseel Raheem Mardan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.388 Dated 29.10.2024

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Original Article

Relationship between Radiation Doses with Anthropometric Characteristics during Cardiac Catheterization Procedures in Iraqi Patients with Ischemic Heart Disease

Marwa Abd AL-Redha Abd Al-Khaliq and Najeeb Hassan Mohammed

ABSTRACT

Objective: To investigate relationship between radiationdose received during cardiac catheterization and patients' anthropometric characteristics, particularly body mass index.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the Single-plane catheterization laboratory at Ibn-Al-Bittar-Centre-Baghdad/Iraq from 1st May 2024 31st August 2024.

Methods: This is a cross-sectional, study with prospective data collection; cardiac-catheterization was done in Single-plane catheterization laboratory at Ibn-Al-Bittar-Centre-Baghdad/Iraq vide letter No. 478 dated 30th April 2024 and 110 patients were enrolled.

Results: There were 39 females and 71 males. 63.6% underwent coronary-angiography and 36.4%underwent percutaneous coronary intervention. The mean age was 58.6 years and body mass index was 30.3±4.9kg/m². The mean dose area product by body mass index group was 19.3, 65.6, 66.1, 112.3, 92.2and 52.1Gy·cm², respectively. The corresponding mean kinetic energy released perunitmass (KERMA) values were: 277.7, 878.2, 1102.4, 1606.8, 1719.9, and 850.9mGy, respectively. Normality tests for both kinetic energy released per unit mass and dose-area-product showed significant differences across body mass index group($p<0.05$), indicating that radiation dose data were not normally distributed. However, ANOVA tests showed no statistically significant differences in dose-area-product ($p=0.415$) or kinetic energy released per unit mass ($p=0.580$) among age groups.

Conclusion: There is a clear association between body mass index and radiation dose. Higher body mass index levels are linked with increased kinetic energy released per unit mass and dose area product values.

Key Words: Cardiac catheterization, dose area product (DAP), kinetic energy released per unit mass (KERMA), Anthropometric characteristics

Citation of article: Abd Al-Khaliq MAR, Mohammed NH. Relationship between Radiation Doses with Anthropometric Characteristics during Cardiac Catheterization Procedures in Iraqi Patients with Ischemic Heart Disease. Med Forum 2025;36(9):49-54. doi:10.60110/medforum.360909.

INTRODUCTION

When coronary arteries become narrowed or blocked, blood cannot adequately supply heart muscle, a condition known as ischemic heart disease (IHD). It's a leading cause of mortality worldwide. The most common causes of coronary artery diseases are hypertension, Diabetes mellitus, hyperlipidaemia, smoking, positive family history of IHD etc.[1]

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Received: January, 2025
Reviewed: February-March, 2025
Accepted: April, 2025

Radiation is increasingly being used in cardiac catheterisation, which necessitates real-time-imaging in both.

Coronary angiography (CA) is an invasive diagnostic procedure in which radio-contrast is injected into the coronary arteries (5-12ml) under X-ray guidance to display the coronary anatomy and possible luminal obstruction.[2]

Percutaneous coronary intervention (PCI) is a widely utilised non-surgical-procedure designed to treat this condition by enhancing blood flow through narrowed arteries. It involves inserting a catheter through a small skin incision to access the coronary arteries. PCI encompasses techniques like balloon angioplasty and stent insertion to relieve arterial narrowing.[3] It exposes patients to high doses of radiation during complicated procedures.[4] Fluoroscopy time affects radiation dose, in addition to many other, such as patients' anthropometric-characteristics, operator experience, the quality of fluoroscopy machines, and other conditions.[5] Radiation dose management is essential to help minimise the health risks associated with the use of

ionising radiation during cardiac catheterisation procedures.[6] This is important for the following reasons: health risks include deterministic effects like skin burns and tissue damage etc.[7] Cumulative exposure, patients with IHD often undergo multiple imaging and interventional procedures over their lifetime, which can lead to significant cumulative dose. Proper dose management helps limit this exposure, reducing the associated risks.[8] Safety of healthcare staff in cardiac catheterisation laboratories are also exposed to radiation. Managing radiation doses ensures that these exposures remain within safe limits, protecting the health of healthcare professionals over their careers.[9]

Image quality and diagnostic accuracy consisted of efficient dose management aims to reduce radiation exposure while maintaining or improving the quality of diagnostic imaging. Following the ALARA "As Low As Reasonably Achievable".[10] The influence of body mass index(BMI) and body surface area(BSA) on radiation exposure during cardiac catheterisation is a topic of considerable research interest, as anatomical variations can severely affect procedural safety and efficacy. Understanding specific patient characteristics that influence radiation doses is imperative for optimising procedural protocols. Studies have attempted to dissect the respective roles of BMI and BSA on radiation exposure during cardiac catheterisation, with a greater focus on BMI. A close study of more than 8,000 patients, for example, showed that both BMI and BSA are related to DAP, This sets up basic links between body measurements and radiation dose.[11]

Radiation dose measurement typically quantified using several different parameters to assess exposure. Key measurements include: DAP also known as KERMAarea product(KAP), this measures the total amount of radiation energy delivered to a specific area. It is a product of the dose and the area exposed, usually expressed in ($Gy \cdot cm^2$). DAP accounts for both the dose at a specific point expressed area exposed, making it useful for assessing overall patient exposure during a procedure.[12]Air KERMA refers to the kinetic energy from ionizing radiation deposited per unit mass of air, typically measured at a reference point in the X-ray beam.It's measured inmGy. Air KERMA provides an indication of the intensity of the radiation field.[13]The kilo voltage (kV) and milli ampere (mA) are two crucial parameters in fluoroscopy, controlling the x-ray beam's energy and intensity, respectively.[14]Fluoroscopy time is the total amount of time that fluoroscopy is used during a procedure. Increased fluoroscopy time correlates with higher radiation exposure. It is often used alongside other measurements to give context to the radiation exposure.[15]

METHODS

This prospective, single centre cross-sectional observational analytical study enrolled 110 patients include both genders who have IHD selected through successive sampling from Ibn-Al Bittar Centre for Cardiac Surgery, Baghdad Iraq from 1st May 2024 to 31st August 2024. Patient of both gender aged >30 years old with IHD were included while pregnant women and children were excluded. Angiogram was done in Single plane catheterisation lab (Philips Allura X per FD & Phillips Azurion release 2.2(L7)).The major coronary arteries were image as followed:

Left anterior descending (LAD) artery major branch of the left coronary artery supplying a significant portion of the left ventricle which is visualizing in a: Cranial (40°): (A view angled towards the cranial end of the patient); Fishbone (40°-40°); and spider (40°-40°).

Left circumflex (LCX) artery another major branch of the left coronary artery supplying the lateral wall of the left ventricle which is visualized in right anterior oblique [RAO] (30°) – caudal (20°); caudal (40°); and spider (40°-40°).

Right coronary artery (RCA) supplying the right ventricle and parts of the posterior left ventricle which visualizing in RAO (30°): right anterior oblique; and left anterior oblique [LAO] (60°-40°): LAO.

Since the fluoroscopy real time imaging its generates more than 40 image per second, Kv and mA change every second, to standardize measurements, the Kv and mA for LAO view was consider. Patient information, including age, gender were collected from self-reported entries in questionnaire filled out at the time of Cardiac catheterization, height was measured by Stadiometer, and weight were measuring using a scale. BMI calculated by ($BMI = weight/height^2$) then divided into 6 groups, underweight<18.5, normal 18.5-24.9, overweight 25-29.9, obesity 30-34.9, obesity 35-39.9, extreme obesity>40.BSA calculated by the DuBois formula (BSA=0.007184×height 0.725m×weight 0.425kg. Patient radiation exposure was measured by the catheterisation lab system the radiation metrics like Air KERMA, fluoroscopy time, and DAP are taken from radiation log of Individual procedure for that particular patient. Statistical Analysis done by IBM SPSS-26, descriptive statistics, Normality tests (Kolmogorov-Smirnov and Shapiro-Wilk), box plot, Spearman's rho correlation and ANOVA test.

RESULTS

The data is categorized by age groups and gender. For females, in the age group under 40 years no participants, in the 40–60 years age group, the number of participants were19 with a mean of 53.05 years, In the over 60 year's age group, the number of participants was also 19, with a mean of 65.73 years. For males, in the age group under 40 years, number of participants was 3, with a mean of 35.33 in the 40–60 years age group, there were 33 participants, with a mean of 50.71. In the over 60 year's age group, number of participants was 36, with a mean of 65.83 (Table 1).

KERMA and DAP values increase with BMI, but Extreme obesity group shows a decrease due to smaller sample size (n=7), making the mean less reliable. This is due to the increasing prevalence of obesity and the need for more accurate measurements. The kV shows a general increasing with increasing BMI, while mA shows less of a clear trend (Table 2).

Most BMI categories and both radiation dose variables, the p-values are <0.05 for both tests. This strongly evident that the radiation dose data within each BMI group is not normally distributed. The exceptions are KERMA, extreme obesity. The p-value is above 0.05 for both tests (n=5), suggesting normality cannot be rejected. However, the small sample size limits the test's power. DAP, extreme obesity. The Shapiro-Wilk test is close to significant (p=0.048), suggesting a possible deviation from normality. The small sample size again needs to be considered (Table 3).

The horizontal axis represents the BMI categories, while vertical axis indicates KERMA values. Each box plot demonstrates the range of radiation exposure for the corresponding BMI group. The boxes highlight the interquartile range (IQR), which encompasses the middle 50% of data points, with the median value marked by a line within the box. Extending from the boxes are vertical lines, which depict the minimum and maximum values within a reasonable range. Outside these lines, outlier'scases with radiation levels that deviate significantly from the majority (Fig. 1).

As BMI increases, there is a general increase in KERMA values, indicating that individuals with higher BMI have greater radiation exposure. This is particularly evident in the obesity- category, where the range of KERMA values is remarkably wide, suggesting significant variability in exposure levels within this group. Similarly, the extreme obesity category shows consistently high KERMA values, although the sample size appears smaller, leading to less variability. In contrast, the underweight and normal groups, exhibit narrower distributions of KERMA values. Nonetheless, even in these groups, a few outliers are present, indicating occasional instances of unusually high radiation exposure. The X-axis represents BMI categories, while the Y-axis represents DAP (Gy/cm²), observed patterns increase in DAP with higher BMI due to the radiation dose increases progressively as BMI moves from underweight to obesity classes. Greater variability in higher BMI categories, Obesity classes, especially II and III, exhibit a broader spread of values and more outliers (Fig. 2). The Spearman's correlation suggests a weak positive association between BSA and KERMA, but the result is non-significant (Table 4).

The strength of the association between BSA and several other variables (KERMA, DAP, kV, mA). KERMA-BSA: A weak positive linear correlation (R= 0.136, $R^2 = 0.018$) is observed. However, the Eta value (0.642, $Eta^2=0.412$) indicates a much stronger association when considering BSA as a categorical variable (perhaps due to binning or grouping). This discrepancy suggests a non-linear relationship or the presence of outliers affecting the linear correlation.

Table No.1: Frequency of male and female according to age

Age (years)	Male	Female
Under 40	3	-
40-60	32	19
Over 60	36	19

Table No.2: Mean KERMA, DAP, KV and mA across body mass index groups

BMI Groups	Mean KERMA (mGy)	Mean DAP (Gy/cm²)	KV	mA
Under weight	277.7	19.33	73	727
Normal	878.2071	65.6572	76.67	699.19
Overweight	1102.4384	66.1011	86.21	747.24
obesity I	1606.8228	112.3007	83.34	777.63
Obesity	1719.8982	92.2175	99.27	722.36
Extreme obesity	850.2857	52.0571	109.86	676.86
Total	1244.5629	80.749	86.25	739.75

Table No.3: Normality Test for KERMA and DAP radiation dose measures across BMI categories

Normality test	BMI	Shapiro-Wilk			
		Statis-tic	P-value.	Statis-tic	P-value
KERMA (mGy)	Underweight	.260			
	Normal	.248	.014	.763	.001
	Overweight	.224	.000	.687	.000
	Obesity I	.234	.000	.628	.000
	Obesity	.302	.001	.746	.001
	Extreme obesity	.269	.200	.830	.138
DAP (Gy/cm²)	Underweight	.260			
	Normal	.307	.000	.761	.001
	Overweight	.249	.000	.709	.000
	Obesity I	.211	.000	.638	.000
	Obesity	.303	.001	.758	.001
	Extreme obesity	.318	.110	.773	.048

Table No.4: Correlations coefficient between BSA and KERMA

Spearman's		KERMA (mGy)	BSA
KERMA (mGy)	Correlation-Coefficient	1.000	.182
	P-value	.	.057
	N	110	110
BSA	Correlation-Coefficient	.182	1.000
	P-value	.057	.
	N	110	110

Table No.5: Measures of association of BSA with radiation-parameters

	R	R^2	Eta	Eta^2
KERMA-BSA	.136	.018	.642	.412
DAP-BSA	.173	.030	.678	.460
KV-BSA	.470	.221	.805	.648
mA-BSA	.005	.000	.698	.487

DAP-BSA: Similar to KERMA, a weak positive linear correlation (R=0.173, R^2 = 0.030) is observed, but the Eta value (0.678, Eta^2=0.460) suggests a much stronger association when BSA is treated categorically. Again, this points to a non-linear relationship or outlier influence. KV-BSA: A moderate positive linear correlation (R=0.470, R^2=0.221) is observed, indicating that 22.1% of the variance in kV is explained by BSA. The Eta value (0.805, Eta^2=0.648) is even higher, suggesting a strong association when BSA is treated categorically. The relatively close agreement between R and Eta suggests a reasonably linear relationship. mA-BSA: A negligible linear correlation (R=0.005, R^2=0.000) is observed. However, the Eta value (0.698, Eta^2=0.487) indicates a strong association when BSA is treated categorically. This large discrepancy strongly suggests a non-linear relationship or the presence of significant outliers (Table 5). This ANOVA results investigating the relationship between a radiation parameter (KERMA, DAP) and age categories: KERMA: Age categories overall effect: There was no-significant difference in the mean KERMA between age groups (p=0.580). DAP-Age Categories Overall Effect: The mean DAP did not significantly differ among age groups (p=0.415) [Table 6].

Table No.6: Relationship between age categories and radiation-parameters

Age categories		Sum of Squares	df	Mean Square	F	P-value
KERMA (mGy)	Between Groups	4023813.972	3	1341271.324	.658	.580
	Within Groups	216129179.968	106	2038954.528		
DAP (Gy/cm^2)	Between Groups	22720.220	3	7573.407	.958	.415
	Within Groups	837758.018	106	7903.378		

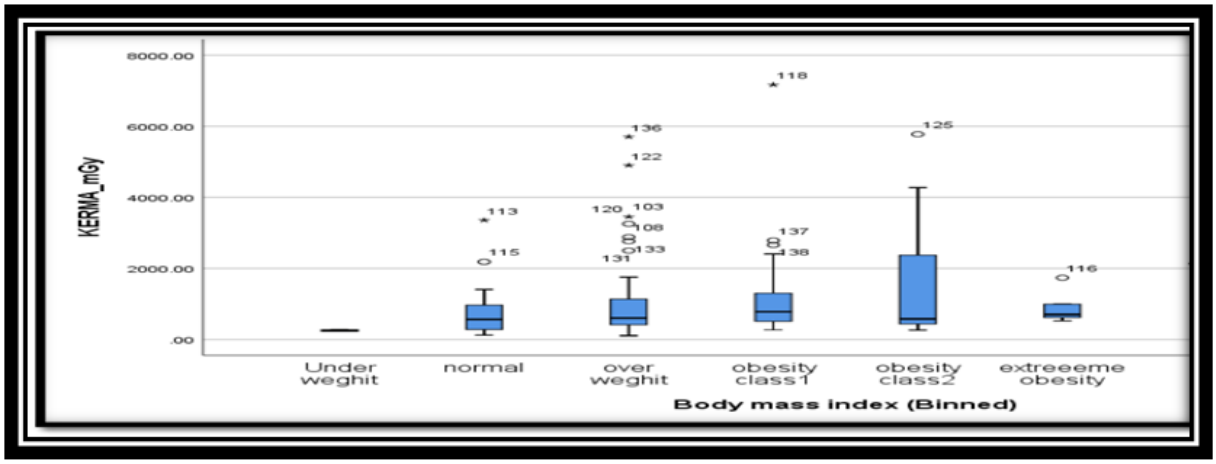

Figure No. 1: KERMA across BMI categories

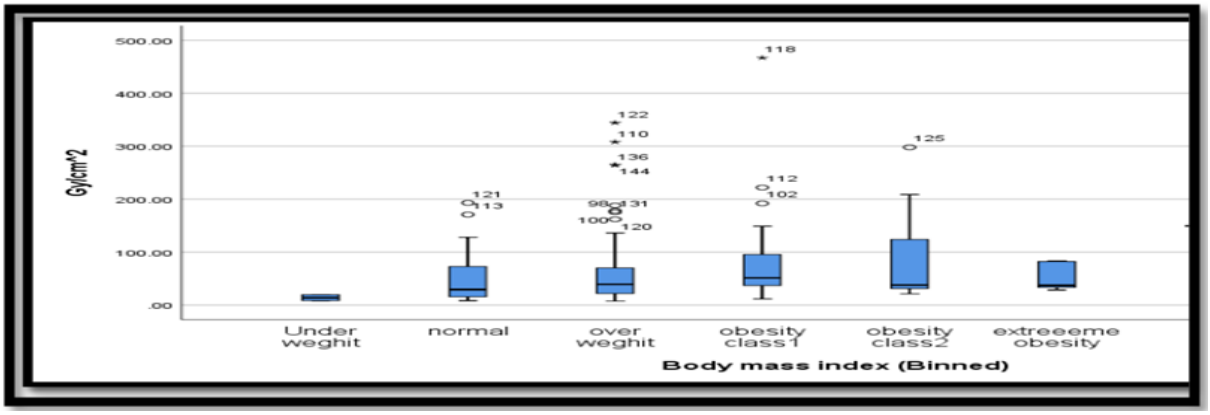

Figure No, 2: DAP (Gy/cm^2) across BMI categories

DISCUSSION

A significant relationship between body mass index (BMI) and radiation exposure, indicating that higher BMI levels are linked to increased KERMA values.[11,16]This observation prompts an exploration of the factors contributing to the wide variability in exposure levels among individuals in obesity. As BMI increase , there is an increase in the thickness of body tissues, including fat and muscle,obesity is often categorized by two types of fat distribution: visceral (around organs) and subcutaneous (under the skin). Visceral fat can lead to increased abdominal girth, while subcutaneous fat affects overall body shape and the proportion of fat that overlays vital organs.[17] The presence of significant fat around the heart (epicardia fat) cases difficulties in accurately imaging cardiac structures because fat has a lower atomic number and density compared to muscle and organ tissues, this leads to a differential attenuation of x-rays, specifically, fat will attenuate x-rays less than muscle tissue. An increased layer of fat may results in diminished visibility of vascular structures during cardiac catheterization impacting the contrast and resolution of the image obtained. Therefore, modern fluoroscopy automatic system raises the KVp subsequently more x-ray produces to obtain more resolution hence leading to greater radiation absorption.[16]

It also important to note that individuals with obesity may have a higher prevalence of chronic health conditions, such as coronary artery disease, diabetes, or respiratory disorders.[18] This association can result in an increased frequency of medical imaging and, subsequently, higher radiation exposure. Understanding these dynamics is crucial for developing tailored imaging strategies that minimize risk while maintaining diagnostic efficacy.

This study found a positive correlation between BMI and radiation doseDAP.[19,20]With higher BMI categories requiring more radiation for effective imaging . Obesity categories exhibit more variability in radiation doses, reflecting the challenges in imaging individuals with higher BMI., Uneven fat distribution may require different imaging settings, leading to dose variability and the outliers indicate significant variation in radiation doses for individuals within the same BMI category. These may result from unique anatomical characteristics, technical challenges, or inconsistent equipment calibration.

Kohet al[11] stated that beyond factors such as sex, BMI, and overall weight, BSA serves as a reliable biomarker for assessing radiation exposure and dose. Furthermore, BSA is identified as a significant anthropometric parameter that varies between sexes.While this study indicates that the linear correlation between BSA and radiation dose is relatively weak. However, a more robust association is observed when BSA is analysed in a categorical manner. In contrast, the relationship between BSA and kilovolt (kV) appears to exhibit a more pronounced and linear correlation.

There is no statistically significant evidence of an overall effect of age category on the KERMA&DAP (Table 5). This suggests that, within the age ranges considered in this study, age does not significantly influence these radiation parameters which provides strong evidence that age does not significantly influence KERMA, DAP within the age ranges and other conditions of this study.

CONCLUSION

Body mass index has been shown to be a significant factor in determining the radiation dose to both the patient and the operator, in such a way that higher BMI raises the DAP and KERMA exposure during cardiac catheterization procedures.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Marwa Abd AL-Redha Abd Al-Khaliq, Najeeb Hassan Mohammed
Drafting or Revising Critically:	Marwa Abd AL-Redha Abd Al-Khaliq, Najeeb Hassan Mohammed
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.478 Dated 30.04.2024

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Original Article

The Role of Iron Deficiency as Trigger for Depression: A Biochemical and Psychological Perspective

Jaafar S Al-Showaily, Mohand Kareem Razzaq, Hussein Flayyih Hassan and Qayssar A. Obaid

ABSTRACT

Objective: To investigates the role of iron deficiency as a determinant of depression as perceived from both a biochemical and a psychological perspective.

Study Design: Case control study

Place and Duration of Study: This study was conducted at the Al-Rifai Teaching Hospital in Dhi Qar, Iraq from 1st November 2024 to 30th June 2025.

Methods: 162 participants were enrolled. Self-inventory instrument has been used which included two domains, psychological domain by using Beck`s depression inventory to assess depression level and the other domain is the biochemical domain which include biochemical sample and analysis of 5 ml venous blood samples were collected from participants and placed in a 2 ml anticoagulated tube for complete blood count measurement, which included white blood cell, red blood cell, Hemoglobin A1c and hematocrit only, and 3 ml in a yellow gel tube for ferritin and B12 measurement. Participants were divided into two groups; control group those with no depression and depressed group (cases) those with depression.

Result: Depression level among depressed group was high 65%. The differences between control subjects either moderate or severe anemia regarding age, gender and body mass index. Ferritin showed significant decreases among patients who had either moderate anemia or severe anemia when compared to participants in the control group ($p<0.01$) which proved iron deficiency as a primary condition within these groups. The anemic groups exhibited diminished levels of all three markers (hemoglobin, hematocrit and red blood cell) which confirm the clinical presentation of anemia.

Conclusion: Biochemical and psychological investigation support highly-converging evidence for a role of iron deficiency in depressive symptoms, and as anemia and depression increase ferritin decrease and as response to the inflammatory process white blood cell also increases.

Key Words: Depression, Iron deficiency, Biochemical perspective, Psychological perspective

Citation of article: Al-Showaily JS, Razzaq MK, Hassan HF, Obaid QA. The Role of Iron Deficiency as Trigger for Depression: A Biochemical and Psychological Perspective, Med Forum 2025;36(9):55-59. doi:10.60110/medforum.360910.

INTRODUCTION

The bio-psycho relationship between iron and depression, the goal of the research is to explore this relationship in an integrated bio-psycho manner and are expected to reveal the biopsychological mechanisms of iron's influence on depression and provide evidence-based references for clinical management.[1-3]

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Received: July, 2025
Reviewed: August, 2025
Accepted: August, 2025

Depression is one of the most commonly reported mental health problems. It is related to the loss of interest in normal daily activities and is characterized by sadness, discouragement, hopelessness, guilt, decreased energy, and concentration and sleep disorders. Depression has been the main cause of disability globally, affecting an estimated 300 million people in 2015.[4] If significant steps are not taken, by 2030, it is anticipated that it will undermine the productive abilities of individuals more than all other diseases, culminating in economic losses at the level of trillions of USD. Depressive disorders have also caused an increase in suicide rates. Globally, more than 800,000 individuals committed suicide in 2012, while kin suicide caused 50% of all deaths. In addition, the direct economic burden for depression therapy is enormous and amounts to millions of dollars.[5,6]

Rapid industrialization has brought about an obvious change in the structure and nutrition of diets, with iron deficiency appearing as the main public health issue. In fact, iron deficiency is the most prevalent public

nutritional problem, impacting anemia's occurrence. It is well established that there is a reciprocal relationship between anemia and depression. In a group of over 2,500 women in the USA, anemia evolved prior to a depressive episode and thus increased the likelihood of having health problems. 3,491 women aged 18-65 in the USA suggested anemia is always likely to be correlated to the symptoms of depression. A similar conclusion was drawn from a longitudinal, epidemiological cohort study of 3,770 men and 4,093 women joining the army in between the years 2000 and 2003, who did not submit psychiatric medical backgrounds before.[7,8]

Currently, mental disorders comprise the leading cause of non-fatal illness worldwide. Depression is a common mood disorder characterized by fatigue, suicidal ideation, anhedonia (reduced pleasure response), feelings of worthlessness and guilt, and dysphoria. Depression causes a significant decrease in people's quality of life, and currently, the pathophysiology of this complex and heterogeneous disorder is still not fully understood.[9,10] Considering the considerable global burden of disability related to depression and iron deficiency, the research not only contributes to a better understanding of negative mood states but also could provide a potential preventative strategy for the clinical treatment of depression, and especially in a country like China where the increasing frequency of smog events is related with oxidative stress and other severe mental health problems.[11] It is interdisciplinary research on the biochemistry and psychology of nutrition in relation to emotional well-being. Reduced costs will improve the efficiency and analytical focus of current biochemical research, while broadening the horizons of this laboratory. Regarding the biochemical perspective, the clinical management of iron deficiency, along with the capacity of specific psychological states to be biochemically quantified, will be investigated. The psychological perspective examines the nutritional factors of mental health. Benefits for students also include an opportunity for mentoring, laboratory training in advanced equipment and techniques, and the development of an original and substantial piece of research. It is anticipated that this study will make a significant contribution to the knowledge and understanding of how iron impacts the depressive state. In each instance, the biochemical and psychological paradigms becomes a lens in understanding issues related to mental health. Such a rigorous two-sided strategy towards depression will serve as a benchmark and many paths for future inquiry.[12-14]

METHODS

This case control study was conducted at Al-Rifai Teaching Hospital in DhiQar, Iraq from 1st November 2024 to 30th June 2025 and 162 samples were collected and using non-random purposive sampling method. The sample size was calculated the standard equation used (z×pq)/d. The samples were divided into three groups: the first group consisted of 41 healthy individuals as a control group (C (male = 21, female = 20), aged 35–65 years); 42 patients with moderate anemia (male = 21, female = 21), aged 37–65 years); and 79 patients with severe anemia (male = 40, female = 39), aged 43–65 years. To collect data, all study participants underwent an annual medical examination at Al-Refai Teaching Hospital. Patients have chronic kidney diseases, chronic liver disease, tuberculosis, arthritis, systemic lupus erythematosus, mononucleosis, Bechet's disease, and cancer have been excluded. A standard scale (Beck`s depression inventory (BDI)has been used, it has been translated and modified according to the Iraqi community standards, it is 21 items scale it has been merged to three levels of diagnosis, control, moderate and high depression (0-13) represent control, (14-28) moderate depression and (29-63) represent high depression. On the other hand, biochemical sample and analysis5 ml venous blood samples were collected from all participants and placed in a 2 ml anticoagulated tube for CBC measurement, which included WBC, RBC, HbA1c, and Hct only, and 3 ml in a yellow gel tube for ferritin and B12 measurement.

The data was analyzed using SPSS-26. The Kolmogorov-Smirnov test was used to divide the variables across the research groups. The one-way ANOVA test was used to compute and compare the means and standard deviations. The P value $P<0.05$ was considered significance, while the receiver operating characteristic curve (ROC) was used to ascertain the ideal specificity and sensitivity of a diagnostic test.

RESULTS

Table 1 demonstrate depression levels among participants which present that about half of the participants have high depression (48.8%) while no depression (control) and moderate depression each with about 25% of all participants which constitutes the other half of participants.

Table 2 showed the parameter age when compared group control with moderate showing no significant ($p<0.05$) also when compared control with high and moderate with high showing no significant ($p<0.05$), also gender and BMI showing no significant when compared control with all groups.

Table 3 showed the parameter B12 when compared group control with moderate showing no significant ($p <0.05$) also when compared control with high and moderate with high showing no significant ($p <0.05$), as well as Ferritin showing highly significant increase when compared control with moderate ($p > 0.01$) also showing highly significant increase ($p>0.01$) when compared control with high and moderate with high showing highly significant increase ($p>0.01$), and WBC and RBC the results showing when compared control

with moderate (p>0.01) also showing highly significant increase (p>0.01) when compared control with high as well as when compared moderate with high showing no significant increase (p<0.05), and Hgb showing highly significant increase with all groups (p>0.01), HCT the results showing when compared control with moderate (p>0.01) also showing highly significant increase (p>0.01) when compared control with high as well as when compared moderate with high showing no significant increase (p<0.05).

Table No.1: Depression level among participants (n=162)

Depression levels	No.	%
Control (no depression)	41	25.3
Moderate	42	25.9
High depression	79	48.8

Table No. 2: Comparison of Age, Gender and BMI among different groups (n=162)

Parameter	C (n=41)	M (n=42)	H (n=79)	Group	P value
Age	31.56±14.51	31.35±15.43	26.98±11.43	C* M	0.997
				C* H	0.180
				M* H	0.204
Gender	1.92±0.26	1.95±0.21	1.83±0.37	C* M	0.927
				C* H	0.285
				M* H	0.126
BMI (Kg/m^2)	24.02±2.50	25.62±3.29	24.63±2.92	C* M	0.036
				C* H	0.525
				M* H	0.183

**p<0.01 is extremely significant, *p<0.05 is significant, and p > 0.05 is no significant.
C for Control, M for Moderate anemia and H for High anemia

Table No.3: Comparison of B12, Ferritin, WBC, RBC, Hgb and HCT among different groups (n=162)

Parameter	C (n=41)	M (n=42)	H (n=79)	Group	P value
B12	272.90±56.77	248.98±112.70	269.80±137.78	C* M	0.615
				C* H	0.989
				M* H	0.615
Ferritin	50.105±28.12	25.72±12.87	14.79±5.29	C* M	0.000**
				C* H	0.000**
				M* H	0.001**
WBC	5.72±2.91	7.52±2.58	7.89±2.60	C* M	0.007
				C* H	0.000**
				M* H	0.755
RBC	4.03±0.60	4.52±0.55	4.50±0.66	C* M	0.001
				C* H	0.000**
				M* H	0.988
Hgb	12.69±1.05	10.09±0.95	8.45±1.39	C* M	0.000**
				C* H	0.000**
				M* H	0.000**
HCT	36.33±4.02	26.41±4.53	23.69±8.32	C* M	0.000**
				C* H	0.000**
				M* H	0.081

**p <0.01 is extremely significant, *p < 0.05 is significant, and p > 0.05 is no significant
C for Control, M for Moderate anemia and H for High anemia

DISCUSSION

In the present study, the control group matched the other groups regarding both age and gender demographics per statistical analysis (p>0.05). Body mass index measurement demonstrated an increased value in the moderate anemia group to the extent that it produced a statistically meaningful correlation versus the control group (p = 0.036) indicating a link between body mass and anemia severity.[15]

The biochemical indicators of table 3 showed significant decreases among patients who had either moderate anemia or severe anemia when compared to participants in the control group (p<0.01), which proved iron deficiency as a primary condition within these groups. The anemic groups exhibited diminished levels of all three markers (Hgb and HCT and RBC) which confirm the clinical presentation of anemia. The elevated white blood cell count in anemic groups can be attributed either to an immune response or the stress

that accompanies anemia state. The Vitamin B12 amounts collected from testing remained equal for all participant groups.[16]

Depression levels among participants as it reveals highest percentage (48%) are high and severe depression which is can be considered as indicator for the connection between depression and anemia, the interplay between mental health and hematological picture, as it has been stated, anemia have high connection with mood disorders, cognitive of the individual and other aspects like fatigue.in fact, having this percentage of depression may make the connection picture is clearer.[17] Liu et al[18] find same result as about half of the participants have high depression level.

The correlation between depression and iron deficiency have been studied in many articles as attempt to prove the exact relationship between depression and iron deficiency.[19,20] Iron deficiency is affecting hippocampus, and some neurotransmitters, and as the hippocampus is responsible for learning and memory and by it is need to iron, any disturbance will lead to psychological disorders. Same idea is a about corpus stratum which is affected by iron shortage and as it is responsible for controlling executive activities like, sustaining attention, regulation of emotion and other purposes. As for neurotransmitters iron deficiency have great impact on dopamine, serotonin and noradrenaline which they have direct impact on mood and play a major role in depression.[20]

The research supports existing evidence for the biopsychosocial model of depression by showing that iron deficiency plays an essential part in initiating and worsening psychological symptoms. Health authorities of developing and industrializing societies should establish integrated screening services which protect both mental wellness and nutritional health because their populations face rising mental health burdens alongside changes in dietary patterns.

CONCLUSION

The results of this biochemical and psychological investigation support highly-converging evidence for a role of iron deficiency in depressive symptoms, advancing the field by revealing this association in a younger population in a country of rapid Westernization. Iron deficiency has long been associated with fatigue, which is a common and characteristic symptom of depression. The important role of iron in the brain has been increasingly acknowledged, with some going as far as to consider the brain a "privileged" organ for iron in terms of metabolism. Iron acts as a co-factor in the synthesis and reuptake of several important neurotransmitters, including dopamine, norepinephrine, glutamate, and serotonin. The elucidation of the close association between iron deficiency and depressive symptoms may have urgent implications for public health, particularly in non-Westernized countries of rapid industrialization that currently have a relatively low prevalence of mental disorders and receive little attention with regard to mental health care and research. It is recommended that further biomedical studies be conducted on a larger scale within the socio-cultural context of such industrializing regions, and that these studies focus on the organization of multi-ethnic cohorts of younger populations as a priority area for the field.

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

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 2/8/2400 dated 18.11.2024

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Original Article

Association of the CYP3A4 Gene (rs2740574 C>A, G, T) with the Risk of Benign Prostatic Hyperplasia in Iraqi Patients

Basim Mohammed Abdul Latif, Ammar Ahmed Sultan and Thikra Ata Ibrahim

ABSTRACT

Objective: To evaluate the association between the genetic polymorphism rs2740574 in the CYP3A4 gene and the risk of developing benign prostatic hyperplasia in a sample of Iraqi patients.

Study Design: Descriptive study

Place and Duration of Study: This study was conducted at the Baqubah Teaching Hospital, Diyala Province, Iraq from October 2023 and May 2024.

Methods: This descriptive study included 30 benign prostatic hyperplasia patients and 10 healthy controls aged between 50 and 70 years. DNA was extracted from the samples, and the target fragment was amplified using polymerase chain reaction. Genetic variations were analyzed using the Geneious software. The sequencing analysis of the rs2740574 polymorphism in the CYP3A4 gene revealed three genotypes: CC, CT, and TT.

Results: A strong association between the genotype distribution of this polymorphism and the risk of BPH. The CC genotype and C allele were highly prevalent among the control group and represented a strong protective factor against BPH, supported by Fisher's exact test (P=0.000) and an odds ratio (OR) of 0.003. In contrast, the CT genotype was significantly more frequent in patients and indicated a very high risk of disease (P=0.000, OR=81.00). The T allele was present in 51.67% of patients versus only 5% in controls, making it a major risk factor with an OR of 20.31. The TT genotype was rare and not statistically significant but was still considered a potential risk factor according to Fisher's test (P=0.721) and OR=1.84. The Hardy–Weinberg equilibrium test showed genetic balance in the control group (P = 0.8678, not significant), indicating a normal distribution unaffected by disease pressure. However, the patient group showed a clear deviation from equilibrium (P=0.000), reflecting the influence of the disease state on genotype distribution in this sample.

Conclusion: The genetic polymorphism of the rs2740574 of the gene could lead to the incidence of autism. A case of benign is that CYP3A4 gene has a huge role in genetic predisposition to benign. Women: prostatic hyperplasia.

Key Words: CYP3A4, Benign prostatic hyperplasia, Single neuclotide polymorphism, Hardy–Weinberg

Citation of article: Latif BMA, Sultan AA, Ibrahim TA. Association of the CYP3A4 Gene (rs2740574 C>A, G, T) with the Risk of Benign Prostatic Hyperplasia in Iraqi Patients. Med Forum 2025;36(9):60-65. doi:10.60110/medforum.360911.

INTRODUCTION

The prostate is part of the male reproductive system, storing and secreting seminal fluid. It typically measures about three centimeters in length and weighs twenty grams.[1] In the pelvis beneath the bladder and in front of the rectum, it surrounds part of the urethra, which carries urine and semen.[2]

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Received: December, 2024
Reviewed: January-February, 2025
Accepted: March, 2025

With age, the prostate enlarges, potentially obstructing urinary flow and causing complications in the bladder, urinary tract, or kidneys.[3]

The prostate's small glands produce about 20% of seminal fluid and are regulated by androgens, mainly testosterone synthesized in the testes and derived from dehydroepiandrosterone (DHEA) from the adrenal glands. DHEA is converted to testosterone by 5α-reductase in the prostate.[4]

The adult prostate has four zones. The peripheral zone (PZ), mainly posterior and lateral, accounts for 70% of glandular tissue, is the most frequent origin of prostate cancer, and is prone to inflammation.[5]

The central zone (CZ) forms 25% of glandular tissue, is more resistant to cancer and inflammation, and contains cells with thick cytoplasm, basophilic staining, and large nuclei.[6]

The transitional zone (TZ) contains short ducts and mucous glands, bounded by collagenous tissue.

After puberty, its glands lack clear definition, but in older men it enlarges in nodular masses of epithelial cells, causing urinary obstruction characteristic of BPH.[3,7]

The periurethral zone includes mucous and submucous glands, which may proliferate during BPH, adding urethral pressure and urinary retention.[5]

Benign prostatic hyperplasia compresses the urethra, thickening and weakening the bladder, leading to incomplete emptying and urinary retention.[8] Proposed causes include hormonal imbalance (estrogen/testosterone). BPH may predispose to prostate cancer in older men, affecting urinary and reproductive systems, with possible metastasis requiring proper management.[9,10]

Polymorphisms in cytochrome P450 genes, such as CYP17A1 and CYP3A4, influence testosterone metabolism and prostate growth, linking them to prostate cancer risk.[11]

CYP3A4 encodes a major enzyme metabolizing endogenous and exogenous compounds, mainly in the liver and intestines, and affects drug metabolism and therapeutic responses.[12,13] Variants, including rs2740574, alter enzyme activity, influencing susceptibility to diseases like BPH and treatment outcomes.[14] Studying these variants enhances understanding of drug response variability and supports personalized medicine.[15]

This study investigates the association between CYP3A4 rs2740574 polymorphism and BPH risk in Iraqi patients, aiming to evaluate its role as a predictive biomarker of disease susceptibility.

METHODS

This research was conducted at the Molecular Genetics Laboratory, College of Education for Pure Sciences, University of Diyala, Iraq. It focused on BPH patients and healthy Iraqi individuals from Diyala province, admitted to Baqubah Teaching Hospital. Blood samples were collected in October 2023 and May 2024.

Genetic polymorphism of the CYP3A4 gene (rs2740574) was analyzed using PCR. Primers were designed with NCBI Primer-BLAST (Forward: CACACCACTCACTGACCTCC, Reverse: GTAGGTGTGGCTTGTTGGGA). The expected product length was 217 bp, with GC contents of 60% and 55%, and melting temperatures of 59.97°C and 59.89°C, respectively. PCR mixtures contained primers, DNA template, master mix, and nuclease-free water in 25 µl volume. Amplification was confirmed by agarose gel electrophoresis.

Thermal cycling included: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 5 min; followed by a final extension at 72°C for 5 min. PCR products were separated on 1% agarose gel at 90 V for 1.5 h. Amplified DNA was sequenced at Macrogen (South Korea) using the Sanger method. Genotypes were classified as protective or causal based on nucleotide sequence analysis with Geneious software, applying Hardy–Weinberg equilibrium.

Quality control included duplicate PCR runs, positive/negative controls, and chromatogram evaluation of sequencing. Ethical approval was obtained from the Institutional Ethics Committee, University of Diyala, and informed consent was provided by all participants, in accordance with the Declaration of Helsinki.

RESULTS

The results of amplifying a segment of the CYP3A4 gene promoter region at the rs2740574 polymorphic site showed that the molecular weight of the resulting bands was 217 base pairs for both patient and control samples after staining with ethidium bromide and visualization under ultraviolet light (Fig. 1).

The nucleotide sequences of all samples subjected to sequencing30 samples from patients with benign prostatic hyperplasia (BPH) and 10 samples from healthy individuals were aligned and compared using a single chart (Fig. 2). The aim was to investigate genetic variations within the amplified region of the CYP3A4 gene, located on chromosome 10, with a molecular size of 217 base pairs, and encompassing the polymorphic site rs2740574. The analysis was conducted using the Geneious software from the National Center for Biotechnology Information (NCBI). Multiple sequence alignment was employed to identify nucleotide substitutions and their precise locations. These sequences were then compared with one another as well as with the reference DNA sequence available. The results revealed the presence of point mutations of the transition type in the BPH patient samples when compared to both the reference DNA sequence and the sequences of the healthy control group, as documented on the NCBI platform.

The genotypic distribution results revealed that the homozygous CC genotype was the most prevalent among the healthy control group, with 9 out of 10 individuals (90%) carrying this genotype. The C allele frequency in this group was 19 (95%). In contrast, only one patient out of 30 with benign prostatic hyperplasia (BPH) exhibited the CC genotype (3.33%), with a corresponding C allele frequency of 29 (48.33%). According to Fisher's exact test, this difference was statistically highly significant (P=0.000), and the odds ratio (OR) was 0.003 with a 95% confidence interval (CI) of 0.000–0.067. These findings suggest that the CC genotype and C allele may serve as strong protective factors against BPH (Table 1).

On the other hand, the CT heterozygous genotype was found in 27 out of 30 patients (90%) compared to only 1 case (10%) in the control group. The associated OR was 81.00, with a 95% CI ranging from 7.76 to

1834.07, and the result was statistically significant (P = 0.000), indicating that the CT genotype represents a very high risk factor for BPH. The TT homozygous genotype was identified in 2 patients (6.66%) and was absent in the control group.

Table No. 1: Evaluation of the Association between Genotypes and Alleles of the CYP3A4 Gene at the rs2740574 (C>A, G, T) Polymorphic Site Across Study Groups

Genotype //rs2740574 C>A,G,T	Control	Patients	Fisher's/P-value	O.R. (C.I.)
CC	9 (90%)	1 (3.33%)	0.00*	0.003 (0.000 - 0.067)
CT	1 (10%)	27 (90%)	0.00*	81 (7.76 - 1834.07)
TT	-	2 (6.66%)	0.721 NS	1.84 (0.09 - 36.60)
Total	10 (100%)	30 (100%)		
Allele	**Frequency**			
C	19 (95%)	29 (48.33%)	O.R. (C.I.) = 0.05 (0.00 - 0.30)	
T	1 (5%)	31 (51.67%)	O.R. (C.I.) = 20.31 (2.69 - 153.61)	
*P≤0.05 (Significant), NS: Non-Significant.				

Table No. 2: Distribution of Genotypes and Allelic Frequencies of the CYP3A4 Gene at the rs2740574 (C>A, G, T) Polymorphic Site in Study Groups According to the Hardy-Weinberg Equilibrium in Benign Prostatic Hyperplasia Compared to the Control Group

Group		**Genotype // rs2740574 C>A,G,T**		**Allele frequencies**			**P value**
		CC	**CT**	**TT**	**C**	**T**	
Patients	Observed	9	1	-	19	1	0.867 (NS)
		90%	10%	-	95	5	
	Expected	9.03	0.95	0.03	Not diagnosed		
		90.25	9.5	0.25			
Controls	Observed	1	27	2	29	31	0.000*
		3.33%	90%	6.66%	48.34%	51.67%	
	Expected	7.01	14.98	8.01	Not Diagnosed		
		23.36	49.94	25.69			

NS = Not significant *P≤0.01 (Significant)

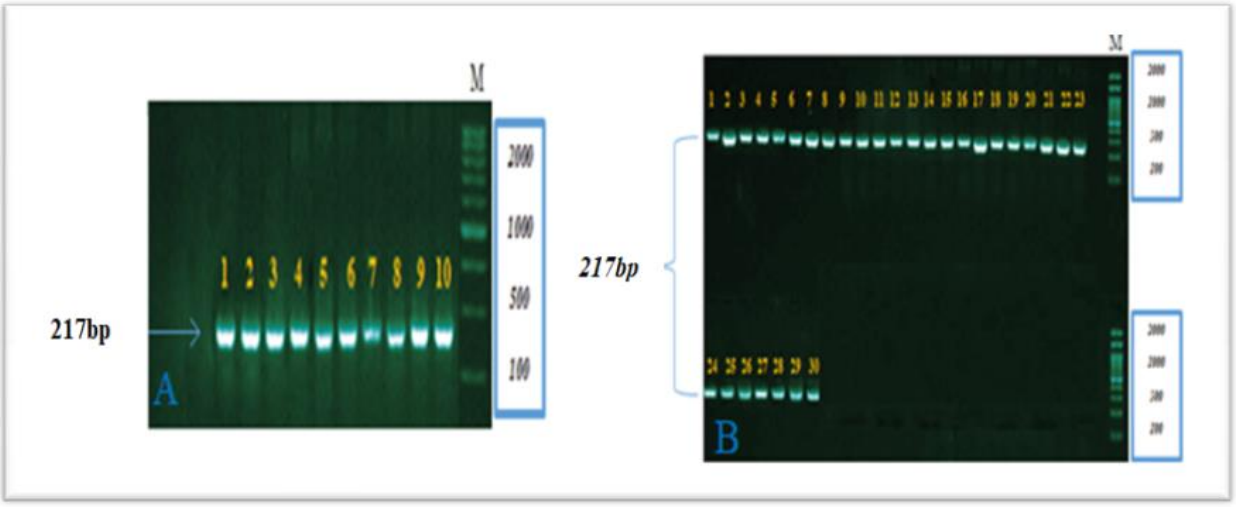

Figure No. 1: The amplification result of a segment from the promoter region of the CYP3A4 gene at the rs2740574 C>A, G, T polymorphic site in (A) the healthy control group and (B) patients with benign prostatic hyperplasia

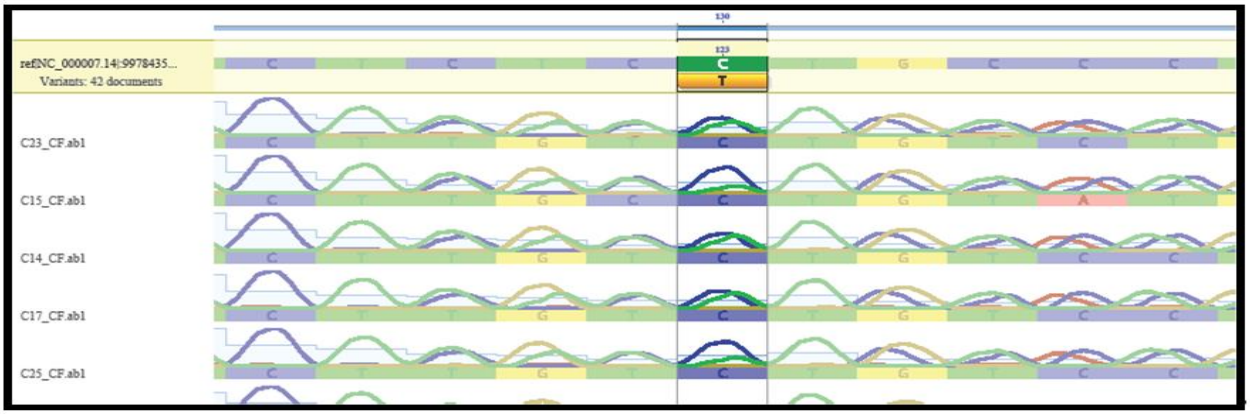

Figure No. 2: Comparative Alignment of Nucleotide Sequences for a Segment of the CYP3A4Gene Among BPH Patient Samples, Healthy Controls, and the GenBank Reference Sequence. This figure illustrates the position of the rs2740574 (C>A, G, T) polymorphism and identifies the type of mutation observed. The alignment was performed using data from NCBI (2024)

The corresponding P-value was 0.721, and the OR was 1.84 with a 95% CI of 0.09–36.60, suggesting a potential risk associated with this genotype, albeit not statistically significant. Regarding overall allele distribution, the T allele was significantly more common among patients (51.67%) compared to only 5% in healthy individuals. This difference was also statistically significant, with an OR = 20.31 and a 95% CI of 0.00–0.30, indicating that the T allele is a major genetic risk factor for developing BPH. Additionally, the Hardy-Weinberg equilibrium analysis, shown in Table (2), demonstrated that the control group was in genetic equilibrium (P = 0.8678; NS), suggesting that genotype frequencies followed expected Mendelian distribution and were unaffected by disease pressure. In contrast, the patient group exhibited a significant deviation from equilibrium (P = 0.000), possibly due to disease-related selective pressure or a direct association between the genotypes at the studied locus and susceptibility to BPH (Table 2).

DISCUSSION

This study was conducted based on a comparative analysis between two groups: patients diagnosed with benign prostatic hyperplasia (BPH) and a healthy control group, to investigate the genetic polymorphism rs2740574 (C>A, G, T) in the CYP3A4 gene. The results of the genotypic distribution presented in Tables (1–2) revealed that the CC homozygous genotype was the most prevalent in the healthy group, with 90% of individuals carrying this genotype, and a C allele frequency of 95%. In contrast, this genotype was observed in only one BPH patient (3.33%), with a corresponding C allele frequency of 48.33%. These differences were statistically significant (P=0.000, OR=0.003), suggesting that both the CC genotype and C allele may serve as protective factors against BPH. Conversely, the CT heterozygous genotype was found in 90% of the BPH patients, compared to only 10% in the control group (OR=81.00, P=0.000), indicating a strong association with increased risk of developing BPH. The TT homozygous genotype was rare, appearing in only 6.66% of patients and not at all in controls, with a non-significant (P=0.721, OR=1.84), suggesting a limited impact due to its low frequency. In terms of allelic distribution, the T allele was markedly more frequent among patients (51.67%) compared to only 5% in controls, with strong statistical significance (OR=20.31), identifying it as a potential risk allele. The Hardy–Weinberg equilibrium test showed a balanced genotypic distribution in the control group (P=0.8678), suggesting a normal, unaffected population. In contrast, there was a significant deviation from equilibrium in the BPH group (P=0.000), possibly reflecting disease-associated genetic pressure or a direct genotype–phenotype correlation.

This polymorphism resides in the promoter of CYP3A4 gene and is synonymous with the -392A>G mutation that is often written CYP3A4*1B. This variant is termed to influence the gene expression of the responsible enzyme to metabolise testosterone to less active forms. In the past, such a change has occurred. It has been implicated to have both knock-on and malignant prostate states groups of population.[15,16]

To prove this point, one study done in Korea illustrated a strong relationship between specific variant forms of CYP3A4, especially CYP3A4-Ht-2 and higher metastatic potential within prostate cancer cells.[17] Also, a recent research article emphasized that CYP3A4*1B (rs2740574) is one of the most commonly investigated polymorphisms regarding relation to prostate-related disorders both with respect to BPH and prostate cancer.[18,19]

In addition, a study done in South Africa indicated that the G allele of rs2740574 was associated with susceptibility to prostate cancer in both Blacks and

White men, having emphasized the significance of this polymorphism in ethnically various ethnics groups.[20]
The research suggests that additional research into bigger and wider research is necessary. This is done by way of populations to confirm such findings and to understand more into the molecular mechanisms through which this genetic variation leads to development of this disease.

CONCLUSION

The genetic polymorphism of the rs2740574 of the gene could lead to the incidence of autism. A case of benign is that CYP3A4 gene has a huge role in genetic predisposition to benign. Prostatic hyperplasia women have T allele is linked with a higher chance of getting BPH and the C allele seems to have an effect of suppressing BPH protective effect. The results outline the possible relevance of genetic screening of finding people who are more risky to BPH and opening the way to the potential use this polymorphism as a prospective biomarker of early diagnosis and disease prevention.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Basim Mohammed Abdul Latif, Ammar Ahmed Sultan
Drafting or Revising Critically:	Basim Mohammed Abdul Latif, Thikra Ata Ibrahim
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 1-9/2021 dated 5.04.2023

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Original Article

Psychological Changes of Patients with Cancer

Psychological Changes with Cancer

Amna Hamid Hussein and Sahar Adham Ali

ABSTRACT

Objective: To assess psychological changes of patients with cancer at oncology teaching hospitals.
Study Design: Descriptive cross sectional study
Place and Duration of Study: This study was conducted at the Hillah city (Al-Imam Al-Sadiq Teaching Hospital and Babylon Cancer Treatment Center from 1st March 2025 to 30th June 2025.
Methods: Two hundred and eighty patients selected to carrying out and distributed as 30 patients who participate in the pilot study, 250 patients who assigned to engage the original study sample.
Results: There were 150 (60%) females and 100 males, married 187 (74.8%), between (51-60) years of age, 155 (62%) lived in urban area. Overall, the mean score indicated a moderate level of psychological distress among participants.
Conclusion: No significant association is observed between psychological changes and educational level or residency.

Key Words: Psychological changes, Anxiety, Depression, Cancer

Citation of article: Hussein AH, Ali SA. Psychological Changes of Patients with Cancer. Med Forum 2025;36(9):66-70. doi:10.60110/medforum.360912.

INTRODUCTION

Uncertainty cancer diagnosis may cause worry, despair, a decline in quality of life, thoughts of suicide, and other possibly severe life changes.[1] Psychological problems such as depression and anxiety persist and can cause an additional burden during their treatment, making it more challenging in terms of its management and control, compliance during the treatment course, duration of hospital stay, and, survival rate in the end. According to previous research, cancer patients are two to three times more likely than the general population to suffer from depressive disorders.[2]

Depression is a common mental illness affecting about 5% of people worldwide. It is characterized by persistent sadness, loss of interest, changes in appetite and sleep, fatigue, and difficulty concentrating. Depression significantly impacts daily functioning and quality of life.

It results from complex social, psychological, and biological factors, with triggers including early-life adversity, loss, and unemployment.[3]

Anxiety is the expectation of a threat in the future that shows up as excessive and ongoing worry or fear.

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Received: July, 2025
Reviewed: August, 2025
Accepted: August, 2025

An essential psychological component of cancer survivorship care is addressing anxiety.[4]

In today's world, anxiety, tension, worry, stress, and strain are all normal emotions. We won't seek a professional for simple stress or anxiety, but when these emotions become chronic and start to interfere with our lives, we need to take action and find strategies to cope in order to continue living our best lives. It is a typical symptom of cancer since it can be described as an unpleasant subjective experience connected to the sense of actual danger. An adaptive response, which is a difficult and unusual feeling, and represents a state of fear, but it is not specified, a feeling that is not commensurate with the situation attributed to it. Adaptive, in response to the risk caused by cancer, it is a normal response that lasts from 7 to 10 days after diagnosis (suspicion, suffering, death).[3]

Anxiety is more common in cancer survivors than in healthy controls, and this effect is true for individuals whose cancer was discovered ten years or more ago. Anxiety can negatively impact quality of life in cancer survivors, impacting mental health and functioning and Increasing the severity of somatic symptoms.[4]

Oncology nurses play a vital role in cancer care through supportive care and patient empowerment. Their broad responsibilities significantly impact outcomes and should be included in national cancer strategies.[5]

Supportive-expressive group psychotherapy, cognitive-behavioral and cognitive-existential therapy, meaning-centered psychotherapy, mindfulness, and mindfulness stress reduction programs are the interventions that have the strongest empirical backing for addressing suffering in cancer patients. Additionally, there is mounting evidence that integrative therapies

particularly mind-body therapies are useful supportive care tactics for cancer patients.[6]

To treat progression anxiety, a number of therapy ideas have been explored. In a (partially) RCT in a rehabilitation hospital context, a group therapy founded on cognitive behavioral principles was found to reduce anxiety of advancement. In a pilot phase for an outpatient setting, it also demonstrated some encouraging outcomes, although with some changes. Another therapy has recently demonstrated notable gains in an RCT among survivors of colorectal, prostate, and breast cancer. It employs behavioral modification, cognitive restructuring, and psycho-education in an individual therapeutic environment.[7,8]

METHODS

This descriptive cross sectional study was conducted at teaching oncology hospitals in Hillah city (Al-Imam Al-Sadiq Teaching Hospital and Babylon Cancer Treatment Center from 1st March 2025 to 30th June 2025. One of the most crucial components of quantitative research is ethical concern, as these studies frequently involve human participants. Depending on the study's objectives, consent is typically sought verbally (orally or in writing). This type of ethical grade may safeguard the study participants' privacy and dignity. Therefore, following outlining the goals of the study, a formal consent form was applied for each participant in order to get their formal consent. The non-probability convenience sample method was used with 280 patients were selected distributed as 30 patients who participate in the pilot study, 250 patients who assigned to engaged to the original study samples. Demographic characteristics consist of 5 items, which include age, sex, educational level, marital status and residency. Psychological changes include 14 items adapted from Osse et al[9], translated to Arabic language to facilities data collection.

Content validity of the questionnaire was obtained by panel of 10 experts from multidisciplinary field, who have not less than 7 years of experience in their specialty. Changes and modification performed according to the advises and opinion of the expert in order to reach the proper degree of understanding, clearness and relevance of questionnaire obtained to facility data collection, factors analysis approach carried out which estimated as 0.748 with statistically accepted. The pilot study is conducted on 30 patients undergoing chemotherapy carried out at Al-Marjan Medical city (Babylon Cancer Treatment Center) to assess the stability of the instrument which is prepared to collect the data. Using a questionnaire (Arabic version), interview and self-report procedures with patients, data was gathered after the cooperation and approval of hospitals administration. After introducing herself and outlining the study's objectives to the participants, the researcher obtained their verbal consent before giving them the questionnaire. The participants (patients) complete the form and provide a response. The participants separately respond to a questionnaire. It takes roughly fifteen to twenty minutes for each self-report. Data collection took place throughout the span of time about (18) days, it started from 15th March 2025 to 1st April 2025. The data was entered and analyzed through SPSS-26.

RESULTS

The study sample consists of 250 participants, with a diverse distribution across various demographic characteristics, regarding age, the majority of the sample fall within the 51-60 years age group 65(26.0%). The sample comprises more females (60.0%) than males (40.0%). while educational attainment varies, recorded that the highest proportion of the sample having an education level of institute and above (30.0%), while 24.0% are illiterate. Marital status reveals that most participants are married (74.8%), while smaller proportions are single (10.0%), widowed (13.2%), divorced (1.6%), or separated (0.4%). Residency-wise, the majority of participants reside in urban areas (62.0%), whereas 38.0% live in rural settings. These demographic insights provide a comprehensive understanding of the study sample's distribution, which may have implications for the study's findings (Table 1).

Table No. 1: Patients responses related to demographical characteristics

Categories	No.	%
Age (years)		
30-40	46	18.4
41-50	42	16.8
51-60	65	26.0
61-70	52	20.8
71-80	45	18.0
Gender		
Female	150	60.0
Male	100	40.0
Education Level		
Illiterate	60	24.0
Educated	30	12.0
Elementary	56	22.4
Intermediate	29	11.6
Institute and above	75	30.0
Marital status		
Married	187	74.8
Single	25	10.0
Divorced	4	1.6
Separated	1	0.4
Widow	33	13.2
Residency		
Rural	95	38.0
Urban	155	62.0

Table No.2: Patients responses related to psychological changes

Questions	No		Somewhat		Yes		Mean	St. Dev	Level
	No.	%	No.	%	No.	%			
Depressed mood	64	25.6	55	22.0	131	52.4	2.27	.843	Moderate
Fearing from physical suffering	91	36.4	35	14.0	124	49.6	2.13	.920	Moderate
Fear of treatments	112	44.8	27	10.8	111	44.4	2.00	.946	Moderate
Fear of death	112	44.8	14	5.6	124	49.6	2.05	.972	Moderate
Fear from metastases	79	31.6	24	9.6	147	58.8	2.27	.913	Moderate
Fear from being alone	107	42.8	22	8.8	121	48.4	2.06	.955	Moderate
Feelings of guilt	103	41.2	22	8.8	125	50.0	2.09	.953	Moderate
Feelings of shame	114	45.6	29	11.6	107	42.8	1.97	.942	Moderate
Loss of control over emotions	74	29.6	72	28.8	104	41.6	2.12	.837	Moderate
Difficulties to accept a changed bodily appearance	80	32.0	26	10.4	144	57.6	2.26	.913	Moderate
Find it difficult and feel unable to deal with future events.	88	35.2	20	8.0	142	56.8	2.22	.936	Moderate
Difficulties in showing emotions.	116	46.4	48	19.2	86	34.4	1.88	.893	Moderate
Difficulties to See positive aspects of the situation.	100	40.0	20	8.0	130	52.0	2.12	.954	Moderate
Being overwhelmed by all decisions that have to be made.	82	32.8	18	7.2	150	60.0	2.27	.926	Moderate
General mean and standard deviation							2.122	0.921	Moderate

Low 1-1.69; Moderate 1.7-2.39; High 2.4-3

Table No. 3: Association between psychological changes of patient with cancer and demographical characteristics

Parameter	Chi square	Degree of freedom	P. value	Significance
Physical needs of patients Age	158.221	112	.003	S
Physical needs of patients Sex	8.945	1	.003	S
Physical needs of patients Educational level	107.817a	112	.594	NS
Physical needs of patients Marital status	142.619a	112	.027	S
Physical needs of patients Residency	16.924a	28	.950	NS

The table 2 presents the distribution of responses regarding the psychological needs of the study sample. It highlights the prevalence of various psychological concerns among participants, with a focus on symptoms such as depressed mood, fear of physical suffering, fear of treatments, fear of death, fear of metastases, feelings of guilt and shame, emotional control issues, difficulties in accepting bodily changes, and challenges in dealing with future events. Across all 14 items, a moderate level of psychological need was reported, with means ranging between 1.88 and 2.27. The highest levels of concern were seen in fear of metastases (58.8% responded "Yes") and fear of death (49.6% responded "Yes"), while difficulties in showing emotions and seeing positive aspects of the situation had slightly lower percentages. Overall, the mean score of 2.12, with a standard deviation of 0.921, indicates a moderate level of psychological distress among the study participants.

Table 3 presents the association between the psychological needs of cancer patients and their demographic characteristics using the Chi-square test. The results indicate a significant association between the physical needs of patients and age ($\chi^2 = 158.221$, df=112, p=0.003), as well as sex ($\chi^2 = 8.945$, df=1, p=0.003), suggesting that these factors play a role in

influencing patients' physical needs. Additionally, marital status is significantly associated with physical needs (χ^2 = 142.619, df=112, p=0.027). However, no significant association is observed between physical needs and educational level (χ^2 = 107.817, df=112, p=.594) or residency (χ^2 = 16.924, df=28, p=0.950).

DISCUSSION

Related to the demographical characteristics of the study sample explain that most patients age undergoing chemotherapy were between (51-60) years. This result supported by result conducted by study in Asian by Wisesrith et al[10], that show majority of terminal ill cancer patients aged between (51 and 60) years. As the point view, the risk of cancer increases between the ages of 51 and 60 due to the accumulation of genetic mutations with age, prolonged exposure to carcinogenic factors such as smoking and pollution, as well as weakened immunity and hormonal changes during this period. Increased screening also contributes to the detection of more cases. In short, it's a combination of time, body changes, and screening.

Regarding to sex, this study show that majority of patients undergoing chemotherapy were female. This outcome followed with Shi et al[11] that demonstrates that most of the patients were female. The logical interpretation of this point go under women are more likely to develop cancer due to their predisposition to specific cancers, such as breast and ovarian cancers, the influence of hormones, increased screening for early detection, and their often longer life spans, which increases their risk of developing the disease.

According to the educational level most of the patients were with high educational level. The finding shows that most of the patients were married. This result follows with Gupta et al[12] that majority of patients were high level of education (high school/college) were married. As the point of view, married people and those with a high school or higher appear at higher rates in cancer statistics and are more likely to develop cancer. This is often attributed to their greater health awareness and interest to seek early screening, which increases the chances of detecting the disease. Additionally, their lifestyle may include work stress, lack of physical activity, or unhealthy habits. Marriage, on the other hand, makes it easier to detect symptoms thanks to the support of a partner, which speeds up diagnosis.

This study showed that 62% of cancer patients undergoing chemotherapy lived in urban areas. This result supported by result conducted by study in Iraq by Ajel[13] that 61.25% the majority of cancer patients undergoing chemotherapy lived in urban areas. To discuss this point, the logical interpretation is that, cancer is a disease that can affect individuals regardless of their geographic location, including both urban and rural areas. While cancer may be more prevalent in urban populations due to factors such as lifestyle choices, environmental exposures, higher levels of air pollution, stress, sedentary lifestyles.

Responses regarding the psychological changes of the study sample. It highlights the prevalence of various psychological concerns among participants. Overall, the mean score of 2.12, with a standard deviation of 0.921, indicates a moderate level of psychological distress among the study participants. This result agree with the result of study conducted by Karunanithi et al[14] reported that psychological distress = 44 (11–98) as the median score, over 62.4% of respondents indicated that they were in moderate distress. The logical interpretation of this point is that Cancer patients experience moderate levels of psychological distress due to a combination of initial shock and gradual adjustment to the disease, along with psychological and social support that alleviates the severity of stress. Fear of the future, the possibility of relapse, and the daily challenges of treatment also contribute to persistent but mild anxiety, which explains the prevalence of moderate levels of psychological distress among this group.

Responses regarding association between psychological changes of patient with cancer and demographical characteristics, the results indicate a significant association between the psychological changes of patients and age, as well as sex, suggesting that these factors play a role in influencing patients' psychological changes. Additionally, marital status is significantly associated with psychological changes. This result of this study is comparable with Hamilton et al[15] illustrated that distress score variations between age groups were significant and participants who were 70 years of age or older showed much less distress than those who were younger, according to post hoc tests. The emotional demands of younger people were substantially greater than individuals in the other age groups and significantly more physical needs than older adult individuals. Women had higher distress greater than men's. The number of needs for men and women was similar. The total distress score showed significant results for marital status. The distress scores of married people were lower than those of unmarried people. Psychosocial requirements were lower among married people. Compared to married people, single people reported substantially higher practical and nutritional needs, according to post hoc studies.

In this study the results indicate a significant association between the psychological needs of cancer patients and age, gender, and marital status. Younger, female, and unmarried patients reported higher psychological needs than others. This is attributed to social support, anxiety about the future, and psychological changes that vary by gender and marital status, underscoring the importance of this factor when providing psychological support to patients.

CONCLUSION

Moderate level of psychological distress among participants reported, no significant association is observed between psychological changes and educational level or residency.

Recommendation: Psychological program therapy may be established in oncology centers to encourage feeling sharing, and counselling to decrease patient emotional impact. This program may be extended to engage patient's family members in the supporting process to enhance patient's responses to treatment.

Author's Contribution:

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Drafting or Revising Critically:	Amna Hamid Hussein, Sahar Adham Ali
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No. 325 dated 24.02.2025

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Original Article

Attitudes of Nursing Staff towards Family Involvement in Patient's Care at Acute Care Units

Ameer Salah Aldeen Abdulrazaq[1], Wefaqq Mahdi Hadi[2], Zaman Ahmed Hrefish[1] and Abbas Lateef Muhe-Aldeen[3]

ABSTRACT

Objective: To explore nurses' attitudes toward family participation and collaboration in the care of hospitalized adult patients. Additionally, it sought to identify any correlations between these attitudes and the nurses' socio-demographic characteristics.

Study Design: A descriptive study

Place and Duration of Study: This study was conducted at the Intensive Care Units at Hilla Teaching Hospital and Imam Sadiq Hospital, Babylon Province from 1st January 2023 to 1st April 2023.

Methods: A descriptive study was carried out using a non-probability convenience sample consisting of 100 nurses employed in intensive care units at Hilla Teaching Hospital and Imam Sadiq Hospital, Babylon Province. Data were collected and divided in two main parts: the first section covered demographic characteristics, while the second included a 16-item scale assessing nurses' perceptions regarding family participation in patient's care. Responses were recorded using a three-point Likert scale (Agree, Neutral, Disagree).

Results: Majority of nurses (58%) was aged between 20 and 25 years, 60% were female with 66% held a college degree in nursing. Additionally, 56% were married, 80% had five years of clinical experience, and 75% resided in urban areas.

Conclusion: Nurses generally maintained a neutral stance toward the involvement of families in patient care within the ICU setting and a significant correlation between nurses' perspectives and various demographic factors, including their age, educational background, years of clinical experience, and area of residence.

Key Words: Attitudes, Nurses, Patient care, Acute care units

Citation of article: Abdulrazaq ASA, Hadi WM, Hrefish ZA, Muhe-Aldeen AL. Attitudes of Nursing Staff towards Family Involvement in Patient's Care at Acute Care Units. Med Forum 2025;36(9):71-75. doi:10.60110/medforum.360913.

INTRODUCTION

Intensive care units (ICUs) are inherently challenging environments, often placing considerable emotional strain on both patients and their families. Actively involving family members in the care process has been linked to notable improvements in patient outcomes and overall care quality. Given their pivotal role in providing direct and continuous care, nurses are uniquely positioned to encourage and support such involvement.

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Received: December, 2024
Reviewed: January-February, 2025
Accepted: March, 2025

Nevertheless, the nature and effectiveness of nurse–family interactions largely depend on nurses' personal attitudes toward the participation of families in patient care.[1]

When families take an active role in patient education and caregiving, they gain a clearer understanding of the patient's health status, allowing them to play a more meaningful role in the recovery journey. This involvement becomes especially important for elderly patients or individuals who are unable to manage their care independently. In these situations, family members often provide essential support with daily care tasks and help ensure that treatment plans, including medication schedules, are followed correctly. Such engagement can significantly enhance recovery speed and improve overall health outcomes.[2]

Nurses are increasingly faced with complex clinical situations that require active engagement with patients' families, who are now recognized as vital partners in the healthcare process. Delivering high-quality care in such settings hinges on effective communication, collaborative teamwork, and shared decision-making-competencies that continue to evolve as core aspects of professional nursing practice.[3] Given that nurses,

among other healthcare providers, tend to offer episodic or support-based care, family members need to take on a more central role in caregiving. This collaborative model that brings together patients, families, and clinicians plays a vital role in continuity and coordination of care.[4,5]

Interacting with family members in the intensive care unit may be particularly challenging, as both parties often experience increased emotional distress, varied responsibilities, and risks for caregiver burnout. As the patient's caregivers, family members become inextricably linked with the seriousness of the illness, even suffering some themselves in the form of adverse psychological impacts that can include post-traumatic stress disorder (PTSD), anxiety, depression, or prolonged grief-conditions that together comprise post-intensive care syndrome-family (PICS-F). The prevalence of such outcomes among caregivers varies considerably, with studies reporting depression rates from 4% to 94%, anxiety from 2% to 80%, and PTSD from 3% to 62%. Despite these difficulties, families remain essential partners in the care process, offering emotional reassurance and actively participating in clinical decisions. The number of stressors in the ICU that add to families' emotional strain, such as the fear and anxiety that come with not knowing what the patient's outcome will be, the upheaval of their daily routines, the unfamiliar and often intimidating medical setting, and the overwhelming emotional toll it all takes.[6-7]

METHODS

The descriptive study was used a non-probability convenience sampling method to select participants. It involved 100 nurses working in the intensive care units of Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon Province from 1st January 2023 to 1st April 2023. Before data collection began, all ICU nurses who took part were fully informed about the study's purpose, procedures, and expected benefits, and each gave their written consent to participate. To protect participants' confidentiality, questionnaires were completed anonymously, and assurances were given that all collected data would be handled exclusively for research purposes, with strict adherence to privacy and data protection standards throughout the study.

The data collection tool was a structured questionnaire developed by the researchers specifically to assess the study variables. The design of the instrument was guided by a comprehensive review of relevant literature and previous related research studies. The questionnaire consisted of two parts: assessment of nurses' attitudes toward family involvement in nursing care: This section includes Sixteen items measured in three point (Agree, Neutral, Disagree). Socio-demographic characteristics: This part evaluates six variables, including educational qualification, marital status gender, age, years of experience, and place of residence. Data analysis was conducted using SPSS-24.

RESULTS

Most nurses (58%) were aged between 20 and 25 years. Females comprised the larger share of the sample, representing 60% of respondents. In terms of education, the majority (66%) held a bachelor's degree. More than half of the participants (56%) were married. Regarding work experience, 80% reported having up to five years of professional practice. Lastly, a significant portion of the sample (75%) resided in urban areas (Table 1).

The ICU nurses' attitudes toward family involvement in patient care, generally neutral overall attitude among the participants. Positive attitudes were observed for items 1, 2, 7, 9, 10, 11, and 12. Items 3, 4, 6, 14, 15, and 16 received neutral mean scores, indicating uncertainty or ambivalence. In contrast, negative attitudes were identified for items 5, 8, and 13 (Table 2).

Table No.1: Demographical information of the patients (n=100)

Variable	No.	%
Age (years)		
20-25	58	58.0
26-30	36	36.0
31-35	4	4.0
35-40	2	2.0
Gender		
Male	40	40.0
Female	60	60.0
Marital status		
Single	43	43.0
Married	56	56.0
Divorce	1	1.0
Educational status		
Diploma	31	31.0
Bachelor's	66	66.0
Postgraduate	3	3.0
Residency		
Urban	75	75.0
Rural	25	25.0
Experience (years)		
<5	80	80.0
6-10	17	17.0
11-15	1	1.0
16-20	2	2.0

The statistically significant relationship between nurses' attitudes with several demographic characteristics, namely age, educational attainment, years of experience, and place of residence ($p \leq 0.05$). No significant association was found with gender. Specifically, age was significantly related to attitudes ($p=0.05$), with the most positive attitudes reported

among nurses aged 20–25 years. Furthermore, a significant association was identified between nurses' educational level and their attitudes (p = 0.04), where those holding a bachelor's degree exhibited more favorable views toward family involvement. The study found a strong link between nurses' attitudes and their years of experience (p=0.012), with those having five years or less of experience expressing more positive views. Additionally, where the nurses lived also played a significant role (p=0.038), as those residing in urban areas tended to be more supportive of involving families in patient care (Table 3, Fig. 1).

Table No. 2: Distribution of ICU Nurses' Attitudes toward family Involvement in patient care

Items		No.	%	Mean±SD	Level
Maintaining a positive relationship with the patient's family enhances my job satisfaction	Disagree	16	16.0	2.44±0.756	Positive
	Neutral	24	24.0		
	Agree	60	60.0		
I encourage the patient's family to actively participate in the patient's care	Disagree	16	16.0	2.54±0.758	Positive
	Neutral	14	14.0		
	Agree	70	70.0		
The presence of the patient's family is significant to me as a nurse	Disagree	25	25.0	2.19±0.813	Neutral
	Neutral	31	31.0		
	Agree	44	44.0		
Having the patient's family present provides me with a sense of security	Disagree	47	47.0	1.73±0.777	Neutral
	Neutral	33	33.0		
	Agree	20	20.0		
The presence of the patient's family helps to reduce my workload	Disagree	52	52.0	1.66±0.768	Negative
	Neutral	30	30.0		
	Agree	18	18.0		
The patient's family should play an active role in planning the patient's care	Disagree	19	19.0	2.32±0.777	Neutral
	Neutral	30	30.0		
	Agree	51	51.0		
The involvement of the patient's family is important for the family members themselves	Disagree	14	14.0	2.47±0.723	Positive
	Neutral	25	25.0		
	Agree	61	61.0		
Engaging with the patient's family makes me feel that I am being helpful	Disagree	54	54.0	1.69±0.825	Negative
	Neutral	23	23.0		
	Agree	23	23.0		
I communicate with the patient's family when the patient is first admitted to my care	Disagree	10	10.0	2.65±0.657	Positive
	Neutral	15	15.0		
	Agree	75	75.0		
I provide updates to the patient's family regarding the patient's condition at the end of the care period	Disagree	13	13.0	2.53±0.717	Positive
	Neutral	21	21.0		
	Agree	66	66.0		
I communicate with the patient's family when there is a deterioration or change in the patient's condition	Disagree	15	15.0	2.54±.0744	Positive
	Neutral	16	16.0		
	Agree	69	69.0		
The presence of the patient's family sometimes interferes with my ability to perform my duties	Disagree	50	50.0	1.67±0.753	Negative
	Neutral	33	33.0		
	Agree	17	17.0		
I often lack sufficient time to provide support to the patient's family	Disagree	17	17.0	2.27±0.737	Negative
	Neutral	39	39.0		
	Agree	44	44.0		
The presence of the patient's family can make me feel as though my work is being evaluated	Disagree	31	31.0	2.15±0.689	Neutral
	Neutral	23	23.0		
	Agree	46	46.0		
The presence of the patient's family makes me feel anxious or uneasy	Disagree	42	42.0	1.90±0.859	Neutral
	Neutral	26	26.0		
	Agree	32	32.0		
General mean and standard deviation	-	-	-	2.20±0.766	Neutral

Table No. 3: The relationship between nurses' attitudes toward family involvement in nursing care and their socio-demographic characteristics

Parameter	Chi-Square Value	DF	Significance	Interpretation
Age	56.000	66	0.055	Significant (S)
Gender	32.274	22	0.073	Not Significant (NS)
Educational Status	82.565	66	0.040	Significant (S)
Years of Experience	42.025	66	0.012	Highly Significant (HS)
Residency	62.019[a]	44	0.038	Significant (S)

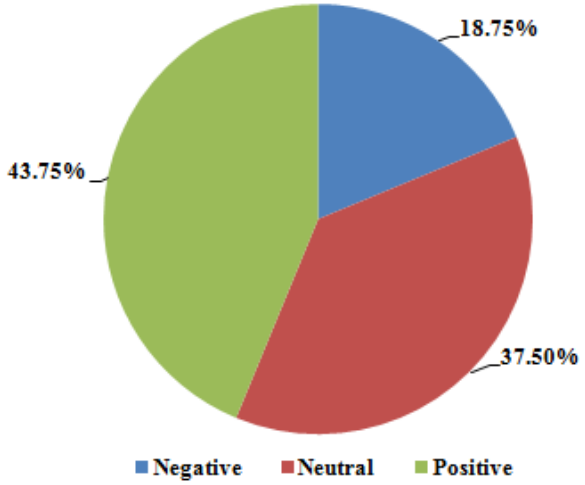

Figure No. 1: General overview of ICU nurses' perspectives on family participation in patient care

DISCUSSION

The results of this study show that most participants were between 20 and 25 years old. This aligns with findings from Imanipour and Kiwanuka[8], where 62% of participants were aged 22 to 30. In contrast, studies by Hagedoorn et al[4] and Halperin et al[9] reported an average participant age of 42. This difference may reflect a tendency in ICU staffing to favor younger nurses, especially those holding bachelor's degrees.

When it comes to gender, the majority of participants in the study were female, making up 60% of the sample. This is in line with findings from Halperin et al[9] and Mason et al[10], and likely reflects a broader trend in which women are more likely than men to enter nursing and healthcare professions.

In terms of marital status, 56% of the participants were married, which is consistent with the findings reported by Halperin et al[9] and Mason et al.[10] Moreover, 66% of the participants held a bachelor's degree in nursing, reinforcing the observations of Mason et al[10] and Luttik et al[3], who highlighted that ICU nurses are generally chosen for their strong academic backgrounds and clinical expertise.

When it comes to ICU experience, the majority of nurses (80%) had fewer than five years of practice, aligning with the findings of Imanipour and Kiwanuka.[8] This study also revealed that nurses generally held neutral attitudes toward involving families in patient care - similar to the results reported by Cranley[2] and Luttik et al.[3] In contrast, other research by Shibily et al[11], Omran et al[12] and Kleinpell et al[13] indicated that nurses tend to have more positive views on family involvement.

The study also revealed notable links between nurses' attitudes and several demographic factors, such as age (especially among those aged 20-25), education level, years of experience (under five years), and living in urban areas ($p \leq 0.05$). Gender, on the other hand, showed no significant association. These findings align with those of Shibily et al[11], who similarly found no meaningful connection between gender and attitudes. However, other research, including studies by Hagedoorn et al[4] and Shamali et al[14], identified gender as a significant factor influencing nurses' perspectives.

The study also found that nurses with higher levels of education tended to express more positive attitudes - a result that aligns with the findings of Luttik et al[3] and Shamali et al.[14] Interestingly, younger nurses (aged 20–25) were more likely to hold favorable views on family involvement in care, supporting the observations of Sampaio et al[15], who reported that younger nurses often show more positive attitudes than their older counterparts. This contrasts with earlier research, which generally links older age and greater experience with more positive attitudes findings that differ from those of the current study.

CONCLUSION

Nurses generally hold a neutral attitude toward family involvement in nursing care additionally, significant differences in nurses' attitudes were observed based on age, educational level, years of experience, and place of residence.

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.4503 Dated 6.12.2022

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Original Article

Assessment of Serum Levels of Vitamin B12 and Folic Acid in Iraqi Patients with Lichen Planus

Ahmed Adel Othman[1], Muntaha Fawzi Salih[3], Ahlam Hameed Majeed[2] and Abdalbseet A. Fatalla[4]

ABSTRACT

Objective: To assess serum levels of vitamin B12 and folic acid in patients with lichen planus.
Study Design: Comparative study
Place and Duration of Study: This study was conducted at the Department of Oral Medicine, College of Dentistry, Uruk University, Iraq from 1st March 2024 to 31st August 2024.
Methods: We examined the serum vitamin B12 and serum folate levels of 44 patients who presented with oral/skin and mixed lichen planus. Blood samples were obtained by venipuncture, sera were separated in sterile tube stored frozen until assay, and an Amersham International vitamin B12/folate DUAL radio assay kit was utilized for the purpose of determining the levels of vitamin B12 and folate in human serum.
Results: 18.1% of the subjects had low levels of serum vitamin B12 and serum folic acid, which is significantly higher than the 6.3% of the subjects who served as controls and the percentage was not markedly distinct from the control group.
Conclusion: Hematological disorders are less likely to increase the risk of developing various types of Lichen planus.

Key Words: B12 Vitamin, Serum folate, Oral lichen planus, Skin lichen planus.

Citation of article: Othman AA, Salih MF, Majeed AH, Fatalla AA. Assessment of Serum Levels of Vitamin B12 and Folic Acid in Iraqi Patients with Lichen Planus. Med Forum 2025;36(9):76-79. doi:10.60110/ medforum.360914.

INTRODUCTION

Serum vitamin B12 and folic acid are important in the biosynthesis of amino and nucleic acids, and therefore; in cell division.[1] Megaloblastic anemia produced by deficiency of either of these vitamins is identical.[2] Serum vitamin B12 and folate deficiency has been reported in many oral mucosal diseases.[3-7]

Patients with recurrent aphthous ulceration (RAU) exhibited a greater prevalence of serum vitamin B12 and folate deficiency. Numerous studies indicate that deficiencies in iron, vitamin B12, and folic acid significantly contribute to the pathophysiology of RAU.[3,4]

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Received: January, 2025
Reviewed: February, 2025
Accepted: March, 2025

All RAU patients should undergo hematological screening. Other studies found that it is uncommon that a hematological abnormality plays a major role in the pathogenesis of RAU.

It is uncertain whether serum folate and vitamin B12 level assays must be done routinely unless there is a dietary deficiency; history of gastrointestinal diseases or abnormalities the blood indices.[5] A study revealed that no one in either the research group or the control group had abnormal serum vitamin levels, and the results showed no statistically significant differences in B12 or serum folate concentration. Patients with RAU do not usually need hematological testing beyond a full blood count and that serum vitamin insufficiency is not a major etiological factor in the disease.[6]

A shortage in folate significantly impacts the function of the hematological system and causes issues with cell turnover in the oral mucous membranes.[7] Folate deficiency may be observed in individuals undergoing treatment with Diphenylhydantoin (DPH).[8] Folic acid can affect the gingival hyperplasia severity; however, the mechanisms by which it operates are not well understood. Additionally, folate deficiencies have been shown to lead to the oral epithelial cells' degeneration.[9]

A Researcher discovered in their research that individuals on long-term anti-convulsant medications should be monitored for folic acid levels, as deficiency predominantly occurs in these individuals, and supplementation with folic acid appears to enhance

gingival health status.[10-11] The significance of hematological abnormalities in relation to non-ulcerative lesions of the oral mucosa is less definitive. The incidence of a hematological deviation was elevated in patients with Lichen Planus (LP), particularly in the erosive variant.[5,12]

The evaluation of vitamin B12 and folic acid in LP has been the subject of a limited number of investigations, as far as we are aware.[5,12] A comparison of the levels of serum folic acid and B12 that were measured with those of control cases is going to be the focus of this investigation.

METHODS

This comparative study was conducted at Department of Oral Medicine, College of Dentistry, Uruk University, Iraq from 1st March 2024 to 31st August 2024 vide letter No.3457/fg/Approval/dkd9872 dated 7th February 2024. Forty-four patients who were referred to the oral medicine clinic of the college of dentistry at the University of Baghdad and diagnosed with oral and/or cutaneous lichen planus were included in our study, together with the outpatients of clinical dermatology clinic, medical city, Baghdad. Additionally, twenty-two healthy controls were included. The diagnosis of oral and skin LP was based on clinical and histopathological criteria in accordance with the WHO recommendation.[13]

Blood samples were obtained by venipuncture, sera were separated in sterile tube stored frozen ' till assay, an Amersham internationals vitamin B12/folate DUAL radio assay kit was utilized for the purpose of determining the levels of vitamin B12 and folate in human serum. The data was entered and analysed through SPSS026. The difference in the prevalence of abnormalities between the study group and the control group, the chi-square test was utilized.

RESULTS

There were 19 females with an age range of 18-66 years (mean=42 year.) and 25 males with an age range of 27-70 year (mean=47.5 year). The lesions have been present for 3 weeks upto 12 years. Clinically a combination of twenty-two reticular LP, eight erosive LP, one pigmented LP, and one atrophy, with nine skin LP and three mixed LP (Table 1).

A serum vitamin B12 below 150 pg/ml was defined as low. A serum folate below 2g/ml was designated as low. Table 1 displays the results of serum folate and vitamin B12 in LP patients. Eight oral LP show a low value for serum folate, four was erosive type, two was reticular type, one skin LP and one mixed LP and all other values were within the accepted laboratory range of normal. Regarding vitamin B12 only three cases show low value, one erosive type, one reticular and one skin LP. The other cases show a normal laboratory value for vitamin B12. The chi square test was used and found no significant correlation between B12 and type of LP nor between folic acid and type of LP in comparison with the control cases (Table 2).

Table No.1: Clinical distribution of lichen planus patients (n=44)

Variable	No.	%
Atrophy	1	2.7
Erosive	8	18.2
Mixed	3	6.7
Pigmented	8	18.2
Reticular	18	40.7
Skin	6	13.5

Table No.2: Prevalence of serum B12 and Folate in patients with Lichen planus

Group	Number test	Mean±SD of Folic acid	Mean±SD of Vit. B12	Low Folate	Low Vit. B12	% of low folate & Vit. B12	P value
Lichen Planus	44	4.3+3.6	459.5+357.2	8	3	18.1%	NS
Controls	22	2.4+0.6	252.04+98.9	2	4	6.3%	NS

NS= Non-significant

DISCUSSION

According to the findings of forty-four individuals diagnosed with LP, the incidence of abnormalities in serum vitamin B12 and serum folic acid was 18.1%. in comparison with a prevalence of 6.3% in a healthy control subject. This result is harmonized with that of other studies.[12,14] Oral mucosa can be negatively impacted by deficiencies in vitamin B12 and folate, according to research findings.[14] In this study eight patients with oral LP show a low value for folic acid and three of them show a low value for vitamin B12. In comparison to the control group as well as other studies, this number did not show any significant differences.[12,14] Therefore in present study we could not find enough evidence to confirm these two findings and it seems unlikely that hematological abnormalities predispose to different type of LP.

The hematological deficiencies found in some patients with LP could be considered incidental to their diseases. In this study, four patients with erosive type of LP show low value for folate with one show low value for vitamin B12 and this result suggest that this depressed level may be secondary to ulceration.

Vitamin B12 is necessary for normal folic acid metabolism, which is responsible for converting the inactive form of folate into the metabolically active form. The value of vitamin B12 and folic acid in our study, as well as their relation between the two results, are in agreement with the fact that vitamin B12 is essential for this process.[15,16]

We agreed with the suggestion of the previous studies that when the blood indices revealed abnormalities or when there is gastrointestinal diseases history or dietary imbalance or deficiency, investigation for vitamin B12 and folate should be carried out.[9,12]

In addition to the relationship with other immunological diseases and the existence of a T-cell infiltrate, there is a substantial amount of information that lends support to the concept that LP manifests itself as an immune-related condition.[17] The similarity to the lichenoid change in the graft-versus-host reaction[18], and the presence in close apposition of lymphocytes to keratinocytes showing early apoptosis in the LP[19] and the demonstration of a LP specific antigen (LPSA) in skin and oral LP.[20-21]

It is worth noting that the present study included 44 patients only; it would be optimal if more sample size was utilized. Future studies with more sample size and comparison between genders would be recommended.

CONCLUSION

Hematological disorders are less likely to increase the risk of developing various types of Lichen planus.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.3457/fg/Approval/dkd9872 Dated 07.02. 2024

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Original Article

The Effect of Vitamin D SNP FokI (rs2228570) and ApaI (rs7975232) on Axial Spondyloarthritis in Patients on Biological Treatment

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ABSTRACT

Objective: Examine associations between FokI and ApaI genotypes and axSpA disease activity, extra-articular manifestations (EAMs), and infliximab (INF) response in Iraqi patients.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Baghdad Tertiary Centers from 1st August 2024 to 3th December 2024.

Methods: This cross-sectional analysis of 150 axSpA patients on INF (≥3 months) at Baghdad Tertiary Centers. Disease activity was staged by ASDAS; genotypes were determined by PCR-RFLP. Logistic models adjusted for age, sex, smoking, and disease duration assessed genotype-phenotype links; serum VDR activity and an INF "effect-length" index were evaluated.

Results: The mean age 38.8±8.9 years; 72% male; smokers 30%. FokI showed CC 60% and CT 40% (TT absent). CC was confined to inactive ASDAS and associated with remission (OR 19.2; p=0.04), whereas CT increased odds of high activity (OR 3.27; p=0.001). CT carriers had lower frequencies of uveitis, dactylitis, and UTI. ApaI genotypes did not associate with ASDAS or EAMs. Neither SNP related to serum VDR activity or INF effect-length.

Conclusions: FokI acts as a phenotypic modifier—CC protective for axial activity; CT associated with higher activity yet fewer select EAMs-while ApaI appears neutral. Pharmacodynamic measures were unaffected.

Key Words: Axial spondyloarthritis; Vitamin D receptor; FokI; ApaI; infliximab

Citation of article: Drewil AH, Rasheed MK, Jassim NA. The Effect of Vitamin D SNP FokI (rs2228570) and ApaI (rs7975232) on Axial Spondyloarthritis in Patients on Biological Treatment. Med Forum 2025;36(9): 80-84. doi:10.60110/medforum.360915.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disorder with sacroiliac and spinal involvement and substantial impacts on function and quality of life.[1] Despite global advances in axSpA management[2,3], regional data from the Middle East remain scarce and diagnostic delay persists, partly due to the lack of specific biomarkers for early detection (unlike in other diseases.[4,5]

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Received: February, 2025
Reviewed: March-April, 2025
Accepted: May, 2025

TNF-α is pivotal in axSpA pathogenesis, underpinning the effectiveness of inhibitors such as infliximab[6,7]; however, a considerable subset shows primary or secondary non-response.[8]

Well-established genetic factors such as HLA-B27 influence axSpA susceptibility and phenotype[9], and variants in other genes including the vitamin D receptor (VDR) may further shape disease activity and extra-articular manifestation profiles.[10] The FokI start-codon polymorphism alters VDR transactivation potential[11], whereas ApaI is an intronic variant with inconsistent clinical associations.[12] We investigated whether FokI and ApaI genotypes relate to ASDAS-defined disease activity, EAMs, and infliximab response in Iraqi axSpA patients.

METHODS

This cross-sectional study conducted at the Rheumatology Clinics of Baghdad Hospital/Medical City and the National Center for Educational Laboratories from 1st August 2024 to 30th December 2024. A total of 150 consecutive adults (≥18 years) with axSpA verified by MRI or radiography, all receiving INF for ≥3 months. Data collection:

Structured interview and examination captured demographics, smoking, and EAMs; laboratory indices included ESR. Disease activity was staged using ASDAS. Genotyping: VDR FokI (rs2228570) and ApaI (rs7975232) were genotyped by PCR-RFLP. Serum VDR activity was quantified by ligand-binding assay. Using SPSS-24, data was analyzed. Between-group comparisons used t-tests/ANOVA where appropriate. Associations with ASDAS stages used Chi-square and logistic regression adjusted for age, sex, smoking, and disease duration. $p \leq 0.05$ was considered significant.

RESULTS

The mean age was 38.8±8.9 years and BMI was 28.9±6.5 kg/m²) (Table 1). The males were predominated (72%). Only 16% had a first-degree family history suggesting mostly sporadic disease and 30% were current smokers (Table 2).

ASDAS stage correlated with gender (p=0.02; all inactive were male), inflammatory back pain (IBP) (p=0.003; 74% overall, peaking 87% in low activity), colitis (p=0.02; clustered in low activity), and smoking (p<0.001; 46.7% of smokers in the high-activity group). Peripheral arthritis (p=0.41), uveitis (p=0.10), dactylitis (p=0.13), and psoriasis (p=0.19) showed no significant stage trends; enthesitis was borderline (p=0.055; highest 12.5% in very high activity). The males were predominance in the inactive stage; IBP and smoking are strong markers of higher activity; colitis clustering at low activity may indicate a distinct phenotype (Table 3).

ASDAS staging: inactive 12 (8%), low 69 (46%), high 45 (30%), very high 24 (16%). Mean axSpA duration was 4.77±4.22 years; infliximab exposure was 3.07±2.23 years. The INF effect index averaged 1.53±0.2, indicating maintained efficacy in most patients (Table 4).

FokI (rs2228570): CC 60%, CT 40%, TT absent; allele frequencies C 80%, T 20% consistent with HWE and no genotyping bias. ApaI (rs7975232): CC 42%, CA 40%, AA 18%; allele frequencies C 62%, A 38% also HWE-consistent, indicating study genetic stability (Table 5).

FokI (rs2228570): Strong stage association (p<0.001). All inactive cases were CC (12/12). CT frequency rose with activity (low 30.4%, high 60%, very high 50%). Regression: CC → remission (OR 19.2; 95% CI 1.11-331.9; p=0.04); CT → high activity (OR 3.27; 95% CI 1.58–6.77; p=0.001). ApaI (rs7975232). No significant (p>0.05) association with ASDAS stage (Table 6). Genotype–EAMs (Tables 1-6 and 1-7): For FokI, CT carriers had lower uveitis and dactylitis (each p=0.04). Enthesitis trended lower in CT (ns). ApaI showed no significant EAM associations. Serum VDR activity and INF effect-length did not vary by genotype (Tables 7-8).

Table No. 1: Descriptive statistics of the patients

Variable	Mean±SD
Age (years)	38.82±8.88
BMI (mg/m^2)	28.92±6.45

Table No. 2: Demographic information of the patients

Variable	No.	%
Gender		
Male	108	72.0
Female	42	28.0
Family of SPA		
Yes	24	16.0
No	126	84.0
Smoking		
Yes	45	30
No	105	70.0

Table No. 3: Association between disease stages with gender, extra-articular manifestations and smoking

Variable		Disease Activity				Total	P value
		Inactive disease	Low disease activity	High disease activity	Very high activity		
Gender	Female	-	24 (34.8%)	12 (26.7%)	6 (25%)	42 (28%)	0.02*
	Male	12 (100%)	45 (65.2%)	33 (73.3%)	18 (75%)	108 (72%)	
Inflammatory back pain	No	6 (50%)	9 (13%)	18 (40%)	6 (25%)	39 26%)	0.003*
	Yes	6 (50%)	60 (87%)	27 (60%)	18 (75%)	111 (74%)	
Arthritis	No	6 (50%)	24 (34.8%)	15 (33.3%)	12 (50%)	57 (38%)	0.41
	Yes	6 (50%)	45 (65.2%)	30 (66.7%)	12 (50%)	93 (62%)	
Enthesitis	No	12 (100%)	66 (95.7%)	45 (100%)	21 (87.5%)	144 (96%)	0.055
	Yes	-	3 (4.3%)	-	3 (12.3%)	6 (4%)	
Uveitis	No	12 (100%)	57 (82.5%)	42 (93.3%)	21 (97.5%)	132 (88%)	0.10
	Yes	-	12 (17.5%)	3 (6.7%)	3 (2.5%)	18 (12%)	
Dactylitis	No	12 (100%)	57 (82.6%)	42 (93.3%)	21 (87.5%)	132 (88%)	0.13

	Yes	-	12 (17.4%)	3 (6.7%)	3 (12.5%)	18 (12%)	
Psoriasis	No	12 (100%)	66 (85.7%)	45 (100%)	24 (100%)	147 (98%)	0.19
	Yes	-	3 (14.3%)	-	-	3 (2%)	
Colitis	No	12 (100%)	63 (91.3%)	45 (100%)	24 (100%)	144 (96%)	0.02*
	Yes	-	6 (8.7%)	-	-	6 (4%)	
Smoking	No	12 (100%)	48 (69.6%)	24 (53.3%)	21 (87.5%)	105 (70%)	<0.001*
	Yes	-	21 (30.4%)	21 (45.7%)	3 (12.5%)	45 (30%)	

Table No.4: Mean duration of axiel spondylarthritis, infliximab usage and infliximab effect

Variable	**Mean±SD**	**SE**	**Range**
Duration of AxSpA	4.77±4.22	0.34	3.00-26.00
Duration of INF.	3.07±2.23	0.18	1.00-9.00
Effect of INF.	1.53±0.21	0.01	1.0-2.0

Table No.5: Vitamin D receptor gene FokI and ApaI polymorphism variant distribution

Variable		**N**	**Mean ± SD**	**SE**	**p-value**
FokI	CT	69	4.75 ± 2.13	0.25	0.92
	CC	90	5.21 ± 3.70	0.39	
ApaI	CC	60	5.16 ± 1.53	0.19	0.93
	AA	27	5.24±1.85	0.35	
	AC	60	5.08±2.98	0.36	

Table No.6: Association between VDR polymorphism variants and disease stage

Variable		**Disease Activity**				**Total**	**P value**
		Inactive disease	**Low disease activity**	**High disease activity**	**Very high activity**		
Fok I	CC	12 (100%)	48 (69.6%)	18 (40%)	12 (50%)	90 (60%)	<0.001*
	Odd	19.2 (1.11-331.9)	1.71 (0.88-3.31)	0.30 (0.14-0.63)	0.61 (0.25-1.47)		
	p	0.04	0.10	0.003	0.27		
	CT	-	21 (30.4%)	27 (60%)	12 (50%)	60 (40%)	
	Odd		0.58 (0.3-1.12)	3.27 (1.58-6.77)	1.62 (0.67-3.9)		
	p		0.10	0.001	0.27		
ApaI	AA	4 (33.3%)	11 (15.9%)	9 (20%)	3 (12.5%)	27 (18%)	0.50
	Odd	2.5 (0.64-9.0040)	0.77(0.33-1.7)	1.20(0.49-2.94)	0.57 (0.15-2.07)		
	P	0.16	0.54	0.60	0.39		
	AC	6 (50%)	29 (42%)	15 (33.3%)	10 (41.7%)	60 (40%)	
	Odd	1.55(0.44-5.07)	1.2 (0.63-2.33)	0.66 (0.31-1.3)	1.08(0.44-2.6)		
	P	0.46	0.58	0.23	0.88		
	CC	2 (16.7%)	29 (42%)	21 (46.7%)	11 (45.8%)	63 (42%)	
	Odd	0.45(0.09-2.12)	1.001(0.52-1.9)	1.13(00.69-2.48)	1.20(0.50-2.89)		
	p	0.31	0.99	0.40	0.60		

Table No.7: Association between FokI genotypes and gender/extra-articular manifestations

Variable		**Fok**		**Total**	**P value**
		CC	**CT**		
Gender	Female	24 (26.7%)	18 (30%)	42 (28%)	0.71
	Male	66 (73.3%)	42 (70%)	108 (72%)	
Inflammatory back pain	No	21 (23.3%)	18 (30%)	39 (26%)	0.41
	Yes	69 (76.7%)	42 (70%)	111 (74%)	
Arthritis	No	33 (36.7%)	24 (40%)	57 (38%)	0.73
	Yes	57 (63.3%)	36 (60%)	93 (62%)	
Enthesitis	No	84 (93.3%)	60 (100%)	144 (96%)	0.08
	Yes	6 (6.7%)	-	6 (4%)	
Uveitis	No	75 (83.3%)	57 (95%)	132 (88%)	0.04
	Yes	15 (16.7%)	3 (5%)	18 (12%)	

Dactylitis	No	75 (83.3%)	57 (95%)	132 (88%)	0.04
	Yes	15 (16.7%)	3 (5%)	18 (12%)	
Psoriasis	No	87 (96.7%)	60 (100%)	147 (98%)	0.27
	Yes	3 (3.3%)	-	3 (2%)	
Colitis	No	84 93.3%)	60 (100%)	144 (96%)	0.08
	Yes	6 (6.7%)	-	6 (4%)	

Table No.8: Association between ApaI genotypes and gender/extra-articular manifestations

Variable		Apa			Total	P value
		AA	AC	CC		
Gender	Female	5 (18.5%)	18 (30%)	19 (30.2%)	42 (28%)	0.45
	Male	22 (81.5%)	42 (70%)	44 (69.9%)	108 (72%)	
Inflammatory back pain	No	10 (37%)	21 (35%)	26 (41.3%)	57 (38%)	0.76
	Yes	17 (63%)	39 (65%)	37 (58.7%)	93 62%)(	
Arthritis	No	10 (37%)	21 (35%)	26 (41.3%)	57 (38%)	0.76
	Yes	17 (63%)	39 (65%)	37 (58.7%)	93 (62%)	
Enthesitis	No	26 (96.3%)	57 (95%)	61 (96.8%)	144 (96%)	0.87
	Yes	1 (3.7%)	3 (5%)	2 (3.2%)	6 (4%)	
Uveitis	No	24 (88.9%)	50 (83.3%)	58 (92.1%)	132 (88%)	0.32
	Yes	3 (11.1%)	10 (16.7%)	5 (7.9%)	18 (12%)	
Dactylitis	No	25 (92.6%)	49 (81.7%)	58 (92.1%)	132 (88%)	0.15
	Yes	2 (7.4%)	11 (18.3%)	5 (7.9%)	18 (12%)	
Psoriasis	No	27 (100%)	58 (96.7%)	62 (98.4%)	147 (98%)	0.44
	Yes	-	2 (3.3%)	1 (1.6%)	3 (2%)	
Colitis	No	26 (96.3%)	58 (96.7%)	60 (95.2%)	144 (96%)	0.91
	Yes	1 (3.7%)	2 (3.3%)	3 (4.8%)	6 (4%)	

DISCUSSION

In this Iraqi study, FokI - but not ApaI was clinically informative for axSpA. The absence of TT and predominance of CC/CT align with regional allele frequencies. Mechanistically, the CC isoform's higher transactivation capacity may augment anti-inflammatory signaling[11], consistent with its restriction to inactive ASDAS and strong remission odds. Conversely, CT tracked with higher axial activity yet coincided with reduced uveitis and dactylitis, suggesting tissue specific or pathway selective immunomodulation. ApaI neutrality mirrors reports with limited clinical translation for this intronic variant.[10,12]

Importantly, serum VDR activity and infliximab effect - length were genotype - independent, implying that while FokI shapes phenotype, pharmacodynamic readouts under TNF blockade remain largely driven by treatment and other non-genetic factors.[13-15] Smoking was concentrated in the high-activity ASDAS group, reinforcing its adverse role in the inflammatory tone.[2] Collectively, these results support using FokI as a prognostic phenotypic marker rather than a predictor of infliximab pharmacodynamics.

CONCLUSION

FokI (rs2228570) is a bidirectional modifier: CC associates with inactive ASDAS (remission odds ↑), while CT associates with high activity but fewer select EAMs. ApaI (rs7975232) shows no meaningful association with activity, EAMs, VDR activity, or INF effect-length. Pharmacodynamic measures under infliximab were genotype-independent.

Recommendations

- Validate findings in larger, longitudinal, multi-ethnic cohorts.
- Undertake functional studies to dissect genotype-specific immune pathways.
- Consider FokI in phenotype profiling and risk stratification; prioritize smoking-cessation strategies in axSpA care.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.44970 Dated 03.07.2024

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Original Article

Impact of Teaching Interventions on Awareness and Complications During Pregnancy in Uterine Leiomyoma Patients

Aasma Nazir, Madiha Mukhtar and Sarfraz Masih

ABSTRACT

Objective: To determine the impact of teaching interventions on awareness about uterine leiomyoma and on complication awareness and to measure the complications of uterine leiomyoma patients during pregnancy.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the Nishter Hospital and Medical University Multan and Khawaja Fareed Social Security Hospital Multan from March 2025 to June 2025.

Methods: A quasi-experimental single-group pre- and post-test design was employed and a total of 74 pregnant women with uterine leiomyomas were selected through convenience sampling. Data were collected using validated tools: a demographic questionnaire, awareness scale (17 items), complication awareness scale (12 items), and a complication checklist (15 items). Following ethical approval, participants completed a pre-test, received a structured teaching intervention comprising five sessions over ten weeks, and were re-assessed post-intervention. Data were analyzed using SPSS V25. Wilcoxon Sign Test and Fisher's Exact Test were used to analyze the data

Results: The findings of study show that among 74 participants, most were aged 36–45 years (63.5%), nearly half had high school education (48.6%), and the majority were housewives (59.5%). Awareness scores significantly improved after the teaching intervention, with median scores rising from 28 to 35 ($p<0.001$), and 97.3% showing improvement. The most frequent complications reported were cesarean delivery (74.3%), labor dystocia (64.9%), and preterm labor (41.9%), while less common complications included placenta previa (10.8%) and peripartum hysterectomy (9.5%). No significant associations were found between complications and demographic factors ($p>0.05$).

Conclusion: The study showed that awareness about uterine leiomyoma significantly improved after the teaching intervention, with 97.3% of participants gaining knowledge. Common complications included cesarean delivery, labor dystocia, and preterm labor, while no significant link was found between demographics and complication severity.

Key Words: Teaching intervention; Awareness; Complications; Pregnancy; Uterine leiomyoma

Citation of article: Nazir A, Mukhtar M, Masih S. Impact of Teaching Interventions on Awareness and Complications During Pregnancy in Uterine Leiomyoma Patients. Med Forum 2025;36(9):85-89. doi:10.60110/medforum.360916.

INTRODUCTION

Uterine leiomyomas, commonly known as fibroids, are the most prevalent benign tumors of the female genital tract and a major cause of gynecological morbidity. They are composed mainly of smooth muscle tissue and can lead to infertility, recurrent abortions, excessive uterine bleeding, pelvic pain, and pressure symptoms on nearby organs.

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Received: July, 2025
Reviewed: August, 2025
Accepted: August, 2025

Globally, fibroids affect up to 60% of women under 45 years of age, with about one-third being symptomatic[1], while in Pakistan, their prevalence ranges between 20–40%[2]. They are among the leading causes of hospital admissions for gynecological problems and the most frequent indication for hysterectomy, with over 70% of women developing fibroids by menopause[3].

Although the exact cause of uterine fibroids remains unclear, hormonal, genetic, and environmental factors play a key role. Risk factors include null parity, obesity, family history, and premenopausal age, while combined oral contraceptive use appears protective[4]. Less than half of the cases are asymptomatic, with common symptoms including abnormal uterine bleeding, pelvic pressure, urinary frequency, subfertility, and abdominal swelling[5]. Fibroids can also complicate pregnancy, leading to miscarriage, preterm labor, and obstructed vaginal delivery[6]. Diagnosis is often incidental during routine gynecological examinations or pregnancy, but can also be made through ultrasound, laparoscopy, hysterosalpingography, CT, or MRI[7].

Management strategies vary depending on age, symptoms, and reproductive desires. Options include medical therapy, uterine artery embolization, myomectomy, or hysterectomy, with hysterectomy being the most definitive treatment but associated with loss of fertility and psychosocial implications[8]. Despite being highly prevalent, awareness about fibroids and their complications remains low, leading to delayed diagnosis and treatment[9]. This lack of knowledge, coupled with the significant financial and social burden, highlights the need for patient education, early detection, and tailored treatment approaches to reduce morbidity and improve quality of life[10].

METHODS

A quasi-experimental single-group pre- and post-test design was conducted at Nishtar Hospital and Nishtar Medical University Multan and Khawaja Fareed Social Security Hospital Multan from March 2025 to June 2025. The study population included 74 pregnant women diagnosed with uterine leiomyomas, selected through convenience sampling, with inclusion and exclusion criteria strictly applied. Data were collected over six months using validated tools, including a demographic questionnaire, an adopted awareness scale[11], a researcher-developed complication awareness questionnaire, and an observation checklist[12], all tested for reliability (Cronbach's alpha ≥0.70) and content validity (CVI ≥0.92). The intervention consisted of five structured educational sessions delivered over ten weeks using interactive lectures, audiovisual aids, booklets, and group discussions. Data were collected in three phases—pre-intervention, intervention, and post-intervention—and analyzed in SPSS v.25 using descriptive and non-parametric inferential statistics (Wilcoxon Signed-Rank Test and Fisher's Exact Test), with a p-value <0.05 considered significant. Ethical approval was obtained from the University of Lahore Research Ethics Board (UOL/IREB//25/6/0004), and informed consent, confidentiality, and anonymity were ensured throughout the study.

RESULTS

Table 1 presents that most of participants were aged 36–45 years (63.5%), followed by 26–35 years (31.1%), while only 5.4% were in the 46–50 years age group. In terms of education, nearly half had completed high school (48.6%), while 32.4% had primary education, 10.8% were illiterate, and only 8.1% had education at the intermediate level or above. Regarding occupation, the majority were housewives (59.5%), followed by others (29.7%), and 10.8% were retired.

Table No.1: Demographics characteristics of participants (n=74)

Demographic Variables	Category	Frequency (f)	Percentage (%)
Age	26-35 Year	23	31.1
	36-45 Year	47	63.5
	46-50 Year	4	5.4
Education Level	Illiterate	8	10.8
	Primary school	24	32.4
	High school	36	48.6
	Intermediate & above Level	6	8.1
Occupation	Housewife	44	59.5
	Retired	8	10.8
	Others	22	29.7

n=number of participants

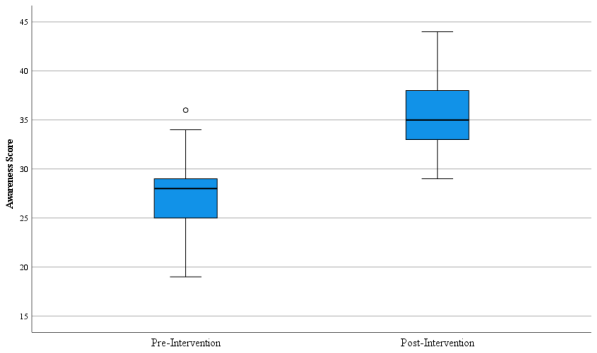

Figure No.1: Comparison of before and after intervention awareness score

Awareness score before teaching intervention was 28(IQR 25-29) and Awareness score after teaching intervention it increases to 35(IQR 33-38). Median Improvement in awareness score was Statistically significant (Wilcoxon Sign Test 7.37, p-value <0.001). Overall awareness score was decreased in 2(2.7%) and improved in 72(97.3%) of the participants.

Thus, the alternative hypothesis (H1-1), stating that teaching interventions have an impact on awareness during pregnancy in uterine leiomyoma patients, is accepted.

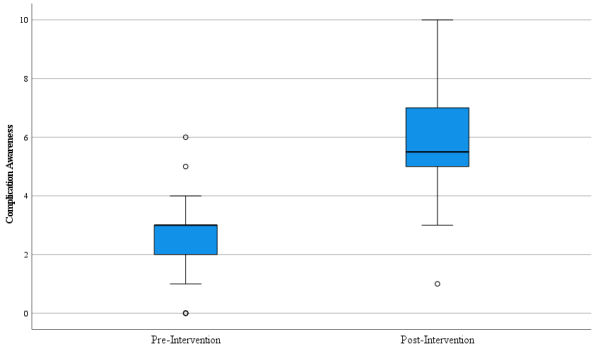

Figure No.2: Comparison of before and after intervention complication awareness score

Median complication awareness score before teaching intervention was 3(IQR 2-3) and after intervention was 5.5 (IQR 5-7). Median difference in complication awareness after intervention was statistically significantly improved from before intervention score (Wilcoxon Sign Test 7.16, p-value <0.001)

Overall complication awareness score was decreased from pre intervention in 5(6.8%) of the participants and no difference was observed in 2(2.7%) and improved score was observed in 67(90.5%) of the participants.

Thus, the alternative hypothesis (H1-2), stating that teaching interventions have an impact on complication awareness during pregnancy in uterine leiomyoma patients, is accepted.

Table No.2: Comparison of complications among patients with uterine leiomyoma (n=74)

Complications	No f (%)	Yes f (%)
Cesarean delivery	19(25.7)	55(74.3)
Malpresentation	39(52.7)	35(47.3)
Labor dystocia	26(35.1)	48(64.9)
Postpartum hemorrhage	48(64.9)	26(35.1)
Per partum hysterectomy	67(90.5)	7(9.5)
Retained placenta	74(100.0)	0(0)
Chorio or endometriosis	74(100.0)	0(0)
IUGR (Intrauterine growth restriction)	62(83.8)	12 (16.2)
Preterm labor	43(58.1)	31(41.9)
Preterm delivery	47(63.5)	27(36.5)
Placenta previa	66(89.2)	8(10.8)
First-trimester bleeding	53(71.6)	21(28.4)
Abruption	62(83.8)	12(16.2)
PPROM (Preterm premature rupture of the membranes)	59(79.7)	15(20.3)

n= number of participant, f=frequency of participants, %= percentage of participants

Table 4.5 shows that most common complication reported was cesarean delivery (74.3%), followed by labor dystocia (64.9%) and preterm labor (41.9%). Other frequent complications included preterm delivery (36.5%), postpartum hemorrhage (35.1%), and first-trimester bleeding (28.4%). Less common complications were intrauterine growth restriction (16.2%), placental abruption (16.2%), and preterm premature rupture of membranes (20.3%). Rare complications such as placenta previa (10.8%) and peripartum hysterectomy (9.5%) were also noted. None of the participants experienced retained placenta or chorio/endometritis.

DISCUSSION

The current study demonstrated a significant improvement in awareness scores after teaching interventions, with median scores increasing from 28 (IQR 25–29) to 35 (IQR 33–38). Almost all participants showed improvement, indicating the effectiveness of structured teaching interventions in enhancing knowledge among uterine leiomyoma patients.[13]

These findings align with recent literature highlighting the role of educational interventions in improving awareness among women with gynecological conditions. A study by Ahmed et al. (2023)[2] found that structured health education programs significantly enhanced patients' understanding of uterine fibroids and their associated complications. Similarly, a systematic review concluded that patient-centered educational interventions were effective in increasing disease-related knowledge and reducing misconceptions, which supports the outcomes of the current study.[14] Another recent study also emphasized that nurse-led educational strategies significantly improved awareness levels and treatment adherence in women with reproductive health issues.

The findings of the current study revealed a significant improvement in complication awareness scores following teaching interventions. These findings highlight the effectiveness of structured educational interventions in enhancing women's knowledge about complications associated with uterine leiomyomas during pregnancy.

Recent evidence supports the effectiveness of nurse-led and structured teaching interventions in improving maternal awareness and risk perception regarding pregnancy complications. For instance, researcher reported that health education interventions significantly enhanced pregnant women's understanding of potential obstetric complications, leading to better preparedness and timely healthcare-seeking behavior.[15] Similarly, another researcher found that tailored patient education programs improved awareness and early recognition of complications among women with high-risk pregnancies. These outcomes align with the current study, reinforcing that education is an essential strategy to mitigate risks in vulnerable populations.[16]

This study evaluated complications among pregnant patients with uterine leiomyomas (n = 74) following a teaching intervention aimed at improving awareness about pregnancy-related risks. The most frequent adverse outcomes were cesarean delivery (74.3%), labor dystocia (64.9%), and preterm labor (41.9%), with additional burdens from preterm delivery (36.5%), postpartum hemorrhage (PPH; 35.1%), and first-trimester bleeding (28.4%). Less common but notable events included

PPROM (20.3%), placenta previa (10.8%), abruption (16.2%), and IUGR (16.2%). Peripartum hysterectomy occurred in 9.5%, while no cases of retained placenta or chorio/endometritis were recorded.

The very high cesarean rate (74.3%) aligns with contemporary evidence that fibroids substantially increase the likelihood of operative delivery. A meta-analysis of 24 studies involving 237,509 pregnancies reported significantly higher odds of cesarean delivery among women with fibroids, as well as increased risks of malpresentation and labor abnormalities[17]. Your malpresentation rate (47.3%) and labor dystocia (64.9%) are directionally consistent with this pooled evidence, although the absolute percentages are higher than most pooled estimates—likely reflecting case-mix, fibroid size, and tertiary-center referral bias. Similar findings were reported in recent case series, which documented higher rates of malpresentation and intrapartum complications among women with uterine fibroids[18].

Preterm labor (41.9%) and preterm delivery (36.5%) were elevated compared with the general obstetric population and lie at the higher end of reported ranges in fibroid-affected pregnancies. A systematic review concluded that fibroids increase risks of preterm birth and premature rupture of membranes[19].

The PPH rate (35.1%) is consistent with global evidence that fibroids impair uterine contractility and predispose women to hemorrhage. Placental complications were also notable: placenta previa (10.8%) and abruption (16.2%), higher than baseline rates. These findings are in line with evidence linking fibroids with increased risks of previa and abruption, particularly when lesions are large or lower-segment[20].

Current evidence emphasizes the importance of structured counseling and shared decision-making in fibroid-affected pregnancies[18].

CONCLUSION

The findings of this study reveal that the majority of participants were women aged 36–45 years, with most being housewives and nearly half having completed high school education. Awareness about uterine leiomyoma significantly improved after the teaching intervention, with 97.3% showing increased knowledge. Cesarean delivery, labor dystocia, and preterm labor were identified as the most frequent complications, while rare complications included placenta previa and peripartum hysterectomy.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Aasma Nazir, Madiha Mukhtar
Drafting or Revising Critically:	Aasma Nazir, Sarfraz Masih
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.UOL/IREB/25/06/004 Dated 17.03.2025

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Original Article

Urban–Rural Disparities in Early Breastfeeding Initiation: A Cross-Sectional Study in Pakistan

Abdul Mannan Mustafa[1], Muhammad Haroon ul Rasheed[2], Ahmed Bilal[2], Rabia Rahat[2], Usama Yaseen[2] and Ghulam Mustafa[1]

ABSTRACT

Objective: To examine breastfeeding patterns among lactating mothers using urban-rural comparison techniques to identify geographical variations and influencing factors.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the Pediatrics department, Nishtar Hospital, Multan, from April 2024 to March 2025.

Methods: Data were collected through structured questionnaires administered to a purposively selected sample of 258 lactating mothers across urban and rural settings. The independent variables included maternal age, residence (urban/rural), child's age, and maternal education level. The dependent variables included the early initiation of breastfeeding.

Results: The overall breastfeeding initiation rate of 16.7% (n = 43) among participants. (Figure. I). Urban mothers' breastfed at a significantly lower rate (13.5%, 27/200) compared to rural mothers (27.6%, 16/58). A chi-square test confirmed a statistically significant association between residence and breastfeeding ($\chi^2(1) = 6.42$, $p = 0.011$), indicating rural mothers were more likely to breastfeed than urban mothers.

Conclusion: Findings of this study reveal lower breastfeeding rates among urban mothers compared to rural counterparts, consistent with global trends. The findings underscore the need for targeted interventions, such as workplace breastfeeding policies in urban areas and community education programs in rural regions.

Key Words: Breastfeeding, Lactating mothers, Rural, Urban, Pattern

Citation of article: Mustafa AM, Rasheed MH, Bilal A, Rahat R, Yaseen U, Mustafa G. Urban–Rural Disparities in Early Breastfeeding Initiation: A Cross-Sectional Study in Pakistan. Med Forum 2025;36(9): 90-93. doi:10.60110/medforum.360917.

INTRODUCTION

Breast milk is an important aspect of infant well-being, as it provides essential nutrients and immunity that nourish development and growth. Despite the widely well-documented benefits of the process, breastfeeding patterns vary across different regions of the globe, which is influenced by cultural, socioeconomic, and environmental factors[1]. It is also significant to understand such disparities to develop targeted measures that increase the rate of breastfeeding and other positive outcomes for mother-baby health[2].

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Received: April, 2025
Reviewed: May, 2025
Accepted: June, 2025

Geographic or urban–rural variation of breastfeeding patterns can indicate the presence of geographic differences and identify areas where the recommended practice is not being followed as much[3].

Breastfeeding varies widely throughout the world, with people in a given region practicing it extensively while others face the challenges of early weaning or the use of supplements with formula[4]. The causes of these inequalities can be attributed to the lack of affordable medical care, the underrepresentation of women in matriculated education, occupational guidelines, and cultural customs[5].

Geographic mapping of these differences can be utilized to identify where clusters of such issues are located and where the implementation of breastfeeding support programs can be most effectively targeted[6]. Through visualization of the breastfeeding percentages, policymakers will be able to allocate their resources more effectively to disadvantaged groups. Such a strategy will make the interventions data-driven, local, and tailored to local needs[7]. The rate of breastfeeding in several low- and middle-income nations is also low, as the population does not avoid poverty, the lack of lactation support, and the aggressive advertising of breast milk substitutes[8]. On the contrary, the barriers to breastfeeding in high-income countries can be various,

with such items as short maternity leave and social disapproval of breastfeeding in the streets[9]. In comparing these divergent conditions, a geographic perspective helps determine the structural and cultural factors influencing breastfeeding practices. It is also possible to monitor e and assess the effectiveness of public health campaigns or policy reforms. Such understanding is important in devising measures that can solve region-specific problems. In the end, however, improving the breastfeeding rate requires a complex solution that considers the spatial, cultural, and socioeconomic aspects[10].

This study aims to identify geographic disparities in breastfeeding practices among mothers to guide us regarding targeted maternal health policies. The results inform the public, and resources could be allocated to mothers who need them the most. By targeting geographical variation in breastfeeding customs, policymakers can improve nutrition and decrease health disparities. This study will add value to international endeavors to ensure optimal breastfeeding, utilizing evidence-based and location-specific solutions.

METHODS

The study employed a cross-sectional design conducted at Pediatrics department, Nishtar Hospital, Multan, from April 2024 to March 2025. Data were collected using structured questionnaires from the 258 lactating mothers from both urban and rural areas, selected through a combination of stratified and purposive sampling. Ethical approval was obtained from the hospital review board, and informed consent was secured from all participants before data collection. Trained enumerators conducted face-to-face interviews to ensure accuracy and minimize recall bias. The collected data were anonymized and stored securely, with access restricted to the research team to protect participant confidentiality.

The target population consisted of lactating mothers with infants aged 0–24 months, with inclusion criteria requiring mothers to be currently breastfeeding, willing to participate, and having infants within the specified age range. Mothers with medical conditions preventing breastfeeding or infants with congenital disorders affecting feeding were excluded from the study. Stratified random sampling was used to ensure representation from different socioeconomic backgrounds, though convenience sampling was applied in areas where random selection proved challenging.

The independent variables examined were residence (rural/urban), maternal age, age of child, and education of mothers. The dependent variables included the early initiation of breastfeeding. Quantitative analysis involved descriptive statistics such as means and percentages, while the Chi-square test was used to compare breastfeeding patterns between rural and urban mothers.

RESULTS

The study included 258 participants, with 46.5% of mothers aged ≤30 years and 53.5% >30 years. Majority of the child, 54.3% were between 2-6 months of age. Among the children, the majority were female (58.9%), while males were at 41.1%.In terms of literacy, maternal education varied, with 37.2% completing 1–5 classes and 19.8% completing 9–10 classes; only 6.6% were graduates. The majority of the participants resided in urban areas (77.5%), with 22.5% in rural regions. (Table. I).

Table No.1: Demographic profile of the study participants

Variable	Category	N	(%)
Mother age (years)	≤30 years	120	46.5
	>30 years	138	535
Child age (months)	≤1 months	57	22.1
	2-6 months	140	54.3
	6-12 months	61	23.6
Gender of the child	Male	106	41.1
	Female	152	58.9
Mother's education	Illiterate	24	9.3
	1-5 class	96	37.2
	6-8 class	45	17.4
	9-10 class	51	19.8
	11-12 class	18	7.0
	Graduation	17	6.6
	MBBS	3	1.2
	Masters	4	1.6
Area of residence	Urban	200	77.5
	Rural	58	22.5

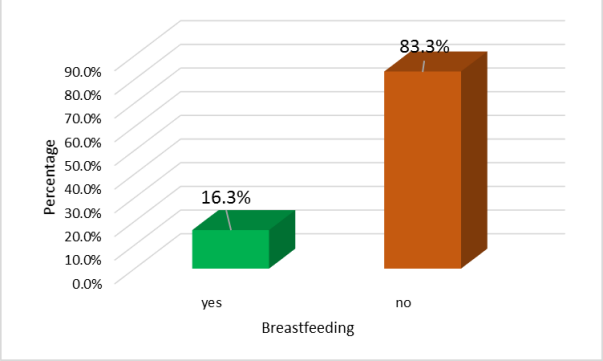

Figure No.I: Prevalence of breastfeeding among the study participants

The overall breastfeeding rate was 16.7% (n = 43) among participants. (Figure. I). Urban mothers breastfed to their children at a significantly lower rate (13.5%, 27/200) compared to rural mothers (27.6%, 16/58). A chi-square test confirmed a statistically significant association between residence and

breastfeeding ($\chi^2(1) = 6.42$, $p = 0.011$), indicating rural mothers were more likely to breastfeed than urban mothers. (Table. 2).

Table No.2: Association of breastfeeding with mothers' area of residence

Area of residence of mothers	Breastfeeding		Test of sig.
	Yes 43 (16.7%)	No 215 (83.3%)	
Urban	27 (62.8)	173 (80.5)	χ^2=6.42, d.f=1,p=0.011
Rural	16 (37.2)	42 (19.5)	
Total	43 (100.0)	215 (100.0)	

DISCUSSION

The present study analyzed breastfeeding patterns among lactating mothers, revealing an overall breastfeeding rate of 16.7%, with significant differences between urban (13.5%) and rural (27.6%) mothers. These findings align with and diverge from previous research on breastfeeding practices, highlighting the influence of sociodemographic and geographical factors.

The low overall breastfeeding rate (16.7%) in this study contrasts with higher rates reported in other regions. For instance, a study in Ethiopia conducted by Tewabe et al[11] found an exclusive breastfeeding rate of 83% among infants under six months, attributed to strong cultural practices and health education programs. Similarly, a Nigerian study conducted by Ogunlesi et al[12] reported a 55% exclusive breastfeeding rate, emphasizing the role of maternal education and healthcare support. The disparity may stem from variations in study populations, as our study included older children (45.3% ≥5 years), whereas others focused on infants.

This study examined breastfeeding patterns among 258 lactating mothers, revealing that 46.5% were aged 30 years or younger, while 53.5% were over 30 years old. The findings align with previous research suggesting that older mothers are more likely to breastfeed longer, possibly due to greater experience and confidence, as seen in a study by Senarath et al[13]. The children were mostly 5 months or older (45.3%), followed by those aged 1 month to 1 year (18.2%), with a majority being female (58.9%). The high proportion of children aged 5 months or older still being breastfed (45.3%) contrasts with global trends where exclusive breastfeeding typically declines after six months, as reported by the WHO (2021)[14]. Haider et al[15] reported that this extended breastfeeding may reflect cultural practices or delayed weaning, or a potential recall bias similar to observations in South Asian studies.

In this study, maternal education levels varied, with 37.2% having completed 1–5 classes, 19.8% completing 9–10 classes, and only 6.6% being graduates. Interestingly, the low percentage of graduate mothers (6.6%) contrasts with studies linking higher education to prolonged breastfeeding, such as Victora et al[16]. However, in some traditional societies, less-educated mothers may rely more on cultural norms that support breastfeeding, as noted by Bhandari et al[17]. Additionally, the predominance of urban mothers (77.5%) in this study differs from research conducted by Hector et al[18]suggesting higher breastfeeding rates in rural areas due to traditional practices. While urbanization often leads to increased formula use, this study suggests urban mothers may still adhere to breastfeeding norms, possibly due to targeted health interventions.

Our findings indicate that rural mothers breastfeed at significantly higher rates (27.6%) than urban mothers (13.5%), consistent with global trends. A study in India conducted by Patel et al[19] found that rural women were 1.5 times more likely to breastfeed exclusively due to traditional practices and limited access to formula milk. Conversely, urban mothers often face work-related barriers, such as employment demands and lack of breastfeeding-friendly workplaces, reducing breastfeeding duration[20]. This aligns with our results, where urban residency was negatively associated with breastfeeding ($\chi^2(1) = 6.42$, $p = 0.011$).

CONCLUSION

Findings of this study reveals lower breastfeeding rates among urban mothers compared to rural counterparts, consistent with global trends. The findings underscore the need for targeted interventions, such as workplace breastfeeding policies in urban areas and community education programs in rural regions. Future studies can highlight the potential cultural, social, and other barriers behind these trends, and help design newer interventions.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.106/2024 Dated 09.03.2024

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Original Article

Epidemiological Insights into Hemoglobinopathies in Basrah, Southern of Iraq

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ABSTRACT

Objective: (1) To elicit evidence of new case detection in Basrah regarding hemoglobinopathy. (2) To study certain characteristics of the newly diagnosed cases. (3) To study characteristics of the newly registered patients in context of the premarital screening program issued since 6 years (4) To detect if the evidence of hemoglobinopathy within the families acts as a deterrent for the families from having further effected children.

Study Design: Retrospective, single-center analysis

Place and Duration of Study: This study was conducted at the Basrah Center for Hereditary Blood Diseases, Basrah Iraq from 1st January 2024 to 31st December 2024.

Methods: This retrospective, single-center analysis was conducted at Basrah Center for Hereditary Blood Diseases, Basrah Iraq vide letter No.18 dated 5th April 2023 and 508 individuals diagnosed with hemoglobinopathies were enrolled.

Results: Sickle cell anemia and sickle β-thalassemia accounted for 31.3% and 34.8% of cases, respectively. Gender distribution was nearly equal across all types, reflecting the autosomal recessive inheritance pattern. Consanguineous marriage was reported in 62% of cases, with the highest familial relation seen among sickle cell anemia patients. Familial clustering was evident, with 144 patients having at least one affected sibling. Educational attainment was low, with 45.5% of patients being illiterate and only 5.1% holding a college degree.

Conclusion: The strong influence of genetic, socio-cultural, and educational factors on hemoglobinopathy prevalence. The high rates of familial clustering and consanguinity highlight the urgent need for enhanced public health interventions, including premarital screening, genetic counseling and community education.

Key Words: Hemoglobinopathies, Epidemiology, Premarital Screening

Citation of article: Ahijaj BAA, Jaber RZ, Radhi AM. Epidemiological Insights into Hemoglobinopathies in Basrah, Southern of Iraq. Med Forum 2025;36(9):94-99. doi:10.60110/medforum.360918.

INTRODUCTION

The most prevalent single gene illnesses in the world are known as hemoglobinopathies, or disorders of hemoglobin.[1,2] Two primary classifications exist for hemoglobinopathies: thalassemia syndromes, and sickle cell diseases which include 2 main variants, homozygous sickle cell anemia and heterozygous sickle cell disease which may include many variants like S/β°, S/β+, S/D.[3]

The World Health Organization estimates that hemoglobinopathies affect about 5% of the human population and that 300,000–400,000 infants are born each year with extremely dangerous hemoglobinopathies.[4,5]

In general, one of the last recent studies indicates that the prevalence of thalassemia, through the data within 16 thalassemia centers in Iraq, was 37.1 per 100,000 in 2015. Between 2010 and December 2015, 11,165 cases of thalassemia were diagnosed, accounting for 66.3% of all hereditary anemia cases that were registered at these facilities. Which consider a significant rate with the need of attention and awareness to this hereditary disease and its burdens on our community.[5]

On the governorates distribution, a lot of studies had been held to elicit the prevalence within their population although separately. For instance, a study in the thalassemia center of Al-Najaf governorate, and by using the collected data of 1,122 patients from October 2019 to March 2020, it concluded that beta thalassemia is the number one diagnosed condition among the hereditary hematologic disorders and accounting for 33.15% of the patients registered in the center in duration of the study. In comparison, SCD account only for 9%.[6]

Another study in Erbil, northern Iraq, also found that beta thalassemia is the dominant condition among the hemoglobinopathies within their population, with a percentage as high as 78.71%. While the SCD account only for 6.23%. Overall, the peak age was between 6-15

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Received: February, 2025
Reviewed: March-April, 2025
Accepted: May, 2025

years account for 44.45%, following by 23.20% for the age group from 1-5 years. The 6-year study showed a steadily increase with all the hemoglobinopathies cases each year.[7]

The sickle-cell disease and thalassemia major from Iraq in the early 1960s, these hereditary disorders gained national recognition as serious health issues, leading to the establishment of centers for their management across the nation.[8]

In Basra Governorate, Southern of Iraq, whilst that the carrier state for both thalassemia (4.6%) and the frequencies of sickle cell trait prevalence vary greatly in different regions of Iraq (0.06–0.07%) in the North whilst it is in its extreme maximization (6.5%) in Basrah.[9]

The time trend of the newly registered patients in Basrah did show an accelerated increment in 2019, with the registration of about 606 new patients differing slightly from that of thalassemia. Iraqi trend for the last five years was published in an Iraqi study in which a steady increase did exist.[10,11] Again the most predominant newly registered were male gender, sickle cell diseases, patients from peripheries and below five years age groups.

METHODS

This retrospective study that had been conducted depending on the patient's records and center data bases had been obtained from the BCHBD after a written approval, data had been processed as numbers and percentages for obtaining the study objectives in highlighting the current situation for hemoglobinopathy in Basrah governorate beside the new patient registry characteristics. All hemoglobinopathy cases registered from January 2024 till end of December 2024 in Basrah center for hereditary blood diseases, hereditary bleeding disorders, query hereditary natures and odd diagnosis had been excluded. The data was entered and analyzed through SPSS-25.

RESULTS

There were 51.38% females and 48.62% males. Sickle thalassemia is the most common condition, accounting for 34.84% of all cases (n=177), with nearly equal distribution between females (17.52%) and males (17.32%). Sickle cell anemia follows closely with 31.30% of cases (n=159), also showing a slight female predominance (16.93%) compared to males (14.37%) These findings suggest that sickle-related disorders are highly prevalent in the studied group and affect both sexes similarly (Table 1).

62.20% of new cases (316 out of 508) occurred in individuals with consanguineous (relative) parents, while 37.80% (192) were from non-consanguineous unions. Sickle cell anemia follows with 31% (n=159) also showing a high number of cases among relatives (107 vs. 52) which suggests a notable genetic component possibly amplified by consanguinity (Table 2).

This raise a fact that 40.75% of the new registered were from an effected families with a complete knowledge of the disease inheritance in their families underscoring the hereditary nature of hemoglobinopathies. Sickle thalassemia (34.84%). The highest number of familial cases: 57 patients (11.22%) had one affected sibling, and 24 patients (4.72%) had two. A small but notable number had three or four affected siblings, indicating strong familial clustering in some households. Similarly, 44 patients (8.66%) had one affected sibling, and smaller percentages had two or more affected siblings. Still, a significant portion (19.69%) had no other affected siblings, suggesting it can also occur in families without known history. 59.25% (n=301) of the registered patients had no affected siblings, suggesting either sporadic inheritance or that the patient is the first diagnosed in the family.28.35% (n=144) had one affected sibling, while a smaller proportion had two (8.66%), three (2.36%), or four (1.38%) affected siblings. This raise a fact that 40.75% of the new registered were from an effected families with a complete knowledge of the disease inheritance in their families underscoring the hereditary nature of hemoglobinopathies. Sickle thalassemia (34.84% of total cases). The highest number of familial cases: 57 patients (11.22%) had one affected sibling, and 24 patients (4.72%) had two. A small but notable number had three or four affected siblings, indicating strong familial clustering in some households. Sickle cell anemia (31.30%) similarly, 44 patients (8.66%) had one affected sibling, and smaller percentages had two or more affected siblings. Still, a significant portion (19.69%) had no other affected siblings, suggesting it can also occur in families without known history. Thalassemia's (α and β types) β-thalassemia major and intermedia mostly appear in families with no affected siblings (5.12% and 3.74%, respectively). However, 6–8 patients per type had one or more affected siblings, reflecting known autosomal recessive inheritance. Fanconi anemia (1.38%), despite being rare, more than half of the cases had affected siblings (4 out of 7), suggesting strong genetic clustering in families with this condition. Nearly 41% of patients had at least one affected sibling, confirming the significant genetic and familial aspect of hemoglobinopathies (Table 3).

Illiteracy is the most dominant category, accounting for 45.47% (n=231) of patients, followed by primary education at 36.61% (n=186).Only 4.13% (n=21) of patients are college graduates, and 0.98% (n=5) institute graduates (Table 4).

70.67% of patients (n=359) are from peripheral areas, while only 29.33% (n=149) are from urban centers. This indicates a notably higher burden of hemoglobinopathies in peripheral areas, possibly due to

a combination of genetic, socioeconomic, and healthcare access factors. The significant concentration of cases in peripheral regions suggests potential factors (Table 5)

The highest proportion of patients (32.28%) was under 1 year old, indicating early onset and possibly neonatal screening or early symptom presentation. Children aged 1-5 years (22.83%) and 6-15 years (13.78%) make up the next largest groups, suggesting that early childhood remains the most common age for detection These disorders also show a small proportion of late diagnoses, with 7-9 patients over age 50 possibly due to milder forms or limited access to diagnosis in earlier life. No cases registered in individuals over 16 years, which aligns with early manifestation of physical or hematological symptoms (Table 6).

Sickle-related disorders (sickle cell anemia and sickle thalassemia) were the most frequent, together comprising over 66% of cases. Blood groups O+ and A+ were the most common among patients across all diagnoses (Table 7).

Table No.1: Categories of hemoglobinopathies towards sex

Type of hemoglobinopathy	Male	%	Female	%	Total	%
β. thalassemia major	19	3.74	15	2.95	34	6.69
β. thalassemia intermedia	16	3.15	17	3.35	33	6.50
α thalassemiaα	32	6.30	33	6.50	65	12.80
Sickle cell anemia	73	14.37	86	16.93	159	31.30
Sickle thalassemia	88	17.32	89	17.52	177	34.84
Fanconi anemia	3	0.59	4	0.79	7	1.38
Others	16	3.15	17	3.35	33	6.50
Sum	247	48.62	261	51.38	508	100.00

Table No.2: Statistics on new registered patients according to kinship

Type of hemoglobinopathy	Relatives	%	Not relatives	%	Total	%
β. thalassemia major	28	7.0	6	7.0	34	7.0
β. thalassemia intermedia	22	6.0	11	6.0	33	6.0
α thalassemiaα	43	13.0	22	13.0	65	13.0
Sickle cell anemia	107	31.0	52	31.0	159	31.0
Sickle thalassemia	93	35.0	84	35.0	177	35.0
Fanconi anemia	6	1.0	1	1.0	7	1.0
Others	17	6.0	16	6.0	33	6.0
Sum	316	100.0	192	100.0	508	100.0

Table No.3: Newly registered patients according to the number of affected saplings in the family

Type of hemoglobinopathy	Effected spilling	%	Effected spilling	%	Effected saplings	%	Effected saplings	%	Effected saplings	%	Total	%
β. thalassemia major	6	1.18	2	0.39	-	-	-	-	26	5.12	34	6.69
β. thalassemia intermedia	8	1.57	4	0.79	1	0.20	1	0.20	19	3.74	33	6.50
α thalassemiaα	10	1.97	2	0.39	-	-	-	-	53	10.43	65	12.80
Sickle cell anemia	44	8.66	11	2.17	3	0.59	1	0.20	100	19.69	159	31.30
Sickle thalassemia	57	11.22	24	4.72	8	1.57	5	0.98	83	16.34	177	34.84
Fanconi anemia	4	0.79	-	-	-	-	-	-	3	0.59	7	1.38
Others	15	2.95	1	0.20	-	-	-	-	17	3.35	33	6.50
Sum	144	28.35	44	8.66	12	2.36	7	1.38	301	59.25	508	100.0

Table No.4: Statistics on newly registered patients according to educational attainment

Type of hemo-globinopathy	Illiterate	%	Primary	%	Secondary	%	Preliminary	%	Institute raduate	%	College aduate	%	Total	%
β. thalassemia major	20	3.94	11	2.17	2	0.39	1	0.20	-	-	-	-	34	6.69
β. thalassemia intermedia	25	4.92	8	1.57	-	-	-	-	-	-	-	-	33	6.50
α thalassemiaα	21	4.13	30	5.91	11	2.17	2	0.39	-	-	1	0.20	65	12.80
Sickle cell anemia	68	13.39	53	10.43	20	3.94	6	1.18	2	0.39	10	1.97	159	31.30

Sickle thalassemia	74	14.57	69	13.58	14	2.76	8	1.57	3	0.59	9	1.77	177	34.84
Fanconi anemia	5	0.98	2	0.39	-	-	-	-	-	-	-	-	7	1.38
Others	18	3.54	13	2.56	1	0.20	-	-	-	-	1	0.20	33	6.50
Sum	231	45.47	186	36.61	48	9.45	17	3.35	5	0.98	21	4.13	508	100.0

Table No. 5: Statistics on newly registered patients by residential area

Type of hemoglobinopathy	Centre	%	Periphery	%	Total	%
β. thalassemia major	8	1.57	26	5.12	34	6.69
β. thalassemia intermedia	12	2.36	21	4.13	33	6.50
α thalassemiaα	24	4.72	41	8.07	65	12.80
Sickle cell anemia	41	8.07	118	23.23	159	31.30
Sickle thalassemia	55	10.83	122	24.02	177	34.84
Fanconi anemia	1	0.20	6	1.18	7	1.38
Others	8	1.57	25	4.92	33	6.50
Sum	149	29.33	359	70.67	508	100.0

Table No. 6: Distribution of various hemoglobinopathies based on age group

Type	<1 year	%	5 years	%	15 years	%	25 years	%	50 years	%	>50 years	%	Total	%
β. Thalassemia major	7	1.38	15	2.95	2	0.39	3	0.59	5	0.98	2	0.39	34	6.69
β.thalassemia intermedia	22	4.33	8	1.57	-	-		-	1	0.20	2	0.39	33	6.50
α thalassemiaα	1	0.20	17	3.35	18	3.54	11	2.17	16	3.15	2	0.39	65	12.80
Sickle cell anemia	-	-	58	11.42	40	7.87	20	3.94	34	6.69	7	1.38	159	31.30
Sickle thalassemia	4	0.79	56	11.02	41	8.07	29	5.71	38	7.48	9	1.77	177	34.84
Fanconi anemia	-	-	2	0.39	5	0.98	-	-	-	-	-	-	7	1.38
others	-	-	8	1.57	10	1.97	7	1.38	5	0.98	3	0.59	33	6.50
Total	34	6.69	164	32.28	116	22.83	70	13.78	99	19.49	25	4.92	508	100.00

Table No.7: Blood group distribution among new hemoglobinopathy cases

Diagnosis	O^-	O^+	AB^-	AB^+	B^-	B^+	A^-	A^+	Total
β-thalassemia major	2 (0.4%)	14 (2.8%)	1 (0.2%)	6 (1.2%)	1 (0.2%)	9 (1.8%)	1 (0.2%)	3 (0.6%)	37 (7.3%)
β-thalassemia intermedia	1 (0.2%)	14 (2.8%)	1 (0.2%)	1 (0.2%)	1 (0.2%)	4 (0.8%)	1 (0.2%)	11 (2.2%)	34 (6.7%)
α-thalassemia	2 (0.4%)	20 (3.9%)	1 (0.2%)	3 (0.6%)	1 (0.2%)	18 (3.5%)	2 (0.4%)	19 (3.7%)	66 (13.0%)
Sickle cell anemia	9 (1.8%)	54 (10.6%)	2 (0.4%)	10 (2.0%)	6 (1.2%)	33 (6.5%)	3 (0.6%)	42 (8.3%)	159 (31.3%)
Sickle thalassemia	12 (2.4%)	71 (14.0%)	1 (0.2%)	10 (2.0%)	1 (0.2%)	41 (8.1%)	1 (0.2%)	41 (8.1%)	178 (35.0%)
Fanconi anemia	2 (0.4%)	1 (0.2%)	-	1 (0.2%)	-	1 (0.2%)	-	2 (0.4%)	7 (1.4%)
Others	1 (0.2%)	9 (1.8%)	1 (0.2%)	4 (0.8%)	1 (0.2%)	7 (1.4%)	1 (0.2%)	11 (2.2%)	35 (6.9%)
Total	29 (5.7%)	175 (34.4%)	7 (1.4%)	35 (6.9%)	11 (2.2%)	113 (22.2%)	9 (1.8%)	129 (25.4%)	508 (100.0%)

DISCUSSION

Sickle cell anemia and sickle β-thalassemia accounted for the largest proportion of cases (31.3% and 34.8%, respectively). This high prevalence is in line with studies from the Middle East and North Africa (MENA) region, where sickle cell disorders are endemic due to historical malaria exposure and high consanguinity rates as in WHO publications, Bahrain[12] and Saudi Arabia.[13] Gender distribution across types was almost balanced, reflecting the autosomal recessive nature of these conditions. This is consistent with studies from Iran and India that observed similar gender ratios like what found in studies done in Iran, Pakistan and West Bengal.[14]

Kinship analysis showed that 62% of new cases came from consanguineous marriages, with the highest familial relation observed among sickle cell anemia (107 out of 159 cases). This supports extensive literature showing that consanguinity remains a major risk factor for autosomal recessive disorders like hemoglobinopathies in regions with traditional intra-familial marriage patterns a similar results found by Tadmouri et al[15] and Denic et al.[16]

This study showed that 144 patients had at least one affected sibling, indicating strong familial clustering. This pattern is frequently documented in hemoglobinopathy registries and reinforces the need for family-based screening and genetic counseling programs a thing elicited also by Cousens et al[17] and Grosse et al.[18]

There is an inverse relationship between educational level and disease prevalence: 231 patients (45.5%) were illiterate, while only 26 (5.1%) had college or institute degrees. Low health literacy has been previously associated with poor awareness about premarital screening and genetic counseling services in Saudi Arabia and Iran.[19] This underscores the importance of targeted awareness campaigns, especially in rural or underserved areas.

Approximately 29.3% of the registered patients were from rural areas. These findings are similar to those from regional surveys where rural residents have higher disease burden due to limited access to diagnostic services and genetic counseling a corresponding results found by Weatherall.[20]

The clustering of cases in consanguineous families with low education levels emphasizes the urgent need to integrate nationwide prevention programs. Premarital screening, early genetic counseling, and newborn screening have been successful in reducing incidence in countries like Cyprus, Iran and Saudi Arabia.[21]

Contrasting findings were reported in a study from India, where B+ was the most common blood group among β-thalassemia patients, followed by O+ and A+, a distribution potentially influenced by regional genetic variation and population structure.[22]

CONCLUSION

Hemoglobinopathies in the region are shaped by genetic, cultural, and socio-economic factors. A significant proportion of new registrants came from families with previously registered cases, reflecting a pattern of cognitive impairment among patients' families about the possibility of recurrence. Continued implementation of education-based and community-driven screening programs is crucial to reduce the incidence and burden of these diseases.

Author's Contribution:

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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.

Source of Funding: None

Ethical Approval: No.18 Dated 05.04.2023

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