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Editorial

Sleep Deprivation – A Serious Public Health Issue

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

Introduction

Sleep deprivation is a serious public health issue that harms both mental and physical health by impairing brain function, emotional regulation, and metabolic processes.

Sleep deprivation is when a person doesn't get enough sleep. This can be a short-term issue, affecting one or a few nights, or it can be a chronic concern that lasts weeks or even months. Sleep deprivation can happen for countless reasons, many of them harmless, but it's also a key symptom of certain health conditions.

Lack of sleep reduces activity in the prefrontal cortex and thalamus, leading to slow reaction times and poor concentration. It disrupts memory consolidation and makes learning new information difficult. Over activity in the amygdala paired with poor prefrontal cortex function causes high irritability and anxiety.

Chronic sleep loss is linked to high blood pressure, stroke, and heart disease. Insufficient rest raises the risk of obesity and type 2 diabetes by disrupting hunger hormones and blood sugar regulation. It increases stress hormones like cortisol and lowers the body's ability to repair itself.

The prevalence of habitual sleep loss has become a major concern today due to night shifts and other work. The consequences of lack of sleep may not have an immediate impact on health, but chronic sleep loss seems to have adverse effects on a person's well-being.[1,2] Several studies have recorded the impact of reduced sleep time on the cardiovascular system, the metabolic system, and even depression and anxiety levels.[3,4] The recommended amount of sleep for adults (18-65 years) should ideally be 7-9 h per day, while older adults (65+ years) should aim for 7-8 h of sleep per day. The amount of sleep required varies with age, with children and adolescents needing more sleep than adults, usually over 8 hours per day.[5] Adults and the elderly, who often sleep less than 7 h, are at increased risk of health issues[6]; however, both sleeping too little (less than 6 h) or too much (more than 9-10 h) appears to negatively influence well-being to varying degrees.[7]

Adverse health effects are a by-product of inadequate sleep duration, with reduced sleep time reported to increase the risk of all-cause mortality among both male and female.[8] Chronic sleep deprivation (SD) has been linked to the development of co-morbidities such as hypertension, diabetes, and obesity.[9] The elderly population is more affected than adults, mainly due to pre-existing health conditions. Sex differences also play a role, with male being at a significantly higher risk of all-cause mortality due to short sleep. In both male and female, the risk of all-cause mortality increases with sleeping more than recommended duration.[10]

Recent studies indicate relationship between sleep time and co-morbidities such as hypertension, cardiovascular diseases (including atherosclerosis, stroke, and coronary artery disease), diabetes mellitus and obesity.[11] Short sleep duration can lead to an increase in overall body weight and influence glucose metabolism. Insufficient sleep also affects homeostasis, leading to food cravings, reduced insulin sensitivity, and altered levels of leptin and ghrelin.[12]

Health outcomes include cancer, diabetes mellitus and cardiovascular diseases like coronary heart disease. There was an increased risk of prostate cancer if sleep was reduced below 6 h per day. Reduced melatonin secretion and circadian rhythm disruption lead to an increased cancer of risk.[13] Short sleep presented increased mortality risk in both the sexes, but the effect of short sleep on diabetes mellitus showed a significant increase in males.[14] Also, males were at a higher risk of all-cause mortality than female when short sleep was reviewed. [10]

Research has extensively focused on hypertension as a primary cardiovascular outcome influenced by sleep deprivation.

Sleep deprivation is very common. Experts estimate between 50 million to 70 million adults in the U.S. meet the medical criteria for sleep deprivation at any point in time. Virtually every human being experiences sleep deprivation at some point in their life. For some people, it's simply a greater or longer-lasting issue, or it happens for a more serious reason.

Stages of Sleep

There are following stages of sleep cycle:

- **Stage 1**: Light sleep. This is a short stage, usually no more than 5% of your total sleep, which begins right after you fall asleep.
- **Stage 2**: Deeper sleep. This stage is deeper and makes up about 45% of all the time you spend sleeping (this number goes up as you get older). Research indicates this stage is key in memory storage and learning.
- **Stage 3**: Deepest sleep. This stage makes up about 25% of the time you spend sleeping (this number goes down with age). There's evidence that this stage is the most important to how your body recovers and maintains itself because the brain prioritizes this stage in people with sleep deprivation. It's very hard to wake someone up from this stage, and they'll usually feel foggy or confused for up to 30 minutes after waking up.

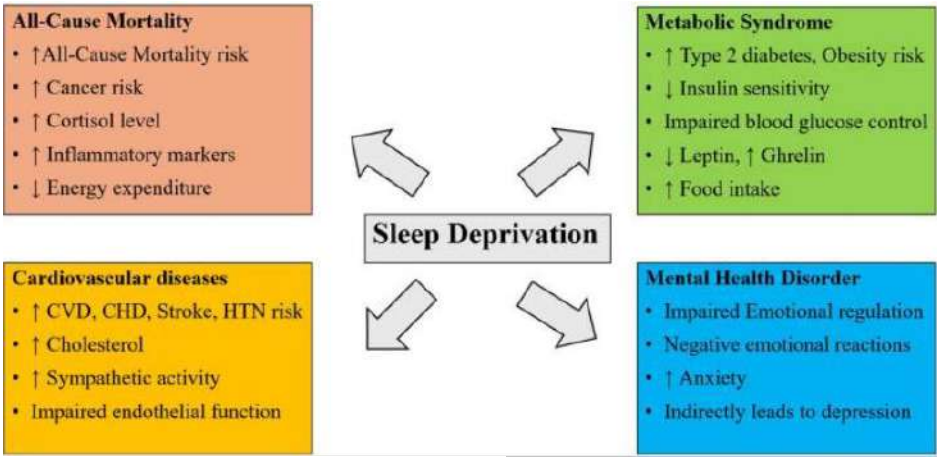

Figure: Effect on body by Sleep Deprivation

- **REM Sleep**: REM stands for "rapid eye movement." This stage is when you dream. When a person is in REM sleep, you can see their eyes moving beneath their eyelids.

When you fall asleep, you typically enter stage 1 and then move in and out of stages 2 and 3. After that, you go into REM sleep and start dreaming. After the first REM cycle, you start a new sleep cycle and go back into stage 1 or 2. One cycle normally takes about 90 to 120 minutes before another begins. Most people go through four or five cycles per night (assuming they get a full eight hours).

Effect of Sleep Deprivation on body:

Sleep deprivation has negative effects in multiple ways throughout your body. Those can affect the following body systems, organs and processes:

- **Heart and circulatory systems**: Sleep deprivation has long-term damaging effects on your heart and circulatory health. People with chronic sleep deprivation are more likely to develop high blood pressure (hypertension) and high cholesterol (hyperlipidemia).
- **Metabolic systems**: People with chronic sleep deprivation are at a much higher risk of developing Type 2 diabetes.
- **Immune system**: Your body's natural defenses against infections can't work properly if you aren't getting enough sleep.
- **Nervous system**: It's common for people who aren't sleeping enough to have higher pain sensitivity, which means they feel pain more easily, the pain is more intense or both.
- **Brain**: Sleep deprivation has very negative effects on how your brain works. While experts don't fully understand sleep's role in brain function, they do know it's a key part of how people learn and remember. There's also some evidence that sleep deprivation could play a role in the development of Alzheimer's disease.
- **Mental health**: Sleep deprivation also negatively affects your mental health, making it harder for you to manage and process your emotions. People with sleep deprivation are more likely to feel symptoms of depression and anxiety.

Sleep deprivation also increases your risk of developing certain conditions or making them worse if you have them. These conditions include Type 2 diabetes, High blood pressure (hypertension), Obesity, Obstructive sleep apnea, Vascular disease, Stroke, Heart attack, Depression, Anxiety and conditions that involve psychosis.

Symptoms of Sleep Deprivation

Some of the most common symptoms include Daytime sleepiness, Fatigue. Irritability, Trouble thinking, focusing and remembering, Slowed reaction times and Headaches.

As sleep deprivation goes on for longer, the symptoms become more severe. Many of the more severe symptoms look like the effects of alcohol intoxication. The severe symptoms of sleep deprivation include "Microsleeps" (when a person briefly falls asleep for only seconds before waking back up), Uncontrollable eye movements (nystagmus), Trouble speaking clearly, Drooping eyelids (ptosis), Hand tremors, Visual and tactile (touch-based) hallucinations, Impaired judgment and Impulsive (or even reckless) behavior.

Stages of Sleep Deprivation

Sleep deprivation stages are:

- **Stage 1**: This is when you go at least 24 hours without sleeping. In this stage, the effects of sleep deprivation are similar to being under the influence of alcohol to the point where it isn't safe for you to drive.
- **Stage 2**: Common symptoms of sleep deprivation intensify. Most people start to experience micro-sleeps in this stage and have trouble thinking or focusing.
- **Stage 3**: People in this stage start to show very severe symptoms like hallucinations. They may

also struggle to communicate with people around them.

- **Stage 4**: The symptoms of sleep deprivation are at their most extreme. The above symptoms worsen to severe or extreme levels. Hallucinations are common and you struggle to tell what's real and what isn't.

Causes of Sleep Deprivation

Sleep deprivation can happen for numerous reasons like Shift work (especially shifts that happen partly or fully during nighttime hours). Alcohol use (especially misuse), Using stimulants like caffeine later in the day, Bad sleep-related habits (known as sleep hygiene), High stress levels, Sleeping in a new or unfamiliar place, such as in a hotel while traveling.

However, sleep deprivation can also happen for medical reasons. Some examples include Lack of quality sleep due to sleep apnea, Degenerative brain disorders such as Alzheimer's disease or Parkinson's disease, Mental health concerns (see below for more about these), Concussions and traumatic brain injuries, Pain, Insomnia, Restless leg syndrome, Parasomnias (disruptive sleep disorders) such as night terrors, sleep paralysis, sleepwalking and more, Medications such as corticosteroids, stimulants and more, Short-term illnesses and infections, such as the common cold, the flu and more.

Mental health issues that can affect sleep include Anxiety, Bipolar disorder, Depression, Mania, Panic disorder, Post-traumatic stress disorder (PTSD) and Somniphobia (fear of sleep).

Diagnosis and Tests

Some possible tests include:

- **Sleep apnea testing**. This can happen in the form of an overnight sleep lab study called a polysomnogram or with an at-home sleep apnea testing device.
- **Electroencephalogram (EEG)**. This test detects and records brain waves. Your healthcare provider, usually a neurologist, can examine your brain activity for signs of unusual brain activity that could contribute to sleep problems or other conditions.
- **Actigraphy**. This test involves wearing a device similar to a watch that tracks sleep patterns to see if you may have a different sleep cycle than is typical. This is key in diagnosing circadian rhythm disorders
- **Multiple sleep latency test (MSLT)**. This test examines whether a person is prone to falling asleep during the daytime. It's often a key part of diagnosing narcolepsy.
- **Maintenance of wakefulness test (MWT)**. This test looks for whether or not a person can resist falling asleep in situations where it would be easy to do so. It's a common part of safety testing for people who drive for a living and may have conditions like sleep apnea.

Management and Treatment

Some of the more common treatments for sleep deprivation and related conditions include:

- **Behavior changes**. Many people can prevent sleep deprivation simply by adjusting their sleep-related behaviors and pre-sleep routine.
- **Medications**. Various medications can help a person fall and stay asleep or change the way they sleep. Some medications can even change the way a person dreams, making it less likely that they'll have severe nightmares or other sleep disturbances. However, many sleep-inducing medications can be habit-forming, so healthcare providers prescribe these cautiously.
- **Breathing support methods**. Conditions that affect breathing during sleep, such as sleep apnea, are treatable with various methods. These include different types of pillows and supports, mouthpieces that adjust your jaw position, surgery to widen your airway, positive airway pressure machines that keep your airway open while you sleep and more.

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Original Article

Role of Serum Biomarkers (Procalcitonin and CRP) in differentiating Bacterial VS Viral Lower Respiratory Tract Infections (LRTIS)

Muhammad Kashif[1], Abdul Hafeez Thebo[2], Arshad Sattar Lakho[3], Akbar Gohar Abro[3], Maryam Ismail[4] and Sajida Haque[5]

ABSTRACT

Objective: To compare the diagnostic accuracy of serum procalcitonin and C-reactive protein, alone and in combination, for differentiating bacterial from viral lower respiratory tract infections.

Study Design: Prospective observational cohort study

Place and Duration of Study: This study was conducted at the Pulmonology Deptt, Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro between March 2024 to February 2025.

Methods: Two hundred patients with LRTIs were recruited (120 bacterial, 80 viral). The admission levels of serum PCT and CRP were determined using chemiluminescent immunoassay and ELISA, respectively. The etiology of bacteria was determined through the culture and sensitivity tests, and the etiology of viral infections was determined by PCR. Receiving operating characteristic (ROC) curves were used to evaluate diagnostic accuracy.

Results: The mean PCT levels were statistically much higher in bacterial LRTIs (5.2 ± 3.1 ng/mL) than viral LRTIs (0.4 ± 0.3 ng/mL). Similarly, mean CRP levels were elevated in bacterial (92.1 ± 34.5 mg/L) versus viral infections (45.2 ± 29.3 mg/L; $p < 0.001$). The PCT area under the curve (AUC) was 0.88 (sensitivity: 85%, specificity: 90% at cut-off >0.5 ng/mL), and compared to 0.76 of CRP (sensitivity: 70%, specificity: 65% at cut-off >50 mg/L). The combination of both biomarkers greatly enhanced diagnostic performance (sensitivity: 92%, specificity: 93%; PPV: 97%, NPV: 91; $p < 0.001$).

Conclusion: Procalcitonin has better diagnostic accuracy than C-reactive protein to differentiate between bacterial and viral LRTIs. Both biomarkers combined improve diagnostic performance and clinical decision making and may lead to fewer unnecessary antibiotic prescriptions. It is suggested that biomarker thresholds need to be validated locally.

Key Words: Procalcitonin, C-reactive protein, lower respiratory tract infections, bacterial infection, viral infection, diagnostic accuracy, biomarker, antimicrobial stewardship.

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INTRODUCTION

Bacterial and viral etiologies of lower respiratory tract infections (LRTIs) continue to be a major cause of morbidity and mortality across the globe, especially among children and immunocompromised adults[1,2].

[1.] Associate Professor Cardiology / Senior Registrar Pulmonology[2] / Assistant Professor Medicine[3] / Women Medical Officer[4] / Specialist Registrar[5], Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro.

Correspondence: Dr. Abdul Hafeez Thebo, Senior Registrar Pulmonology, Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro.
Contact No: 0321-4842788
Email: doc2k7@ymail.com

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Clinical distinction between bacterial and viral LRTIs is clinically vital because in bacterial cases, antimicrobials help whereas in viral ones, they do not, making timely and accurate diagnosis pivotal to effective antimicrobial stewardship and patient outcomes[3]. The use of serum biomarkers like procalcitonin (PCT) and C reactive protein (CRP) to differentiate between these causes are often not reliable due to overlapping symptoms and signs, necessitating the inclusion of serum biomarkers in diagnostic algorithms[4].

Procalcitonin, a precursor to the hormone calcitonin, is barely expressed in normal individuals and instead is strongly expressed in systemic bacterial infections due to endotoxin and pro-inflammatory cytokine stimulation, whereas viral infections tend to stimulate interferon 7 that suppresses PCT release[5]. This selective regulation causes PCT to be a more specific predictor of bacterial infection compared to many conventional markers. PCT increases rapidly within 2-6 hours of bacterial insult and falls rapidly with resolution, in

association with disease activity and supporting its use as an active infection marker[6].

C reactive protein, an acute-phase reactant, which is produced in the liver in response to inflammation, increases in both bacterial and viral infections but tends to have a less pronounced and delayed response than PCT[7]. Although high CRP levels are through association with the severity of infection and may aid in the detection of inflammatory reactions, CRP alone does not exhibit enough specificity to reliably differentiate between bacterial and viral LRTIs without the contextual clinical interpretation and adjunct biomarkers[8].

Multiple studies have shown that PCT typically has a higher diagnostic accuracy than CRP in differentiating between bacterial LRTIs, with procalcitonin thresholds (e.g. of the order of 0.5 ng/mL) having a higher sensitivity and specificity than the traditional CRP cut-offs[9,10]. Combined measurement strategies that combine both PCT and CRP are also effective in improving diagnostic performance because they capitalize on the advantages inherent in each of these biomarkers, constraining the use of unnecessary antibiotics and informing rational therapy[11].

Although these advances have been made, there is heterogeneity in the performance of biomarkers due to differences in patient populations, types of infections, and assay thresholds. Recent evidence suggests that a combination of biomarkers and clinical prediction models or new panels may be useful in the differentiation of bacterial versus viral LRTIs, compared to traditional single-marker-based methods[12]. To idealise the diagnostic accuracy and clinical impact, further research and standardisation of the application of PCT and CRP are essential.

METHODS

This was a prospective, observational cohort study, which was carried out in the Pulmonology Deptt, Liaquat University of Medical & Health Sciences (LUMHS) Jamshoro, between March 2024 and February 2025.

The study involved 200 patients with lower respiratory tract infection (LRTIs). The sample size was calculated by referring to the past studies that had established the optimal sample size to use in testing the diagnostic biomarkers with reasonable statistical power[13]. Inclusion criteria comprised of patients who presented with symptoms of acute LRTI which entailed cough, fever, shortness of breath and abnormal chest radiographs. The exclusion criteria were those with the history of chronic respiratory diseases including asthma or chronic obstructive pulmonary disease (COPD) or one with an immunocompromised condition or known allergy to diagnostic reagents.

At the time of patient enrolment, a comprehensive clinical history was obtained, and physical examination was performed by the attending pulmonologists. Blood samples were taken at admission and the serum concentration of PCT and CRP were measured based on the standard diagnostic blood tests. The use of chemiluminescent immunoassay and enzyme-linked immunosorbent assay (ELISA), respectively, were used to decide the levels of PCT and CRP.

Bacterial etiology of LRTIs was determined by culture, sputum and blood sensitivity tests with isolations of bacterial pathogens being taken as positive. Clinical presentation, radiological findings, and viral nucleic acid in throat swabs detected using polymerase chain reaction (PCR) tests were used to diagnose viral infections. Patients, with infections that could not be identified as either being bacterial or being viral were not further analyzed.

All the data was measured and analyzed using SPSS 26.0, to evaluate the sensitivity, specificity as well as predictive value of PCT and CRP level in differentiating between bacterial and viral infections. This study received ethical approval by the Institutional Review Board of the University. All the participants gave informed consent to take part in the study.

RESULTS

The study enrolled 200 patients with 120 (60% of the patients) diagnosed with bacterial LRTIs and 80 (40% of the patients) diagnosed with viral LRTIs. The average age of the participants was 45.6 years (range: 18 to 82 years), with 58% of the patients being males and 42% females. The demographic information of the research participants will be summarized in Table 1.

Table No. 1: Demographic Data of Study Participants

Age Group (Years)	**Total Patients (n)**	**Bacterial LRTI (n, %)**	**Viral LRTI (n, %)**	**Male (n, %)**	**Female (n, %)**
18–30	50	30 (60%)	20 (40%)	30 (60%)	20 (40%)
31–45	60	40 (67%)	20 (33%)	40 (67%)	20 (33%)
46–60	70	40 (57%)	30 (43%)	40 (57%)	30 (43%)
>60	20	10 (50%)	10 (50%)	8 (40%)	12 (60%)
Total	200	120 (60%)	80 (40%)	118 (59%)	82 (41%)

Table 2 shows the mean serum levels of procalcitonin (PCT) and C- reactive protein (CRP) in patients with bacterial and viral LRTIs. The concentration of the two biomarkers was significantly increased in the patients affected by bacterial infections as compared to those affected by viral infections.

Table No. 2: Mean Serum Levels of Procalcitonin and C-reactive Protein in Bacterial vs. Viral LRTIs

Biomarker	Bacterial LRTI (Mean ± SD)	Viral LRTI (Mean ± SD)	p-value
Procalcitonin (ng/mL)	5.2 ± 3.1	0.4 ± 0.3	<0.001
C-reactive Protein (mg/L)	92.1 ± 34.5	45.2 ± 29.3	<0.001

The diagnostic value of procalcitonin (PCT) and C-reactive protein (CRP) in the differentiation of bacterial and viral LRTIs was evaluated using receiver operating characteristic (ROC) curves. The procalcitonin (AUC) was determined to be 0.88 which means that it has excellent discriminatory capacity between bacterial and viral infections. The AUC of CRP was 0.76 yet it still showed moderate discriminatory power. The following were the cut-off values of both biomarkers to differentiate between bacterial infections and viral infections: PCT > 0.5 ng/mL (sensitivity: 85%, specificity: 90%) and CRP > 50 mg/L (sensitivity: 70%, specificity: 65%). Table-3

Table No. 3: Diagnostic Accuracy of Procalcitonin and C-reactive Protein for Differentiating Bacterial from Viral LRTIs

Biomarker	Cut-off Value	Sensitivity (%)	Specificity (%)	AUC
Procalcitonin	>0.5 ng/mL	85%	90%	0.88
C-reactive Protein	>50 mg/L	70%	65%	0.76

Statistical test showed that both procalcitonin and C-reactive protein were significantly elevated in bacterial LRTI cases in comparison to viral LRTIs ($p < 0.001$ both markers). Also, when the two biomarkers were combined together during a diagnostic algorithm, the accuracy of differentiating between bacterial and viral LRTIs improved and this had a sensitivity of 92% and specificity of 93%. Table-4

Table No. 4: Sensitivity and Specificity of Combined Use of Procalcitonin and C-reactive Protein

Biomarker Combination	Sensitivity (%)	Specificity (%)	p-value
PCT + CRP	92%	93%	<0.001

The positive predictive value (PPV) and negative predictive value (NPV) for procalcitonin were 95% and 80%, respectively, while for C-reactive protein, the PPV and NPV were 72% and 78%, respectively. The combined use of both biomarkers led to a PPV of 97% and an NPV of 91%, suggesting that the use of both markers together significantly improved clinical decision-making regarding the need for antibiotic therapy. Table-5

Table No. 5: Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for Procalcitonin, C-reactive Protein, and Their Combination

Biomarker Combination	PPV (%)	NPV (%)
Procalcitonin	95%	80%
C-reactive Protein	72%	78%
PCT + CRP	97%	91%

DISCUSSION

The utility of serum procalcitonin (PCT) and C reactive protein (CRP) in differentiating between bacterial and viral lower respiratory tract infections (LRTIs) in a one year timeframe was tested in this prospective observational cohort study. The findings revealed that mean levels of both PCT and CRP were significantly higher in bacterial LRTIs than in cases of viruses ($p < 0.001$), and PCT is even associated with greater diagnostic accuracy in comparison with CRP. These findings are generally consistent with a variety of recent studies also highlighting the usefulness of PCT in differentiating bacterial and viral respiratory infections, however some differences between the marker thresholds and predictive values[14,15].

The considerably high levels of PCT in bacterial LRTIs (mean 5.2 ± 3.1 ng/mL) in comparison to viral infections (mean 0.4 ± 0.3 ng/mL) are in line with the established pathophysiological mechanisms and previously known evidence. As an example, Yamamoto et al[16] in a study noted that the PCT level in bacterial-induced infections was significantly higher as compared to the level of the viral etiology. Similarly, a study by Kamat et al[17] has reported that PCT >0.5 ng/mL had high sensitivity and specificity (83% and 88%, respectively) to bacterial pneumonia which closely mirrored our findings. These similarities across studies reinforces the biological rationale that bacterial endotoxins and cytokines stimulate PCT synthesis more strongly than viral interferons, which suppress PCT release.

Conversely, the diagnostic discrimination of CRP levels, although considerably high in bacterial infections, was lower. In our study, mean CRP levels of 92.1 ± 34.5 mg/L in bacterial and 45.2 ± 29.3 mg/L in viral LRTIs and a diagnostic specificity of 65% at the cut off point of 50 mg/L. These values are in line with the results of Povoa et al[18] who found a wide range of variability in the CRP accuracy, which depended often on the population of patients and the severity of the infection. CRP may increase significantly in both bacterial and viral etiologies in mild to moderate infections, decreasing its specificity when used alone.

This is probably one of the reasons why CRP in our study had moderate diagnostic power compared to the higher diagnostic power of PCT.

Combining the results by simultaneously using both biomarkers increased the diagnostic performance with a sensitivity and a specificity of over 90%. This effect of synergy has been reported in other studies. A study by Linder et al[19] demonstrated that combining biomarkers with clinical algorithms improved the precision of infection classification and reduced unnecessary antibiotic prescriptions. This integrative strategy is supported by our increased PPV and NPV values of the combined markers, indicating that combined assessment is a more effective method of reducing risk of misclassification than single markers.

However, there are also some studies that reported somewhat different optimal cut off thresholds, which could follow the variation in the study design, patient demographics, prevalence of specific pathogens and calibration of laboratory assays. Using the example of the raise in baseline CRP and PCT, which may also be elevated in elder populations with multiple comorbidities, which can affect interpretative thresholds "Beaumont et al"[20]. The proportion of community versus hospital acquired infections may also vary, which can also affect the biomarker kinetics because more severe or nosocomial infections may result in higher baseline levels of inflammatory responses.

In general, the findings of this study support the existing literature that PCT is a more valid biomarker than CRP to differentiate between bacterial and viral LRTIs, and that a combination of biomarker measurements is superior to a single biomarker to improve diagnostic accuracy. The differences in absolute cut off values and predictive strength are most probably due to population characteristics, assay methods, and infection spectrum, which emphasizes the importance of contextual interpretation and local validation of diagnostic thresholds.

CONCLUSION

Our findings suggest that PCT has better sensitivity, and specificity than CRP, and serves as a more reliable biomarker of bacterial infections. Whereas CRP is still effective in determining the presence of bacterial or viral etiologies, it does not have the diagnostic specificity needed to effectively differentiate the presence of bacterial and viral etiologies. A combination of PCT and CRP is a very useful tool in clinical decision-making, especially when it comes to decreasing the number of unnecessary antibiotic prescriptions. These results indicate that the integration of PCT and CRP measurements into everyday clinical practice may enhance the management of patients, particularly in those settings, which need a rapid determination of infection etiology. It is advised that further research using larger and more varied groups of patients, as well as inclusion of pathogen-specific assays, should be carried out to help to refine biomarker thresholds and optimal diagnostic strategies in the diagnosis of LRTIs.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muhammad Kashif, Abdul Hafeez Thebo, Arshad Sattar Lakho
Drafting or Revising Critically:	Akbar Gohar Abro, Maryam Ismail, Sajida Haque
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Pattern and Severity of Pulmonary Function Impairment in Patients with Post-Tuberculosis Lung Disease

Arshad Sattar Lakho[1], Muhammad Kashif[2], Abdul Hafeez Thebo[3], Akbar Gohar Abro[1], Sajida Haque[4] and Maryam Ismail[5]

ABSTRACT

Objective: To determine the pattern and severity of pulmonary function impairment in patients with post-tuberculosis lung disease.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Pulmonology, Liaquat University of Medical and Health Sciences, Jamshoro, from Dec 2022 to Jan 2024.

Methods: The patients were selected using non-probability consecutive sampling, totalling 185 patients who had previously undergone anti-tuberculous (AT) therapy for pulmonary tuberculosis for at least six months. A structured proforma was used for the collection of demographic and clinical data. Spirometry was performed to determine pulmonary function, and ventilatory patterns were classified as obstructive, restrictive, mixed or normal.

Results: Mean age of the patients 43.7 ± 12.5 years and 112 (60.5%) were male. Forty-nine (29.5%) patients had pulmonary function abnormalities and 36 (19.5%) had normal pulmonary function. The most common spirometric pattern was the obstructive ventilatory defect, seen in 72 (38.9%) patients, followed by the restrictive defect, seen in 49 (26.5%) patients and the mixed ventilatory defect, seen in 28 (15.1%) patients. The majority of severity were moderate pulmonary impairment. There was significant association between smoking history, recurrent tuberculosis and extensive radiological fibrosis with the presence of abnormal pulmonary function ($p<0.05$). Mean FEV1 values were significantly lower among smokers compared to non-smokers (58.4 ± 14.7% vs 66.9 ± 13.2%, $p=0.003$).

Conclusion: Pulmonary function impairment is very common in patients with PBLD, and is most commonly characterized by an obstructive ventilatory defect. The impairment is worsened by smoking, recurrent TB and fibrotic lung changes. The post-tuberculous management should include routine spirometric assessment and long-term respiratory follow up for early diagnosis and treatment of chronic pulmonary sequels.

Key Words: Pulmonary tuberculosis, Post-tuberculosis lung disease, Spirometry, Pulmonary function impairment, Obstructive ventilatory defect, Chronic respiratory disease.

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INTRODUCTION

Tuberculosis (TB) is one of the leading infectious diseases causing morbidity and mortality worldwide and is still a significant public health problem globally[1].

[1]Assistant Professor Medicine / Associate Professor Cardiology[2] / Senior Registrar Pulmonology[3] / Specialist Registrar[4] / Women Medical Officer[4] / Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro.

Correspondence: Akbar Gohar Abro, Assistant Professor Medicine, Liaquat University of Medical & Health Sciences (LUMHS), Jamshoro.
Contact No: 03332644702
Email: akbargoharabro@gmail.com

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Although diagnosis and treatment have progressed considerably, tuberculosis of the lungs often leads to persistent lung damage and disease despite being cured of the infection. There is growing evidence that many patients who have completed an effective anti-tuberculous therapy still have ongoing respiratory symptoms, exercise intolerance and chronic pulmonary impairment, which is now known as Post tuberculosis Lung Disease (PTLD) [2,3].

Post-tuberculosis lung disease refers to a wide range of residual pulmonary abnormalities, such as fibrosis, bronchiectasis, cavitary destruction, pleural thickening, airway stenosis and emphysematous changes. These pathological alterations may lead to irreversible impairment of pulmonary function and substantial deterioration in quality of life. Pulmonary TB survivors have been shown to have obstructive, restrictive or combination (mixed) ventilatory defects on spirometry[4]. The burden of pulmonary impairment among TB survivors is a significant one, as noted by a recent systematic review and meta-analysis[5,6]. A high

percentage of treated TB patients still had abnormal spirometric results, with obstructive and mixed ventilatory patterns in particular being common, according to Ivanova et al[2]. In addition, there was evidence of significant lung damage in around 10-15% of the TB survivors, highlighting the long term respiratory impacts of TB[8]. In another recent meta-analysis, it was found that patients with TB had significantly lower FEV1 and FVC following the recovery than healthy controls, suggesting that the pulmonary reserve was lost and there is a persistent airflow limitation. [9]

PTLD is of special relevance in countries with high tuberculosis prevalence and limited healthcare resources in low- and middle-income countries. Factors such as delayed diagnosis, recurrent infection, smoking, multidrug-resistant tuberculosis, malnutrition, and poor socioeconomic conditions may further increase the risk and severity of post-tuberculous pulmonary dysfunction[10]. With the growing number of tuberculosis survivors in the South Asian countries like Pakistan, the provision of long-term respiratory health services becomes a significant challenge. However, there is a lack of recognition and study of the sequelae of TB after successful treatment in the local population.

PFTs, especially spirometry, are a simple, non-invasive and reliable way of quantifying the nature and distribution of lung impairment in post-TB patients. Spirometric abnormalities may help identify patients who are at risk of developing chronic respiratory disability earlier in the disease process, for further follow-up, and for targeted interventions to prevent chronic respiratory disability.[11]

There is little information available in the area regarding the severity or pattern of changes in pulmonary function after TB treatment. Several international studies have been conducted to evaluate lung damage following TB treatment; nevertheless, there is a dearth of information regarding the pattern and severity of anomalies in pulmonary function in individuals with post-TB lung illness, particularly in Pakistan. In order to improve respiratory care following TB with long-term therapy, this study sought to evaluate the pattern and degree of pulmonary function decline in post-TB lung illness using spirometric measurement.

METHODS

This descriptive cross-sectional study was conducted in the Department of Pulmonology, Liaquat University of Medical and Health Sciences, Jamshoro from December 2022 to January 2024. The study included patients who were between the ages of 18 and 70, had a history of pulmonary TB, had finished anti-tuberculous treatment at least six months before being enrolled, and had a history of respiratory symptoms or radiological suspicion of post-tuberculosis lung disease. Those with active TB, bronchial asthma diagnosed prior to TB, known ILD, lung malignancy, acute respiratory infection less than 4 weeks prior to study, past thoracic surgery, severe cardiac disease and who didn't have satisfactory spirometry were excluded.

The sample size was determined from the single population proportion formula with a presumed proportion of post tuberculosis patients with pulmonary function impairment of 50%, 95% level of confidence and a margin of error of 7.5% based on limited local data[12]. The calculated sample size was 171 patients, with additional allowance for incomplete spirometry or missing data, the final sample size was raised to 185 patients. Eligible participants were recruited using non-probability consecutive sampling. Written informed consent was obtained from all participants before enrolment.

The data collection was done using a structured proforma, which included details of age, gender, place of residence, smoking habit, BMI, duration since the completion of TB treatment, past treatment category, TB recurrence, current respiratory symptoms, and relevant radiological features. Standard acceptability and reproducibility criteria were used for the measurement of pulmonary function with a calibrated spirometer. Forced vital capacity, forced expiratory volume in one second and FEV1/FVC ratio were taken. Spirometric patterns were classified into obstructive, restrictive, mixed or normal. Severity was classified based on percentage predicted values.

SPSS version 26.0 was used to analyze the data. Data for quantitative variables were provided as mean ± standard deviation, whereas data for qualitative factors were presented as frequencies and percentages. The relationship between pulmonary function impairment and clinical variables was evaluated using the chi-square test. The independent sample t test was used to evaluate and compare continuous data. To find variables that were independently linked to poor pulmonary function, multivariable logistic regression was used. A statistically significant p-value was regarded as ≤ 0.05. The institutional review board of LUMHS, Jamshoro, approved the ethical considerations.

RESULTS

There were 185 patients in all. The majority of patients were between the ages of 31 and 50, with a mean age of 43.7 ± 12.5 years. There were 73 (39.5%) females and 112 (60.5%) males. Of the patients, 68 (36.8%) had a history of smoking. The mean BMI was 22.1 ± 4.3 kg/m².

Persistent respiratory symptoms were common among study participants. Dyspnea was the most frequently reported symptom and was observed in 128 (69.2%) patients, followed by chronic cough in 111 (60.0%), sputum production in 84 (45.4%), wheezing in 51

(27.6%), and hemoptysis in 14 (7.6%) patients. Radiological abnormalities suggestive of post-tuberculous sequelae were identified in the majority of participants, with fibrotic changes and bronchiectatic lesions being the most common findings. Table-2

Table No. 1: Demographic and Clinical Characteristics (n=185)

Variables	Frequency (%) / Mean ± SD
Age (years)	43.7 ± 12.5
18–30 years	38 (20.5%)
31–50 years	96 (51.9%)
>50 years	51 (27.6%)
Male	112 (60.5%)
Female	73 (39.5%)
Smokers	68 (36.8%)
Non-smokers	117 (63.2%)
BMI (kg/m²)	22.1 ± 4.3
Duration since TB treatment completion	3.8 ± 2.1 years

Table No. 2: Clinical Symptoms and Radiological Findings Among Study Participants (n=185)

Variables	Frequency (%)
Dyspnea	128 (69.2%)
Chronic cough	111 (60.0%)
Sputum production	84 (45.4%)
Wheezing	51 (27.6%)
Hemoptysis	14 (7.6%)
Fibrotic changes on imaging	102 (55.1%)
Bronchiectatic changes	74 (40.0%)
Cavitary lesions	29 (15.7%)
Pleural thickening	21 (11.4%)

Table No. 3: Spirometric Patterns and Severity of Pulmonary Function Impairment (n=185)

Variables	Frequency (%)
Normal spirometry	36 (19.5%)
Abnormal spirometry	149 (80.5%)
Obstructive pattern	72 (38.9%)
Restrictive pattern	49 (26.5%)
Mixed ventilatory defect	28 (15.1%)
Mild impairment	51 (34.2%)
Moderate impairment	61 (40.9%)
Severe impairment	37 (24.8%)

Pulmonary function abnormalities were detected in 149 (80.5%) patients, whereas only 36 (19.5%) patients demonstrated normal spirometric findings. Obstructive ventilatory defect was the predominant spirometric pattern observed in 72 (38.9%) patients, followed by restrictive defect in 49 (26.5%) and mixed ventilatory defect in 28 (15.1%) patients. Among patients with abnormal pulmonary function, moderate impairment was the most frequent severity category, identified in 61 (40.9%) patients, while severe impairment was observed in 37 (24.8%) patients. Table-3.

The mean forced expiratory volume in one second (FEV1) was significantly reduced among smokers compared to non-smokers (58.4 ± 14.7% predicted vs 66.9 ± 13.2% predicted, p=0.003). Similarly, patients with recurrent tuberculosis demonstrated significantly lower mean FEV1 and FVC values compared to those without recurrence ($p<0.05$). A statistically significant association was observed between duration since completion of tuberculosis treatment and severity of pulmonary impairment, with greater impairment noted among patients with longer disease duration (p=0.021). Table-4

Table No. 4: Comparison of Pulmonary Function Parameters According to Clinical Variables

Variables	Mean FEV1 (% Predicted)	Mean FVC (% Predicted)	p-value
Smokers	58.4 ± 14.7	64.2 ± 13.5	0.003
Non-smokers	66.9 ± 13.2	71.6 ± 12.8	
Recurrent TB	54.7 ± 15.1	60.3 ± 14.4	0.001
Non-recurrent TB	67.1 ± 12.9	72.8 ± 12.1	

Smoking history, recurring tuberculosis, and severe radiological fibrosis were found to be independent predictors of poor pulmonary function using multivariable logistic regression analysis. While recurrent tuberculosis was linked to a 2.9-fold greater risk of severe pulmonary impairment (AOR: 2.9, 95% CI: 1.4–5.8, p=0.006), smoking increased the likelihood of pulmonary function impairment by around 2.4 times (AOR: 2.4, 95% CI: 1.2–4.9, p=0.014). Table 5

Table No. 5: Multivariable Logistic Regression Analysis for Predictors of Pulmonary Function Impairment

Variables	Adjusted Odds Ratio (AOR)	95% Confidence Interval	p-value
Smoking history	2.4	1.2–4.9	0.014
Recurrent tuberculosis	2.9	1.4–5.8	0.006
Extensive fibrosis	3.1	1.5–6.2	0.002
Duration since TB treatment >5 years	1.8	1.1–3.7	0.021

DISCUSSION

The current study revealed that the burden of pulmonary function impairment was high in patients with post tuberculosis lung disease and abnormal spirometry was seen in 80.5% of patients. Chushkin et al[13] found pulmonary impairment in 47.7% of treated tuberculosis patients, which was lower than our findings. Similarly, Lema et al found abnormal pulmonary function in 47.3% of patients following completion of treatment[14]. This may be due to higher numbers of patients in our study being recruited from hospital with symptoms of disease, delayed presentation, recurrent disease, and higher structural lung damage.

The most common spirometric defect in our study was obstructive ventilatory defect followed by a restrictive and mixed pattern. This is in line with the results of Xing et al who found obstruction as the most common abnormality in post-tuberculosis patients[15]. Gupta et al also introduced the concept of post-tuberculosis obstructive lung disease as a specific clinical phenotype that needs special consideration[16]. Airway fibrosis, bronchiectasis, narrowing of the airways and chronic inflammatory remodeling due to pulmonary tuberculosis could be responsible for the predominance of obstruction. Other studies, however, have found that the predominant pattern is a restrictive one particularly in those with extensive fibrosis and destroyed lung[17]. These differences could be due to differences in the severity of the disease, smoking history, radiological extent and the time of assessment of lung function by a spirometer among different populations.

Uncontrolled smoking was found to be strongly correlated with reduced FEV1 in the current study, indicating that the harmful effect of cigarette smoke is additive to the effect of the already damaged lung parenchyma. This is biologically plausible as smoking and TB both cause chronic airway inflammation and airflow limitation. In a study by, Ralph et al showed that the risk of chronic airflow obstruction is significantly higher in people with pulmonary tuberculosis [18]. Hnizdo et al in contrast did not find a strong link between smoking and the residual respiratory disability in their population[19]. The difference may be attributed to the differences in smoking intensity, environment exposures, nutritional status and socioeconomic status of the study population.

Another important factor associated with pulmonary impairment in our study was recurrent TB. Those with recurrent disease had significantly lower FEV1 and FVC than patients with non-recurrent disease. The same was observed by Woldesemayat et al, who showed that pulmonary TB had cumulative damages to the lungs and chronic pulmonary function impairment in the patients[20]. Chronic and recurrent infections are likely to result in progressive fibrosis, cavitation, airway distortion and permanent destruction of the parenchymal tissue, thus compromising the long-term pulmonary prognosis.

Abnormal spirometry was significantly correlated with radiological fibrosis in the present study. Nightingale et al also found that there was a significant relationship between cavitary and fibrotic lesions and pulmonary function decrease[21]. Lee et al also found that the structural sequelae following TB treatment are present for many years after treatment, and lead to long-term respiratory disability[22]. Fibrosis causes a decrease in the compliance of the lung and can result in restrictive or obstructive abnormalities depending on the amount and distribution of lung involvement.

The most common symptoms were dyspnea and chronic cough, similar to Ashraf et al findings in a Pakistani population[23]. However, the severity of the symptoms is not always directly related to spirometric impairment. After treatment, persistent respiratory symptoms were observed with gradual improvement in lung function, as reported by Meghji et al[24]. This may account for the fact that some patients do not improve even with only mild spirometric abnormalities, perhaps because of the bronchiectasis, deconditioning, recurrent infections or psychological factors.

There are some limitations in the present study, which needs to be addressed. The results of the present study were obtained from a single center, therefore the findings may not be applicable to the general population and causal relationship could not be drawn. Selection bias might have been introduced due to non-probability consecutive sampling and did not have baseline pre-tuberculosis spirometric data to assess the actual extent of lung function damage caused by tuberculosis. Furthermore, the importance of the factors of biomass exposure, occupational hazards, environmental pollution and detailed radiological quantification were not evaluated comprehensively. No long-term follow-up was done to evaluate the progression or recovery of pulmonary impairment over time.

CONCLUSION

According to the current study, patients with post-tuberculosis lung disease had a significant incidence of pulmonary function abnormalities, with obstructive ventilatory defects being the most prevalent spirometric pattern. Significant factors influencing the severity of pulmonary impairment were smoking history, recurrent tuberculosis and radiological fibrosis. The results indicate that successful completion of anti-tuberculous therapy does not necessarily lead to normal pulmonary function and call for regular post-treatment follow-up of respiratory function after tuberculosis, early evaluation of pulmonary function using spirometric tests, and long term follow-up of TB survivors to

minimize chronic respiratory morbidity and enhance quality of life.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Arshad Sattar Lakho, Muhammad Kashif, Abdul Hafeez Thebo
Drafting or Revising Critically:	Akbar Gohar Abro, Sajida Haque, Maryam Ismail
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Frequency of Endometrial Carcinoma among Women presenting with Abnormal Uterine Bleeding at a Secondary Care Hospital

Kiran Ikram[1], Naila Raziq[2], Salma Naz[2] and Munazza Yousaf[2]

ABSTRACT

Objective: To determine the frequency of endometrial carcinoma among women presenting with abnormal uterine bleeding at a secondary care hospital.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Gynecology and Obstetrics, Maqsood Medical Complex, Peshawar, from 1st March 2025 to 28th February 2026.

Methods: The number of women, who presented with abnormal uterine bleeding, were included in the study by non-probability consecutive sampling, totalling 247 women. Information about age, parity, menopausal status, bleeding pattern, comorbidities, endometrial thickness and histopathological diagnosis were recorded. Biopsy, dilatation and curettage or hysterectomy specimen provided the source of endometrial tissue when clinically indicated.

Results: The mean age of patients was 44.8 ± 10.7 years. Mean duration of symptoms was 5.9 ± 3.4 months. Heavy menstrual bleeding was the most common presentation 93 (37.7%). Overall, 13 patients were diagnosed with the endometrial carcinoma, with a frequency of 5.3%. Bleeding pattern, endometrial thickness, age, post-menopausality, diabetes mellitus, hypertension and obesity were significantly associated with carcinoma.

Conclusion: A significant number of women with abnormal uterine bleeding were found to have endometrial carcinoma. Older age, postmenopausal bleeding, and elevated endometrial thickness and metabolic comorbidities should lead to prompt evaluation of the endometrium and subsequent histopathological confirmation.

Key Words: Endometrial Carcinoma, Abnormal Uterine Bleeding, Women

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INTRODUCTION

Abnormal uterine bleeding (AUB) is one of the most frequent gynecological complaints among women of reproductive, perimenopausal and postmenopausal age groups. It is defined as bleeding which differs from normal menstruation in regularity, frequency, duration or volume and is usually classified according to the FIGO PALM-COEIN system into structural and non-structural causes[1]. While many cases are benign, AUB can be the initial or only symptom of premalignant endometrium or endometrial carcinoma, especially in postmenopausal and women over the age of 40.[2]

[1.] Assistant Professor / Senior Registrar[2], Department of Obs & Gyne, Maqsood Medical Complex (MMC) Peshawar.

Correspondence: Dr. Munazza Yousaf, Senior Registrar, Obs & Gyne, Maqsood Medical Complex (MMC) Peshawar.
Contact No: 03365891670
Email: munazzamalik78@gmail.com

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Endometrial carcinoma is one of the most frequently occurring gynecologic cancers in the world, and rising incidence is partly due to obesity and diabetes, nulliparity, late menopause, and unopposed estrogen use[3]. The new FIGO staging system focuses on histological type, the grade of the tumor, the presence of lymphovascular invasion, and molecular characteristics, which highlights the significance of early diagnosis and accurate risk stratification[4]. International guidelines also emphasize that abnormal bleeding is a critical warning symptom that should be promptly assessed by endometrial evaluation, particularly in women who are either postmenopausal or high-risk premenopausal[5].

Age and bleeding patterns are risk factors for endometrial carcinoma. Published data highlight that the overall risk for endometrial cancer in premenopausal women with AUB is low, but higher for women who have experienced intermenstrual bleeding and more advanced age[6]. In Pakistan, delayed health-seeking behavior, limited screening facilities, lack of awareness and restricted access to specialist gynecological services may contribute to late diagnosis. Local studies have revealed that AUB is a frequent

cause of gynecological consultation and structural causes, hyperplasia and malignant lesions are encountered with varying prevalence[7,8].

There are also a number of Pakistani studies which confirm the need for pathological evaluation in selected cases of AUB[9]. A wide range of endometrial pathologies have been reported among Pakistani women with AUB, such as endometrial carcinoma. Among women with abnormal uterine bleeding, Khan et al[10] focused on the study of endometrial carcinoma and stressed the importance of early diagnostic evaluation. More recently, Sharif et al[11] found a higher frequency of endometrial cancer among postmenopausal women compared with premenopausal women presenting with AUB. Therefore, determining the frequency of endometrial carcinoma among women with AUB at a secondary care hospital is important for local evidence generation, timely referral, appropriate biopsy decisions and prevention of advanced-stage disease.

METHODS

The descriptive cross-sectional study was carried out in the Department of Gynecology and obstetrics Maqsood Medical Complex, Peshawar from 1st March 2025 to 28th February 2026. Non-probability consecutive sampling was used to select 247 women with abnormal uterine bleeding for the study. Abnormal uterine bleeding was defined as any bleeding from the uterine corpus that was abnormal in frequency, regularity, duration or amount, including heavy menstrual bleeding, intermenstrual bleeding, irregular bleeding, frequent or prolonged bleeding and postmenopausal bleeding. Women of reproductive, perimenopausal and postmenopausal age groups who presented to the outpatient department or were admitted with abnormal uterine bleeding and underwent clinical evaluation with endometrial sampling were included. Patients with bleeding during pregnancy, known bleeding disorders, cervical carcinoma, bleeding from any apparent lesions in the vagina/vulva, patients with incomplete clinical or histopathological records were excluded.

Detailed history was taken after informed consent was obtained and included details of age, parity, menstrual pattern, duration of symptoms, menopausal status, hormonal therapy, diabetes mellitus, hypertension, obesity, nulliparity, lack of pregnancies, polycystic ovary syndrome and family history of cancer in the gynecological tract. All patients were subjected to general physical examination, abdominal examination and pelvic examination. Baseline investigations relevant to the clinical situation were recommended as appropriate: complete blood count, pregnancy test (when appropriate), blood sugar profile, coagulation profile (when indicated), and pelvic ultrasonography to evaluate the uterus, adnexa and endometrial thickness.

Endometrial tissue was collected from either an endometrial biopsy or from the dilated and curettage or hysterectomy specimen as clinically indicated. Properly labelled container with formalin was used to send all samples to the histopathology laboratory. Histopathological findings were recorded as normal proliferative or secretory endometrium, disordered proliferative endometrium, endometrial polyp, endometrial hyperplasia with or without atypia, chronic endometritis, atrophic endometrium, endometrial carcinoma or other pathology. The main outcome variable was the frequency of endometrial carcinoma among women presenting with abnormal uterine bleeding.

The data were inputted and analysed using SPSS version 26. The quantitative variables age, parity and duration of the symptoms were presented as the mean and standard deviation, and median and interquartile range, depending on the distribution of the data. Qualitative variables like menopausal status, bleeding pattern, ultrasonographic findings and histopathological diagnosis were shown as frequencies and percentages. The frequency of endometrial carcinoma was calculated as the number of histopathologically confirmed cases of endometrial carcinoma divided by the total number of women included in the study. Age group, menopausal status, parity, diabetes mellitus, and hypertension with endometrial thickness was performed for stratification purposes to determine the distribution of endometrial carcinoma across clinically relevant variables. Post-stratification chi-square test or Fisher's exact test was applied where appropriate, and a p-value of ≤ 0.05 was considered statistically significant. Ethical approval was obtained from the institutional ethical review committee before commencement of the study.

RESULTS

Table No. 1. Baseline demographic and clinical characteristics of patients

Variable	Frequency / Mean	Percentage / SD
Total patients	247	100.0
Age, years	44.8	±10.7
Duration of symptoms, months	5.9	±3.4
Age <40 years	92	37.2
Age 40–49 years	76	30.8
Age 50–59 years	52	21.1
Age ≥60 years	27	10.9
Reproductive age group	134	54.3
Perimenopausal	61	24.7
Postmenopausal	52	21.1
Nulliparous	34	13.8
Parity 1–3	83	33.6
Parity ≥4	130	52.6
Diabetes mellitus	64	25.9
Hypertension	72	29.1

Variable	Frequency / Mean	Percentage / SD
Obesity	88	35.6

Total 247 women with abnormal uterine bleeding were analyzed. The mean age was 44.8 ± 10.7 years, and the mean duration of symptoms was 5.9 ± 3.4 months. The reproductive age group was the most predominant. Baseline characteristics are presented in table 1.

The most frequent presentation was heavy menstrual bleeding. Table 2 shows that bleeding pattern was significantly related to endometrial carcinoma, with women who had postmenopausal bleeding having the highest frequency (overall p=0.0002).

Table No. 2: Pattern of abnormal uterine bleeding and association with endometrial carcinoma

Bleeding pattern	Total	Endometrial carcinoma n (%)	No carcinoma	Overall p-value
Heavy menstrual bleeding	93 (37.7)	1 (1.1)	92 (98.9)	0.0002
Intermenstrual bleeding	38 (15.4)	1 (2.6)	37 (97.4)	
Irregular/prolonged bleeding	64 (25.9)	2 (3.1)	62 (96.9)	
Postmenopausal bleeding	52 (21.1)	9 (17.3)	43 (82.7)	
Total	247 (100.0)	13 (5.3)	234 (94.7)	

Table No. 3: Endometrial thickness and association with endometrial carcinoma

Endometrial thickness	Total	Endometrial carcinoma	No carcinoma	Overall p-value
<8 mm	76 (30.8)	0 (0.0)	76 (100.0)	0.0024
8–11 mm	68 (27.5)	2 (2.9)	66 (97.1)	
12–15 mm	57 (23.1)	4 (7.0)	53 (93.0)	
>15 mm	46 (18.6)	7 (15.2)	39 (84.8)	
Total	247 (100.0)	13 (5.3)	234 (94.7)	

Table No. 4: Histopathological findings among women with abnormal uterine bleeding

Histopathological diagnosis	n (%)
Proliferative endometrium	54 (21.9)
Secretory endometrium	29 (11.7)
Disordered proliferative endometrium	42 (17.0)
Endometrial polyp	31 (12.6)
Endometrial hyperplasia without atypia	27 (10.9)
Atypical hyperplasia / EIN	10 (4.0)
Chronic endometritis	14 (5.7)
Atrophic endometrium	18 (7.3)
Other benign pathology	9 (3.6)
Endometrial carcinoma	13 (5.3)
Total	**247 (100.0)**

Table No. 5: Association of demographic and clinical factors with endometrial carcinoma

Variable	Total n	Endometrial carcinoma n (%)	No carcinoma n (%)	p-value
Age <40 years	92	0 (0.0)	92 (100.0)	0.0024
Age 40–49 years	76	3 (3.9)	73 (96.1)	
Age 50–59 years	52	6 (11.5)	46 (88.5)	
Age ≥60 years	27	4 (14.8)	23 (85.2)	
Reproductive age group	134	1 (0.7)	133 (99.3)	0.00003
Perimenopausal	61	3 (4.9)	58 (95.1)	
Postmenopausal	52	9 (17.3)	43 (82.7)	
Diabetes mellitus present	64	8 (12.5)	56 (87.5)	0.0058
Diabetes mellitus absent	183	5 (2.7)	178 (97.3)	
Hypertension present	72	8 (11.1)	64 (88.9)	0.0228
Hypertension absent	175	5 (2.9)	170 (97.1)	
Obesity present	88	9 (10.2)	79 (89.8)	0.0149
Obesity absent	159	4 (2.5)	155 (97.5)	
Nulliparous	34	0 (0.0)	34 (100.0)	0.1437

Variable	Total n	Endometrial carcinoma n (%)	No carcinoma n (%)	p-value
Parity 1–3	83	3 (3.6)	80 (96.4)	
Parity ≥4	130	10 (7.7)	120 (92.3)	

The endometrial thickness was significantly correlated with endometrial carcinoma (p=0.0024). Table 3 shows the highest frequency for patients having endometrial thickness >15 mm.

Histopathological examination revealed that benign endometrial pathology occurred more frequently than malignant pathologies. As presented in Table 4, there was a total of 13 patients (5.3%) with endometrial carcinoma.

Increasing age, post-menopause status, diabetes mellitus, hypertension and obesity were found to be significantly associated with endometrial carcinoma. No significant association with carcinoma was found for parity. The interaction of demographic and clinical factors with endometrial carcinoma is presented in table 5.

DISCUSSION

The present study reported that 5.3% of the women who attend secondary care hospital with abnormal uterine bleeding were diagnosed with endometrial carcinoma. This means that while most of the cases of AUB are caused by benign endometrial problems, a significant number of cases have malignancy, especially in those cases with high-risk features. The findings are similar to those reported by Parveen et al[12] who found that endometrial cancer was observed in 6.51% of women with AUB. This minor discrepancy might be attributed to differences in the setting of studies, the age distribution, menopausal status, and referral procedure and indications for endometrial sampling.

The overall frequency rate in our study is less than the rates that have been reported in studies primarily on postmenopausal bleeding, where the risk of carcinoma is thought to be greater [13,14]. In a study by Verbakel et al[15] found a pooled endometrial cancer risk of ~9% among women with postmenopausal bleeding. The overall frequency in this study was 5.3%, but it was 17.3% for women with postmenopausal bleeding, which confirms the important alerting symptom of postmenopausal bleeding. The higher frequency in this subgroup may be due to selective biopsy of clinically suspicious cases, delayed presentation, or limited early evaluation at primary-care level.

There was a low incidence of endometrial carcinoma in younger women, and an age-related rise thereafter. This is in line with Nees et al[16], who stated that the risk of endometrial cancer or atypical hyperplasia was low in premenopausal women with AUB. This difference in reproductive-age women might be attributed to more functional, hormonal and benign structural causes of bleeding in this age group. Increased age, however, is linked with estrogen exposure for longer periods of time, with anovulatory cycles, metabolic comorbidities, and with an increased risk of endometrial neoplasia, accounting for the increased incidence of carcinoma among women aged ≥50 years.

Benign lesions were the predominant histopathology in this study, such as proliferative and disordered proliferative endometrium, endometrial polyp, and hyperplasia without atypia. This pattern is similar to local histopathological studies, including Riaz et al[17], who reported a wide spectrum of benign endometrial pathologies among Pakistani women presenting with AUB. Minor differences in the relative frequency of individual lesions may be due to differences in age group, inclusion of postmenopausal women, ultrasound-based selection before biopsy, and whether hysterectomy specimens were included along with endometrial biopsies.

There was a significant association between endometrial thickness and carcinoma, with a frequency of more than 15 mm of endometrial thickness in our study. This is biologically plausible because the thickened endometrium also may be a result of hyperplasia, polypoid growth or malignancy, particularly in peri- and postmenopausal women. The measurement of endometrial thickness should be used in conjunction with other factors, however, because other benign conditions can also result in a thickened endometrium, especially in premenopausal women. Thus, the clinical risk profile and bleeding pattern are still relevant for the consideration of biopsy.

In our study, the diabetes mellitus, hypertension and obesity were significantly associated with endometrial carcinoma. These results corroborate those from the literature that associate the metabolic risk factors to endometrial malignancy.[18,19] Obesity contributes through peripheral conversion of androgens to estrogen in adipose tissue, leading to prolonged unopposed estrogenic stimulation of the endometrium. Diabetes and hypertension may be indicative of a more general picture of an insulin resistant state, chronic inflammation and hormonal imbalance. This would help justify a low threshold for endometrial evaluation in AUB patients with metabolic comorbidities.

Parity was not significantly associated with endometrial carcinoma in this study. This differs from literature where nulliparity is considered a recognized risk factor.[20] The difference may be explained by the small number of carcinoma cases, predominance of multiparous women in the sample, and stronger influence of age, postmenopausal status, obesity and diabetes in this cohort.

The strengths of this study include a reasonable sample size, complete one-year data collection and histopathological confirmation of endometrial findings. Important clinical variables such as age, menopausal

status, bleeding pattern, endometrial thickness and metabolic comorbidities were also assessed. However, the study was single-center with non-probability sampling, limiting generalizability. The limited number of cases of carcinoma precluded robust subgroup analysis and some risk factors like hormonal therapy, infertility and family history were not evaluated in detail.

CONCLUSION

Endometrial carcinoma was identified in a clinically important proportion of women presenting with abnormal uterine bleeding at a secondary care hospital. The frequency was higher among older women, postmenopausal patients, those with postmenopausal bleeding, increased endometrial thickness and metabolic comorbidities. These findings emphasize the importance of timely endometrial evaluation and histopathological confirmation in high-risk women with abnormal uterine bleeding to facilitate early diagnosis and appropriate management.

RECOMMENDATION

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:	Kiran Ikram, Naila Raziq
Drafting or Revising Critically:	Salma Naz, Munazza Yousaf
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Evolution the Antibacterial Activity of Ag Nanoparticles Prepared by Green Tea Extract (Camellia Sinensis) Against Salmonella Typhi

Mushtaq Talip Hussein

ABSTRACT

Objective: Estimation of antimicrobial activity of AgPNs using green tea leaves extracts against Salmonella typhi
Study Design: True and controlled in vitro laboratory experimental study
Place and Duration of Study: This study was conducted at the Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Qadisiyah Governorate, Iraq from 10th Aug 2025 to 4th April 2026.
Methods: There were several key procedural steps: preparation of the green tea extract, green synthesis of Ag NPs using the extract, and characterization of the resulting Ag nanoparticles by visual color change and Ultraviolet-Visible Spectroscopy (UV-vis). The morphology and particle size of the Ag nanoparticles were determined by SEM. The antibacterial properties of the GT-AgNPs were estimated using the agar well-diffusion test.
Results: UV-visible spectroscopy provided key confirmation of AgNP synthesis through a visible color change, arising from collective oscillation of the silver nanoparticles' free electrons in resonance with the incident light wave; this produced a characteristic absorption peak at 440 nm. SEM analysis revealed spherical nanoparticles ranging in size from 20 to 30 nm, with a uniform size distribution and consistent morphology. GT-AgNPs tested at concentrations of 20, 40, and 80 mg/ml produced inhibition zones of 9.5, 14, and 20 mm, respectively, against S. typhi.
Conclusion: Green-tea-synthesized Ag nanoparticles show strong, dose-dependent antibacterial activity and represent a promising eco-friendly inhibitor of bacterial growth.

Key Words: antibacterial, tea, synergism, catechins, EGCG

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INTRODUCTION

Nanotechnology is an important field for the scientific and technological development. In recent years, the biomedical sciences and nanotechnology have an intersected and act to develop novel substances with outstanding therapeutic capacity. Among these, AgNPs have appeared as promising materials in antibacterial, catalytic application, and anticancer because of their high surface area-to-volume ratio and unique chemo-physical characters. The synergism action of green tea and silver nanoparticles yields a composite bioactive materials.

[1.] Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Diwaniyah, Iraq.

Correspondence: Mushtaq Talip Hussein, Department of Basic Science, College of Dentistry, Al-Qadisiyah University, Diwaniyah, Iraq.
Contact No: +9647812403390
Email: mushtaqtaliphussein@gmail.com

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These biomaterials were effective against both Gram-positive and Gram-negative and exhibit antibacterial and disease-preventing properties by multi-targeted antibacterial mechanisms like membrane disruption, intracellular oxidative stress generation, and the vital enzyme inhibition.

Conventionally, AgNPs are synthesized through chemical reduction of silver ions in solution or through gas-phase methods at high temperature[1]; however, the use of plant extracts, including green tea leaf extract, has proven successful as a safer, greener alternative route for AgNP synthesis.

Given the combined advantages of eco-friendly nanoparticle synthesis and the intrinsic antibacterial properties of green tea, this study was designed to evaluate the antimicrobial activity of green-tea-synthesized AgNPs against Salmonella typhi isolated from stool samples

Tea is one of the most widely consumed beverages in the world, particularly across tropical and subtropical regions, and is produced from the plant Camellia sinensis, which is cultivated commercially in atleast 30 countries[2]. Green tea, in particular, offers numerous well-documented health benefits: it acts as an

antioxidant, an anti-inflammatory, anticarcinogenic agent, and it contributes positively to cardiovascular and oral health, alongside possessing notable antimicrobial activity[2,1].

Among the different forms of this beverage — including green tea, oolong tea, and black tea — green tea contains the highest concentration of catechins. catechins is natural compound act as powerful anti-oxidant. Numerous studies worldwide have reported the antibacterial activity of green tea and its contribution to disease prevention. This antimicrobial effect is derived mainly from the action of catechins, particularly epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG)[3].

Salmonella enterica species have more than 2,500 serotypes. Infection of human with Salmonella typhi (S. typhi). Other, non-typhoidal Salmonella serotypes can resemble typhoidal Salmonella owing to the similarity of their clinical presentation[4]. Green tea exerts a direct antimicrobial effect against this pathogen and additionally prevents its attachment to intestinal epithelial surfaces. A wide range of bacteria, together with certain fungi and viruses, are susceptible to the antimicrobial activity of green tea[5]. Its principal antibacterial action arises from catechin-mediated damage to the bacterial plasma membrane, alongside additional mechanisms such as inhibition of fatty acid synthesis, suppression of essential enzyme activity, and attenuation of inflammation — including inflammatory processes driven by oxidative stress[6], inactivation of angiotensin II and IL-6-induced C-reactive protein expression[7], suppression of IL-6 (Inter Lecukin-6) and RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand) production in osteoblast-like cells during infection[8], inhibition of IL-8 production[9], and deactivation of hyaluronidase[10]. Owing to their potent antimicrobial activity, silver nanoparticles (AgNPs) are now widely incorporated into consumer products, including medical materials, cleaning agents, and textiles.

This study aims to evaluate the green synthesis, characterization, and biomedical activity of GT AgNPs. By optimizing reaction conditions including pH, temperature and concentrations, this research gives a good evaluation of their structural properties and enhanced antimicrobial activity. this work contributes to addressing the escalating global challenge of microbial resistance.

METHODS

a) Green tea extract preparation: There are many treatments for tea leaves, such as cleaning them with water and alcohol (30%). These leaves are cut into small pieces, ground, and turned into powder through mortar and pestle and dried in the shade. Then store the powder in a dry container until use. The powder (15gm) was mixed with 100ml distilled water in flask to prepare the aqueous extract. Boiling process to the mixer for (5 min), shaking and reduced in temperature. Finally, extract filtered through Whatman No.1 and stored in an airtight container at 4oC until use.

b) Green Tea synthesis of Ag NPs: Several concentrations were (1%, 5%, 10%, 25%, 50% and 100%) (v/v) made from the stock extract solution of tea act as reducing agent. To synthesize the AgNPs, 750 µL of silver nitrate (10 mM) was added drop wise at rate of one drop per second to 15 mL of each concentration of tea extract. The products were stirring constantly at 25, 40 and 55 ◦C, respectively to accelerate the reduction processes. After synthesis, each solution was transferred to centrifugal ultrafiltration (Millipore, Amicon Ultra-15 3k, USA) for purification and concentration. The solutions were centrifuged at 4000 ×g for 2 hrs. Finally AgNPs were rinsed with Milli-Q water (Millipore, 18.2 MΩ cm, USA) to eliminate the unreacted materials.

c) Characterization of Ag nanoparticles: Investigation of Ag nanoparticles can be performed through two methods:

1. Ultraviolet-Visible Spectroscopy (UV-vis): The bio-reduction Ag^+ to Ag^0 was monitored by Ultraviolet-Visible Spectroscopy (UV-vis) (SpectraMax 340 PC, Molecular Devices, Munich, Germany) and reading the absorption spectra (UV-vis) ranged from 300 to 700 nm to detect the surface plasmon resonance peak. The Results were recorded twice after 15 min.

2. Infrared spectrophotometer and XRD: Infrared spectrophotometer (Bruker tensor 27 IR spectrometer) was used to identify the Functional group of green tea. these groups were responsible for capping and reduction of nanoparticles

To analyze the crystalline structures of the samples by an X-ray diffractometer (XRD) (Shimadzu model: XRD 6000 with CuKα radiation with 1.5406ƛ, operated at 40 kV and30 mA). Analysis of the samples shape of particles was performed by a particle size analyzer (Sympatec GmbH,NANOPHOX).

3. The morphology and particle size analysis of Ag nanoparticles by SEM: The particle size and a surface morphology of GT-AgNPs were determined by Scanning Electron Microscopy (SEM). After sonication of GT-AgNPs for 15 min, a small droplet of sample was deposited onto sterilized glass substrate and left to dry at 25^0C to form thin film. The dried material was mounted onto a standard aluminum stub using conductive double-sided carbon tape. The specimen was then examined by using SEM at accelerated voltage of 5.0kV.[11,12,13]

d) Antibacterial properties of GT-AgNP: Briefly, the antibacterial activity of the GT-AgNP solutions was determined using the agar well-diffusion method, as described by Sharma et al[14] with a minor modifications. The bacterial strain was subcultured on Mueller-Hilton agar overnight at 37 °C. The turbidity of the bacterial

suspension was determined and adjusted to match a 0.5 McFarland standard. The antimicrobial activities of AgNPs with green tea extract in different concentratios (20, 40 and 80 µg/ml) were tested and compared with antibiotics (Cefixime 5 µg, Azithromycin 15 µg and Chloramphenicol 30 µg) (as positive control) by using the agar well diffusion method. The strain was plated on a sterile Mueller-Hinton agar plate, and wells were punched into medium and wells were filled with 100 µl of antibiotic or AgNPs with extract, distilled water was represented a negative control in one well. They were made in sterile conditions in a laminar cabinet, with three replicates . Aerobically, all plates were incubated for 24 hrs at 37 °C. Inhibitory Zone of antibiotics, and GT AgNPs were read in mm.

e) Deterimination of MIC and MBC: The broth microdilution method was used to determine the lower antibacterial concentration of AgNPs has ability to inhibited or kill bacteria.

f) Statistical analysis: Statistical analysis was performed by usage of standard error, 3 replications for all antibacterial assays were performed.

RESULTS

a) Color changing to dark brown with in 30 min refer to rapid reduction of silver ions

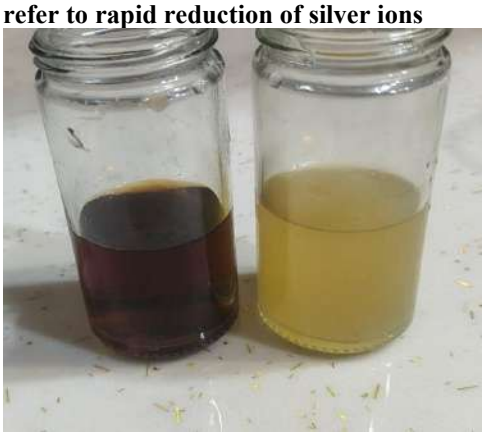

Figure No. 1: The color changing of solution to brown due to the reduced process of Ag+- Ag nanoparticle.

b) UV–Vis spectral analysis of silver nanopartical with green tea: UV-visible spectroscopy provided key confirmation of AgNP synthesis and was accompanied by a visible change in color. Mixing the green tea extract with $AgNO_3$ in aqueous solution produced this color change, which resulted from collective oscillation of the silver nanoparticles' free electrons in resonance with the incident light wave, giving rise to a characteristic absorption peak. Figure (2) shows this peak at 440 nm.

c) Functional groups analysis: The FT-IR spectra of the AgNPs with green tea extract are shown in figure 3. Table 1 showed several absorption bands were obtained from the FTIR spectrum; each band is a functional group of extract surrounding Ag particles, which plays role in their reduction and fixation of them.

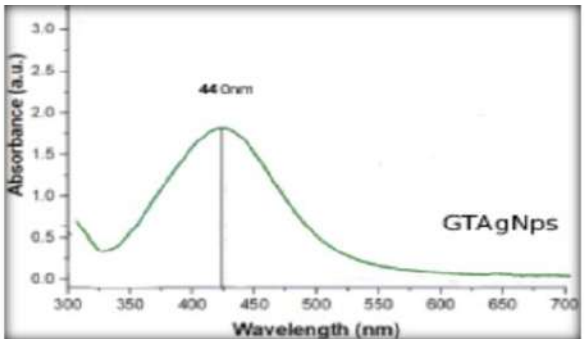

Figure No. 2: The peak (440nm) was indicated on the UV-visible spectrum between 350–500 nm showed GT-AgNPs formation

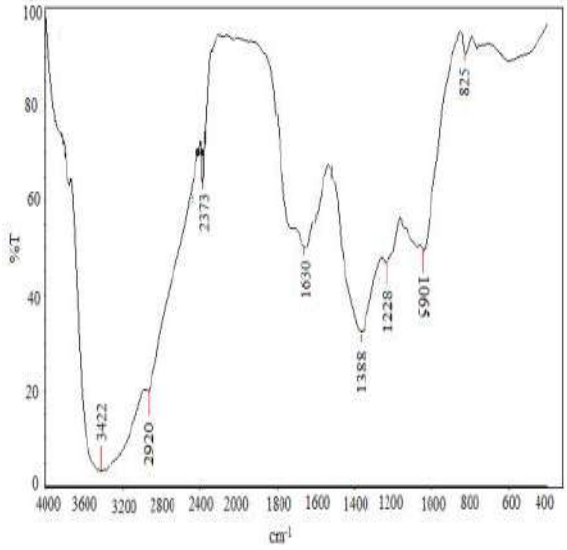

Figure No.3: FTIR spectrum of GT AgNPs shows peaks and their corresponding functional groups

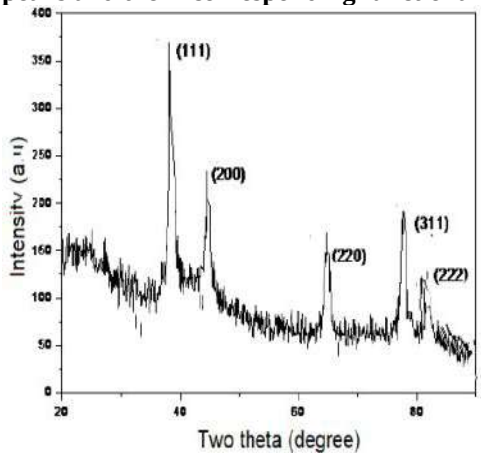

Figure No. 4: The XRD spectra of AgNPs with green tea extracts

d) XRD analysis: Fig. 4. shows the XRD spectra of AgNPs with green tea extracts . Many peaks appeared at 2θ values of 38.2, 44.4, 64.6, 77.6 and 81.6 compatible to the (111), (200), (220), (311) and (222) crystallographic planes respectively. This confirms the

face-centered cubic structure of silver which match ES the standard reference data of (JCPDS No. 87-0720).

Table No. 1: FTIR peak assignment and corresponding functional groups for GT AgNPs

Observed wave number (cm^{-1})	Functional groups	Vibration type	Extend bimolecular source in green tea extract
3422	-OH	Stretching	Phenols, flavonoids, and alcohols
2920	C-H	Stretching	Hydrocarbon compound and fatty acide
2373	N-H	Stretching	Protein and bio amines
1630	C=O	Stretching (or Amide Iband)	Protein (enzyme) and amino acide
1388	C-N	Stretching	Aromatic amines or nitrogenous compound
1228	C-O	Stretching	Ether and ester
1065	C-O	Stretching	Polyos (polysaccharides) and phenols
825	Aromatic C-H	Out of plane bending	Aromatic rings of polyphenols

e) Scanning Electron Microscopy (SEM)

SEM was used to determine the size and shape of the biogenic AgNPs. Figure (5) shows the appearance of spherical nanoparticles ranging in size from 20 to 30 nm, with no direct contact between adjacent particles an effect attributable to the capping action of phytochemicals in the extract, which also mediate reduction and stabilization of the metal nanoparticles. The SEM results further showed a uniform size distribution among the silver nanoparticles

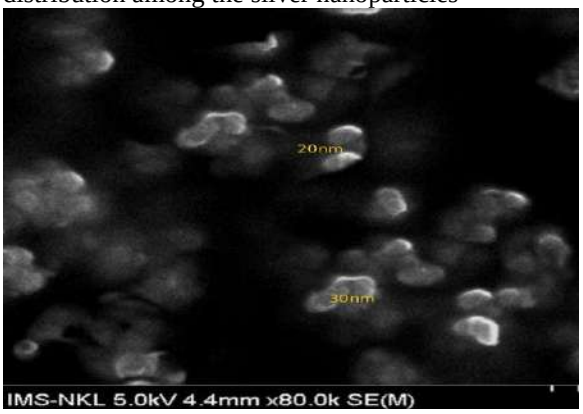

Figure No. 5: Detection of the morphology and size of biogenic AgNPs. The appearance of spherical nanoparticles in several sizes extended from 20 - 30 nm

f) Antibacterial activity of solutions: As summarized in Table 2, GT-AgNPs at concentrations of 20, 40, and 80 µg/ml were tested against S. typhi, producing inhibition zones of 9.5 ± 0.3, 14 ± 1.5, and 20 ± 1.15 mm, respectively. GT-AgNPs showed good inhibitory activity at all concentrations tested, with the lower concentrations producing comparatively slight inhibition against Salmonella Typhi. Figure 6 & 7.

Table No. 2: Inhibition zone diameters produced by GT-AgNPs against S. typhi

GT-AgNP concentration	Inhibition zone (mm)± SR
20 µg/ml	9.5 ± 0.3
40 µg/ml	14 ± 1.5
80 µg/ml	20 ± 1.15

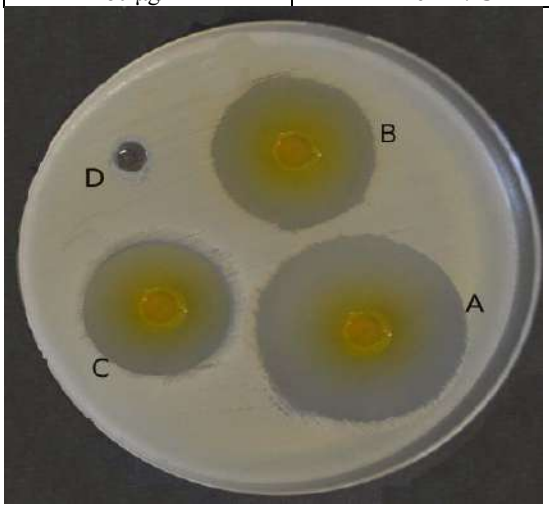

Figure No. 6: Inhibitory zone produced by GT AgNP in three concentrations : (A) 80 mg (B) 20 mg C) 40 mg (D) control

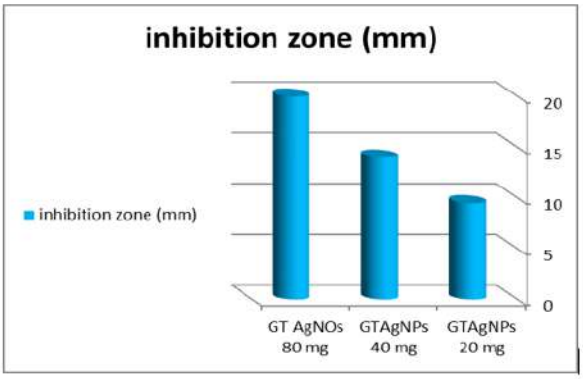

Figure No. 7: antibacterial activity of green tea with Ag nanoparticles in different concentrations (80 mg 40mg and 20 mg) against S. typhi.

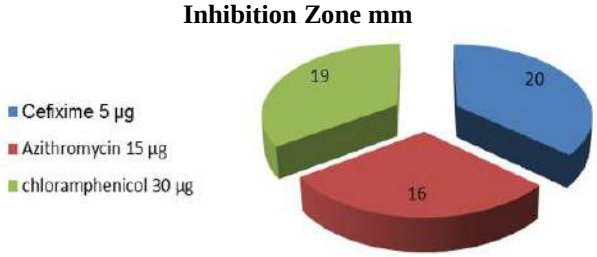

Figure No. 8: The results of the antibiotic sensitivity test towards Salmonella Typhi.

The antibacterial activity of three antibiotics commonly used in the treatment of S. typhi infection was also evaluated by the disk-diffusion method, and the results are shown in the and figure (8). The zone of inhibition produced by Cefixime 5 µg (20 mm) was larger than that of the other antibiotics tested (Azithromycin 15 µg (16 mm) and chloramphenicol 30 µg(19mm), and when compared with GT-AgNPs at 80 µg, the GT-AgNPs showed a comparably strong inhibitory action against bacterial growth. Table (3).

Table No. 3: Inhibition zone diameters of GT-AgNPs (80 mg/ml) compared with standard antibiotics against S. typhi

Antimicrobial agent	Zone (mm)
Cefixime 5 µg	20
Azithromycin 15 µg	16
Chloramphenicol 30 µg	19
GT-AgNPs 80 mg/ml	**20**

g) Determination the MIC and MBC of SA AgNPs.

The MIC and MBC of GT AgNPs were done by well diffusion method to estimated their activity. The concentrations action of the AgNPs were registered in Table (4). The inhibitory activity was started at 10 µg/ml with visible inhibitory growth while MBC is represent in concentration 20µ/ml. The results of MIC and MBC were showed GT AgNPs can be suggested as control and prevent materials against teeth caris and periodontal defects.

Table No. 4: MIC and MBC of SA AgNPs toward Salmonella typhi

AgNPs	Conc. µg/ml
	2.5
	5
MIC	10
MBC	20
	40
	80

DISCUSSION

The UV-visible absorption spectrum confirmed that the surface plasmon resonance peak of the green-tea-synthesized AgNPs at 440 nm. Silver nanoparticle absorption bands are typically reported in the 400–450 nm range, and the peak obtained in the present study falls within this range and this in agreement with Grand et al[15], who attributed such bands to the excitation of surface plasmon vibrations. Likewise, the spherical morphology and average particle size of 20–30 nm obtained by SEM are in full agreement with Agnihotri et al[16], who reported comparably sized, well-dispersed AgNPs synthesized by a similar green-reduction approach. The present findings also agree with Zhang et al[17], who reported that smaller AgNPs exert a stronger antibacterial effect than larger particles; this is consistent with the pronounced inhibitory activity observed here at the smallest effective particle size range.

Identification of the biomolecules was performed by the FT-IR analysis. These molecules were responsible for the reduction of (Ag^+) and the subsequent capping of the synthesized silver nanoparticles (AgNPs) as well as prevent AgNPs aggregation and made them more stabilizing agents. The presence of diverse functional groups of the green tea extract represent by absorption bands. This result was in agreement with Worakitjaroenphon[18].

The alignment of the peaks at the specific angles with the standard reference confirms that the resulting material is pure silver. The arrangement of the peaks reflects the crystalline behavior of the silver produced through chemical reduction.

The sharpness of the peaks shown in the figure indicates that the nanoparticles possess a high degree of crystallinity and are not amorphous.

The absence of unusual or additional peaks indicates that the material is of high purity and that the extract acts as a surface-capping agent without being incorporated into the silver crystal structure. This result corresponding with result of Selvaraj.[19]

Recently, Lara et al[20] reported that non-hazardous AgNPs can be synthesized by a low-cost, biologically active route and applied as effective antibacterial agents, a conclusion the present results support. Another study similarly demonstrated antimicrobial action of AgNPs against Gram-negative bacteria, evidenced by clearly visible inhibition zones. The present study likewise showed a direct, concentration-dependent relationship between AgNP dose and inhibition zone diameter, in full agreement with the dose–response relationship reported by Sondi and Salopek-Sondi[20].

Several theories have been proposed to explain the antimicrobial activity of AgNP solutions. Some attribute the effect to increased permeability of the bacterial cell membrane, leading to leakage of cellular components and eventual cell death, while another theory attributes it to the generation of free radicals that disrupt the cell membrane by dissipating the proton motive force[21]. These mechanistic accounts are only partially consistent with one another, and the precise mode of action of AgNPs therefore remains an open question that the present study, being focused on activity rather than mechanism, cannot resolve.[22,23]

CONCLUSION

AgNPs consider as promising candidate materials in antibacterial field especially with Green tea extract. It served as an effective, low-cost, and eco-friendly substance. AgNPs (20–30 nm) exhibited clear, dose-dependent antibacterial activity against Salmonella typhi, with the 80 µg/ml concentration producing an excellent inhibition zone comparable to that of the standard antibiotic Cefixime.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mushtaq Talip Hussein
Drafting or Revising Critically:	Mushtaq Talip Hussein
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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

Source of Funding: None

Ethical Approval: No.2481 Dated 10.08.2025

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Original Article

Effectiveness of applying Intradialytic Stretching Exercises on Reducing Lower Limbs Muscle Cramps among Haemodialysis Patients

Abdallah Jabbar Mahmood AL-Mtiwti[1] and Saad Hussein Murad AL-Shammary[2]

ABSTRACT

Objective: To evaluate the effectiveness of intradialytic stretching exercises in reducing lower-limb muscle cramps among patients undergoing maintenance hemodialysis.

Study Design: Quasi-experimental pre-test–post-test control group study.

Place and Duration of Study: This study was conducted at selected hemodialysis centers in Mosul, Iraq, from 25th February 2026 to 25th April 2026.

Methods: A purposive sample of 60 adult patients receiving maintenance hemodialysis was recruited and allocated into a study group (n = 30) and a control group (n = 30). The study group performed a structured intradialytic stretching exercise program during the second hour of each dialysis session in addition to routine nursing care, whereas the control group received routine care only. Muscle cramp severity was assessed using the Muscle Cramp Questionnaire, and pain intensity was measured using the Visual Analogue Scale (VAS). Data were analyzed using descriptive and inferential statistics, including the Friedman, Wilcoxon signed-rank, and Mann–Whitney U tests, with statistical significance set at $p < 0.05$.

Results: Patients who participated in the stretching exercise program demonstrated significant reductions in muscle cramp severity and pain intensity compared with the control group ($p < 0.001$). At the final assessment, 83.3% of participants in the intervention group reported no muscle cramps, while only minimal improvement was observed in the control group. The intervention also improved patient comfort and tolerance of hemodialysis sessions.

Conclusion: Intradialytic stretching exercises are a safe, simple, and effective nurse-led intervention for reducing lower-limb muscle cramps among maintenance hemodialysis patients. Incorporating this intervention into routine hemodialysis care may improve symptom management and enhance patients' quality of life.

Key Words: Hemodialysis; Intradialytic Stretching Exercises; Muscle Cramps; Non-Pharmacological Intervention; Nursing Care; Quasi-Experimental Study.

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INTRODUCTION

Chronic kidney disease (CKD) is a major global public health challenge, affecting more than 850 million people worldwide and contributing substantially to morbidity, mortality, and healthcare expenditures [1]. As CKD progresses to end-stage kidney disease (ESKD), renal replacement therapy becomes essential, with hemodialysis remaining the most widely used treatment modality despite advances in kidney transplantation and peritoneal dialysis[2,3]. Although life-sustaining, hemodialysis is frequently accompanied by intradialytic complications that negatively influence treatment tolerance, patient comfort, and quality of life[4].

Muscle cramps are among the most common and distressing complications experienced during hemodialysis, with reported prevalence ranging from 30% to 80% depending on patient characteristics and diagnostic criteria[5].

These painful involuntary contractions predominantly affect the calf and foot muscles, often resulting in premature termination of dialysis sessions, inadequate fluid removal, reduced dialysis efficiency, sleep disturbances, and diminished health-related quality of life [6]. The underlying mechanisms are multifactorial and include rapid ultrafiltration, intradialytic hypotension, electrolyte imbalance, impaired muscle perfusion, and neuromuscular dysfunction [7].

Pharmacological treatments such as vitamin supplementation, L-carnitine, gabapentin, and

[1]MSc Student / Assistant Professor[2], College of Nursing, University of Mosul, Mosul, Iraq

Correspondence: Mr. Abdallah Jabbar Mahmood AL-Mtiwti, MSc Student, College of Nursing, University of Mosul, Mosul, Iraq.
Contact No: +964-7510667405
Email: abdallah.24nup4@student.uomosul.edu.iq

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hypertonic solutions have demonstrated inconsistent effectiveness and may be associated with adverse effects or increased treatment costs [8]. Consequently, non-pharmacological interventions have gained increasing attention. Intradialytic stretching exercises represent a simple, inexpensive, and nurse-led intervention designed to improve muscle flexibility, enhance local circulation, reduce neuromuscular excitability, and alleviate cramp severity during dialysis sessions[9]. However, high-quality evidence supporting their routine implementation remains limited, particularly in Middle Eastern dialysis settings. Therefore, this study aimed to evaluate the effectiveness of intradialytic stretching exercises in reducing lower-limb muscle cramps among patients undergoing maintenance hemodialysis[10].

METHODS

Study Design: A quasi-experimental study with a pre-test–post-test control group design was conducted to evaluate the effectiveness of intradialytic stretching exercises in reducing lower-limb muscle cramps among patients undergoing maintenance hemodialysis. The study was carried out in selected hemodialysis centers in Mosul, Iraq, between 25 February to 25 April 2026. Patients in the intervention group received a structured intradialytic stretching exercise program in addition to routine hemodialysis care, whereas the control group received routine care alone.

Study Setting: The study was conducted in the pediatric wards of five public healthcare institutions in Mosul City, Iraq. Mosul General Hospital, Ibn Al-Atheer Teaching Hospital, and Al-Khansa Teaching Hospital were assigned to the experimental group, while Al-Salam Teaching Hospital and Ibn Sina Teaching Hospital served as the control group. These hospitals provide specialized pediatric healthcare services and employ registered nurses responsible for monitoring and managing hospitalized children.

Participants and Sampling: A purposive sample of 60 adult patients receiving maintenance hemodialysis was recruited and equally allocated into a study group (n = 30) and a control group (n = 30). Eligible participants were adults (≥18 years) diagnosed with end-stage kidney disease, receiving regular hemodialysis for at least three months, experiencing recurrent lower-limb muscle cramps during dialysis, able to communicate effectively, and willing to participate by providing written informed consent. Patients were excluded if they had unstable cardiovascular disease, severe cognitive impairment, acute neurological or musculoskeletal disorders limiting lower-limb movement, recent lower-limb surgery or trauma, severe peripheral vascular disease, or any contraindication to stretching exercises.

Intervention: Participants in the intervention group underwent a standardized intradialytic stretching exercise program performed during the second hour of each hemodialysis session under the supervision of trained nurses. The program consisted of passive and active stretching exercises targeting the calf, hamstring, quadriceps, ankle, and foot muscles. Each stretching position was maintained for approximately 20–30 seconds and repeated three times with short rest intervals. The intervention was implemented during every dialysis session throughout the study period. The control group continued to receive routine nursing and dialysis care without structured exercise.

Outcome Measures: The primary outcome was the severity of lower-limb muscle cramps, assessed using the Muscle Cramp Questionnaire. Pain intensity associated with muscle cramps was evaluated using the Visual Analogue Scale (VAS). Demographic characteristics, clinical history, hemodialysis-related variables, chronic medical conditions, lifestyle factors, and laboratory electrolyte values were also collected using a structured questionnaire.

Data Collection Procedure: Baseline assessments were completed before implementation of the intervention. Follow-up assessments were conducted immediately after completion of the intervention (post-test I) and again during the subsequent evaluation period (post-test). All assessments were performed by trained researchers using standardized data collection procedures to ensure consistency across both groups.

Validity and Reliability: The study instrument was reviewed by an expert panel specializing in nephrology nursing, medical-surgical nursing, and research methodology to establish content validity. Reliability testing demonstrated satisfactory internal consistency and stability, indicating that the instrument was appropriate for assessing muscle cramps and pain among hemodialysis patients.

Statistical Analysis: Data was analyzed using IBM SPSS Statistics version 27. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were summarized using frequencies and percentages. Baseline characteristics between groups were compared using the independent-samples t-test or Mann–Whitney U test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Changes in muscle cramp severity and pain scores over time were analyzed using the Friedman test and Wilcoxon signed-rank test for within-group comparisons, while between-group differences were evaluated using the Mann–Whitney U test. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Patients who participated in the stretching exercise program demonstrated significant reductions in muscle cramp severity and pain intensity compared with the control group ($p < 0.001$). At the final assessment, 83.3% of participants in the intervention group reported no muscle cramps, while only minimal improvement was observed in the control group. The intervention also improved patient comfort and tolerance of hemodialysis sessions.

Table No. 1: Baseline Characteristics of the Participants

Variable	Study	Control	Total	P value
Age (years), Mean ± SD	40.37 ± 8.89	41.87 ± 10.01	41.12 ± 9.46	0.54
Male, n (%)	20 (66.7)	19 (63.3)	39 (65.0)	0.79
Urban residence, n (%)	18 (60.0)	17 (56.7)	35 (58.3)	0.80
Normal BMI, n (%)	17 (56.7)	15 (50.0)	32 (53.3)	0.86
Overweight, n (%)	9 (30.0)	12 (40.0)	21 (35.0)	
Obese, n (%)	3 (10.0)	2 (6.7)	5 (8.3)	
Underweight, n (%)	1 (3.3)	1 (3.3)	2 (3.3)	

Table No. 2: Changes in Muscle Cramp Outcomes in the Study Group

Outcome	Pre-test	Post-test I	Post-test II
No muscle cramps, n (%)	0 (0.0)	11 (36.7)	25 (83.3)
Muscle cramps present, n (%)	30 (100.0)	19 (63.3)	5 (16.7)
Moderate pain (VAS 4–6), n (%)	18 (60.0)	9 (30.0)	0 (0.0)
Severe pain (VAS 7–10), n (%)	12 (40.0)	0 (0.0)	0 (0.0)
No pain, n (%)	0 (0.0)	11 (36.7)	25 (83.3)
Warm lower limb, n (%)	0 (0.0)	25 (83.3)	30 (100.0)
Unbearable discomfort, n (%)	11 (36.7)	0 (0.0)	0 (0.0)

Table No. 3: Summary of the Effectiveness of Intradialytic Stretching Exercises

Outcome	Study Group	Control Group	P value
Participants without muscle cramps at final follow-up	25 (83.3)	Minimal change	<0.001
Moderate/Severe muscle cramps	Markedly reduced	No significant reduction	<0.001
Pain severity (VAS)	Significant reduction	No significant change	<0.001
Muscle discomfort	Significant improvement	No significant change	<0.001
Overall intervention effect	Highly effective	Routine care only	<0.001

DISCUSSION

The present quasi-experimental study demonstrated that intradialytic stretching exercises significantly reduced the severity of lower-limb muscle cramps and associated pain among patients undergoing maintenance hemodialysis. Participants who received the stretching exercise program showed progressive improvement from baseline to both post-intervention assessments, whereas the control group exhibited little or no meaningful change. These findings support the effectiveness of structured intradialytic stretching exercises as a safe, simple, and cost-effective nursing intervention for managing one of the most frequent complications experienced during hemodialysis.

Muscle cramps remain a common and distressing intradialytic complication, affecting approximately one-third to four-fifths of hemodialysis patients worldwide. They frequently lead to discomfort, premature interruption of dialysis sessions, inadequate ultrafiltration, reduced dialysis efficiency, and impaired quality of life. Although the exact mechanisms remain incompletely understood, muscle cramps are believed to result from rapid fluid removal, intradialytic hypotension, electrolyte disturbances, tissue hypoperfusion, and neuromuscular hyperexcitability. Therefore, interventions capable of improving muscle flexibility and local circulation have considerable clinical importance [11,12.]

The substantial reduction in muscle cramp severity observed in the intervention group is consistent with previous studies evaluating exercise-based interventions during hemodialysis. Stretching exercises improve muscle extensibility, increase range of motion, enhance peripheral blood circulation, and reduce muscle stiffness, thereby decreasing the susceptibility of skeletal muscles to involuntary contractions. Furthermore, regular stretching may improve neuromuscular coordination and delay muscle fatigue, resulting in fewer cramp episodes during dialysis sessions [13,14].

Pain intensity also declined markedly following implementation of the stretching program. This finding is clinically relevant because pain associated with muscle cramps often contributes to treatment intolerance, anxiety, and decreased adherence to prescribed dialysis sessions. Stretching exercises may reduce pain through improved muscle oxygenation, decreased ischemia, enhanced venous return, and activation of endogenous pain-modulating mechanisms. The reduction in pain observed in this study agrees with previous investigations reporting that intradialytic exercise effectively alleviates musculoskeletal

discomfort and improves patient comfort without increasing adverse events [15,16.]

Another important finding was the progressive improvement between the first and second post-intervention assessments, suggesting that the beneficial effects of stretching exercises were sustained over time. Continuous implementation during successive dialysis sessions likely promoted gradual physiological adaptation, increased muscle flexibility, and improved tolerance to ultrafiltration-induced physiological stress. This observation supports recommendations that exercise interventions should be incorporated into routine dialysis care rather than applied as isolated sessions. Similar long-term benefits have been reported in previous exercise studies involving hemodialysis patients [17,19].

The favorable outcomes observed in this study also highlight the important role of nurses in delivering evidence-based non-pharmacological interventions. Unlike pharmacological therapies such as quinine, gabapentin, vitamin supplementation, or L-carnitine, stretching exercises require minimal resources, are inexpensive, and carry virtually no risk of medication-related adverse effects[20,21]. Nurse-supervised exercise programs can therefore represent an effective strategy for improving patient outcomes while reducing healthcare costs and medication burden. Recent nephrology guidelines increasingly encourage individualized physical activity programs as part of comprehensive dialysis care [22-23]

The effectiveness of the intervention may also be explained by the structured nature of the stretching protocol. Performing exercises during the second hour of hemodialysis allows patients to complete the activities before substantial ultrafiltration-related physiological changes occur, while maintaining adequate hemodynamic stability. Stretching of the calf, hamstring, quadriceps, ankle, and foot muscles directly targets the muscle groups most frequently affected by dialysis-related cramps, thereby maximizing therapeutic benefit. Similar protocols have demonstrated improvements in muscle function, physical performance, and symptom control in patients receiving maintenance hemodialysis [24.]

Despite these encouraging findings, several limitations should be acknowledged. The study was conducted in a limited number of dialysis centers using purposive sampling and a relatively small sample size, which may reduce the generalizability of the findings. Additionally, longer follow-up periods would be valuable to determine whether the beneficial effects of stretching exercises persist over several months. Future multicenter randomized controlled trials with larger populations and objective physiological outcome measures are recommended to further establish the effectiveness of intradialytic stretching exercise programs [25.]

Overall, the present findings indicate that intradialytic stretching exercises constitute an effective nursing intervention for reducing lower-limb muscle cramps and pain among patients undergoing maintenance hemodialysis. Incorporating structured stretching exercises into routine dialysis care has the potential to improve treatment tolerance, enhance patient comfort, and promote better clinical outcomes.

CONCLUSION

The findings of this study demonstrate that intradialytic stretching exercises are an effective non-pharmacological intervention for reducing lower-limb muscle cramps among patients undergoing maintenance hemodialysis. Patients who participated in the structured stretching exercise program experienced significant reductions in muscle cramp severity and pain intensity compared with those receiving routine care. The intervention also contributed to improved patient comfort and greater tolerance of hemodialysis sessions. Because stretching exercises are simple, safe, inexpensive, and can be performed under nursing supervision without specialized equipment, they represent a practical strategy for routine clinical practice. Integrating intradialytic stretching exercises into standard hemodialysis care may enhance symptom management, improve patients' quality of life, and support more effective dialysis treatment.

Recommendations: IPEWS should be integrated into routine pediatric nursing practice to support early recognition of clinical deterioration and improve patient safety. Ongoing education through training programs, workshops, and competency assessments is recommended to enhance nurses' knowledge and skills. Incorporating IPEWS into nursing curricula may further strengthen evidence-based pediatric care.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Abdallah Jabbar Mahmood AL-Mtiwti, Saad Hussein Murad AL-Shammary
Drafting or Revising Critically:	Abdallah Jabbar Mahmood AL-Mtiwti, Saad Hussein Murad AL-Shammary
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Dydrogesterone Use and Pregnancy-Associated Urinary Tract Infection: Associations with Hormonal, Cytokine, Renal and Bacterial Profiles

Lina Qays Yaseen[1], Reyam F.Saleh[1], Shaymaa M.Murshed[2], Firas Faris Rija[1] and Bilal A Alarabi[1]

ABSTRACT

Objective: To examine the association between dydrogesterone use and UTI in pregnant women, with attention to reproductive hormones, the cytokine markers Inter Leukin-17 (IL-17) and Matrix Metalloproteinase-1 (MMP-1), renal indices, bacterial etiology, and antimicrobial susceptibility.

Study Design: Experimental study.

Place and Duration of Study: This study was conducted at the Department of Microbiology, College of Science, Tikrit University, Salah al-Din, Iraq, from 12th May 2025 to 30th August 2025.

Methods: In this experimental study at Tikrit University and Tikrit Teaching Hospital, pregnant women were grouped by UTI status and dydrogesterone exposure. Serum estrogen. The levels of progesterone, IL-17, MMP-1, urea and creatinine were measured with ELISA. Urine from UTI Positive women was cultured, the isolates identified with the VITEK 2 system and susceptibility data was obtained. Was interpreted according to CLSI guidelines.

Results: Results: Estrogen was significantly elevated in the dydrogesterone group, especially when considering the UTI group. This difference remained after taking account of maternal age and pregnancy into the positive sub-group. stage. No significant difference was found for the other five metabolites: progesterone, IL-17, MMP-1, urea, and creatinine. UTI The occurrence and distribution of bacteria were not significantly related to susceptibility patterns. exposure.

Conclusion: The use of dydrogesterone was not significantly associated with UTI or renal, immune, or other complications. or microbiological change. The immune-endocrine profile is most likely to be high because of the increased oestrogen level of this type of profile. The interaction is not as a direct effect of the drug, but as an interaction occurring while the baby is in the mother's womb.

Key Words: Dydrogesterone, Duphaston, UTI, Pregnancy, IL-17, MMP-1, estrogen.

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INTRODUCTION

One of the most common bacterial complications of pregnancy is urinary tract infection or UTI. has clinical significance as undetected asymptomatic bacteriuria may lead to cystitis or Pyelonephritis and fetal and maternal effects. Pregnancy makes one more vulnerable: urinary: In the presence of stasis, dilatation of the ureters, incomplete bladder emptying and host defense changes in pregnancy. bacterial persistence.

The focus of UTI here is not just an issue that happened locally but is a result of the virulence of the bacteria, susceptibility to antimicrobial agents, immune regulation, endocrine status, and renal function adaptation.[1-3] Escherichia coli remains the predominant uropathogen, but Staphylococcus aureus, Streptococcus agalactiae, Klebsiella spp., and other organisms occur at frequencies varying by local epidemiology and prior antibiotic exposure.

Because agent choice is constrained by fetal safety, gestational age, and regional resistance, culture-based identification and susceptibility testing remain indispensable.[3-5] The oral synthetic progestogen, dydrogesterone (Duphaston), is used in early. Threatened miscarriage, recurrent pregnancy loss, and luteal support are all pregnancy indications for which

[1] Assistant Professor, Department of Biology, College of Science, Tikrit University, Salah al-Din, Iraq.
[2] Assistant Professor, Department of Microbiology, College of Pharmacy, Tikrit University, Salah al-Din, Iraq.

Correspondence: Reyam F. Saleh, Assistant Professor, Department of Microbiology, College of Science, Tikrit University, Salah al-Din, Iraq.
Contact No: +964 772 19 3013
Email: reyamfarissaleh@gmail.com

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CM is indicated. [6-8] Its biological reach may go further than supplementation: reproductive immunology has placed it. As a candidate immunomodulator via cytokine controls and tolerization of the mother-fetal axis.[9-13] Successful gestation is achieved by finely-tuned immune regulation, not by general However, hormones, cytokines, and immune cells maintain fetal tolerance, and suppression is what is needed to avoid it. Preserving antimicrobial defense.[14] Most of such evidence, however, is from reproductive immunology cohorts (not infection) and a direct link between dydrogesterone and UTI. It has not been determined if it has been established.

To assess whether dydrogesterone exposure occurs at a time of inflammation, measurements of IL-17 and MMP-1 were taken. or tissue-remodeling activity. In contrast, IL-17 promotes recruitment and mucosal defence of neutrophils but in, when considering the use of pregnancy, maternal-fetal tolerance must be taken into account.[13,14] MMP-1, an interstitial collagenase, involved in matrix turnover, placental remodeling, and inflammation, and cervical remodeling. Both the intrauterine cytokine levels are associated with metalloproteinase activity.[15-17] Estrogen Progesterone and androstenedione, in addition to their reproductive functions, also affect immune function and are interconverted. Signaling by cytokines during gestation.[14,18] The present study, therefore, evaluated the association of dydrogesterone with the bacteria in UTI is not known in pregnant women, and antimicrobial resistance is not known. Susceptibility, IL-17, MMP-1, urea, creatinine, estrogen, and progesterone.

METHODS

Study design and participants: This experimental study was conducted at Tikrit University and Tikrit City. Teaching Hospital was approved by the institutional ethics committee (Ref. SCITUH-0012, 05 - 1-2025) written informed consent was obtained from all participants. The study included 35 pregnant women classified by UTI status and dydrogesterone exposure. Dydrogesterone users included 20 UTI-positive women and 5 women with no bacterial growth; non-users included 6 UTI-positive women and 4 women with no bacterial growth. The UTI status has been created bacteriologically; dydrogesterone exposure was considered to be if the use was documented or reported. Maternal Adjusted analyses accounted for age and stage of pregnancy. Data on treatment indication, dose, duration, prior UTI, obstetric history, and recent antibiotics were incompletely available and considered when interpreting results.

Sample collection and assays: Peripheral venous blood was drawn aseptically, allowed to clot, and centrifuged to recover serum. Midstream clean-catch urine from women with suspected or confirmed UTI was processed promptly; the sediment was examined microscopically, and quantitative culture confirmed significant bacteriuria. Serum MMP-1, IL-17, estrogen, progesterone, urea, and creatinine were measured by enzyme-linked immunosorbent assay (ELISA) using kits from Sunlong Biotech Co., Ltd. following the manufacturer's instructions, with concentrations read from analyte-specific standard curves.

Bacterial identification and susceptibility: Isolates from UTI-positive women were identified with the VITEK 2 automated system. Susceptibility was classified as sensitive, intermediate, or resistant according to Clinical and Laboratory Standards Institute (CLSI) criteria. Susceptibility was tested against a 15-agent panel (detailed in figure 1).

Statistical analysis: Analyses were performed in SPSS version 26. Continuous variables were expressed as mean ± standard deviation and categorical variables as frequencies and percentages. Groups were compared with independent-samples or Welch's t-tests, and categorical variables with chi-square or Fisher's exact tests. The dydrogesterone-UTI association was summarized as odds ratios (ORs) with 95% confidence intervals (CIs); multivariable logistic and linear regression adjusted for maternal age and pregnancy stage, and Hedges' g provided standardized mean differences. Given the exploratory design and small subgroups, P-values were interpreted alongside effect sizes and confidence intervals. All tests were two-tailed, with $P < 0.05$ regarded as significant.

RESULTS

Across the measured serum markers, dydrogesterone use was associated with a significantly higher estrogen concentration, whereas progesterone, IL-17, MMP-1, urea, and creatinine did not differ significantly between users and non-users. The nonsignificant immune and renal comparisons showed small effect sizes and wide confidence intervals, supporting cautious interpretation rather than evidence of a clear systemic inflammatory or renal shift.

Within the UTI-positive subgroup, the estrogen difference remained statistically significant in both unadjusted analysis and after adjustment for maternal age and pregnancy stage. This indicates that the estrogen signal was not fully explained by these two maternal characteristics, although residual confounding cannot be excluded.

Dydrogesterone use was not significantly associated with UTI occurrence, and the confidence interval around the odds ratio was wide. Among women with UTI, bacterial distribution also did not differ significantly by dydrogesterone use; Staphylococcus aureus was the most frequently recovered organism overall, followed by Escherichia coli and Streptococcus agalactiae.

Table No. 1: Hormonal, immune, and renal parameters according to dydrogesterone use.

Parameter	Dydrogesterone users (Mean ± SD)	Non-users (Mean ± SD)	Mean difference	95% CI	P-value	Hedges' g
Estrogen	203.04 ± 145.36	114.60 ± 92.78	88.44	3.53 to 173.35	0.042*	0.65
Progesterone	66.44 ± 33.67	54.20 ± 21.33	12.24	-7.35 to 31.83	0.210	0.39
MMP-1	29.39 ± 13.26	31.41 ± 18.79	-2.01	-16.10 to 12.07	0.762	-0.13
IL-17	90.24 ± 20.62	94.87 ± 17.37	-4.63	-18.97 to 9.72	0.508	-0.23
Urea	28.68 ± 4.31	27.90 ± 5.22	0.78	-3.21 to 4.77	0.681	0.17
Creatinine	0.62 ± 0.11	0.58 ± 0.15	0.04	-0.07 to 0.15	0.451	0.33

Values are presented as mean ± standard deviation unless otherwise indicated. CI, confidence interval. *P < 0.05.

Table No. 2: Unadjusted and adjusted association between dydrogesterone use and estrogen among UTI-positive women.

Analysis	Comparison	Group 1 value	Group 2 value	Difference	95% CI	P-value	Hedges' g
Unadjusted	UTI + dydrogesterone (n=20) vs UTI + no dydrogesterone (n=6)	210.45 ± 156.41	99.33 ± 64.67	111.12	19.98 to 202.26	0.019*	0.76
Adjusted	UTI + dydrogesterone vs UTI + no dydrogesterone	-	-	139.75	20.55 to 258.95	0.022*	-

The adjusted model included maternal age and pregnancy stage. Welch's t-test was used for the unadjusted comparison. CI, confidence interval. *P < 0.05.

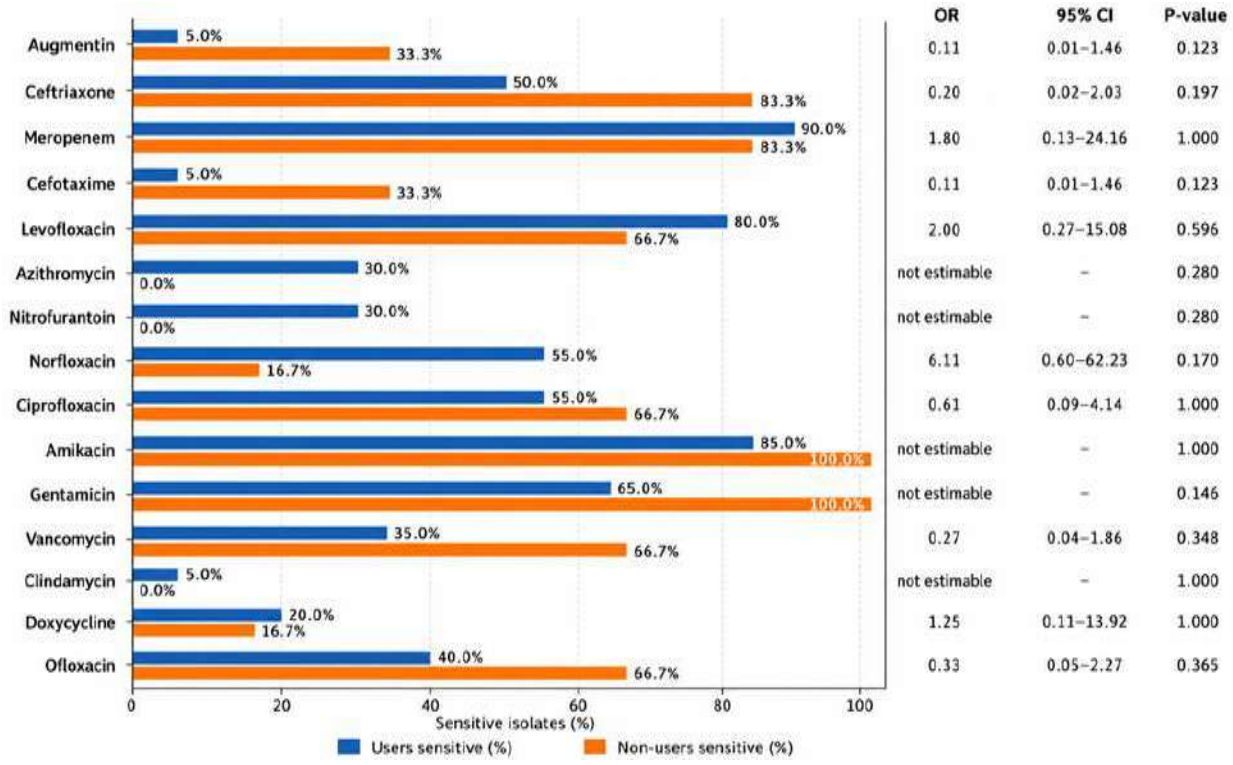

Figure 1: Antimicrobial susceptibility according to Duphaston use (UTI-positive)

Fisher's exact test; some ORs not estimable (zero cells).

No statistically significant between-group difference in antimicrobial susceptibility was observed for any tested agent. The highest sensitivity proportions among dydrogesterone users were observed for meropenem, amikacin, and levofloxacin, whereas the small number of non-users produced several zero-cell comparisons and imprecise odds ratios.

Table No.3: Association between dydrogesterone use, UTI occurrence, and bacterial isolate distribution.

Outcome / isolate	Dydrogesterone users n/N (%)	Non-users n/N (%)	Total n (%)	Effect / statistic	95% CI	P-value
UTI positive	20/25 (80.0%)	6/10 (60.0%)	26/35 (74.3%)	OR 2.67	0.54-13.21	0.393
Control / no growth	5/25 (20.0%)	4/10 (40.0%)	9/35 (25.7%)	Reference	-	-
Escherichia coli	5 (25.0%)	0 (0.0%)	5 (19.2%)	-	-	-
Staphylococcus aureus	14 (70.0%)	6 (100.0%)	20 (76.9%)	Overall distribution	-	0.310
Streptococcus agalactiae	1 (5.0%)	0 (0.0%)	1 (3.8%)	-	-	-
Total UTI-positive isolates	20 (100%)	6 (100%)	26 (100%)	-	-	-

The UTI association is presented as an odds ratio (OR) with 95% CI. Bacterial distribution was compared among UTI-positive women only.

DISCUSSION

The main finding is that estrogen levels are increased among dydrogesterone consumers, particularly in the central observation. Women with UTI's had a similar increase in white blood cell counts, but this was not the case for progesterone levels, immune markers, renal function or renal clearance. Indices, UTI occurrence, Bacterial distribution, or susceptibility. The most prevalent signal was thus Not endocrine, renal or in general inflammatory in nature. It is important to note that this estrogen finding is best interpreted as an association with endocrine activity, and not as a direct estrogenic effect of dydrogesterone. It may be caused by a different hormonal environment, the indication for treatment, Even after adjustment for placental activity or residual confounding, it was still present, suggesting that age; This is not just accounted for by and pregnancy stage. The reproductive hormones are strongly intertwined with Immune regulation, and hormone regulation of humoral and cytokine mediated immune responses. Responses.[14,18] Monteiro et al. found that pregnancy level estrogen and progesterone bolster humoral immunity via the TFH/B-cell axis.[18] Here, however, it was the estrogen rise that was not mirrored by increases in IL-17/MMP-1, no detectable systemic cytokine or tissue remodeling response.

The fact that there is not a progestogenic difference does not rule out being progestogenic, as routine Specific progesterone immunoassays are able to identify endogenous progesterone, but not dydrogesterone or its metabolites; and this is not a measure of the serum progesterone effects of the drug, but of the serum progesterone level. Likewise, Serum IL-17 is serum IL-17 together with the stable IL-17 and MMP-1 levels, which do not support a dramatic systemic shift towards Th17. may not reflect mucosal immunity in the urinary tract. Reported cytokine modulation by hormones. The majority of their data come from reproductive-immunology rather than culture-confirmed UTI cohorts.[9, 13, 20]

Urea and creatinine were comparable suggesting no renal disturbance; renal indices were also comparable. Detect subtle injury from infection, rather than exclude established renal involvement. biomarkers might be more sensitive. There was no association between dydrogesterone and UTI, and the range of confidence is broad which does not allow a definitive conclusion. UTI during pregnancy is multifactorial, Influenced by urinary stasis, gestational physiology, previous infection, exposure to antimicrobial agents and other factors. These data indicate that dydrogesterone is not an independent risk factor for bacterial virulence. [2,3]

Key strengths include the small, unevenly distributed groups, the case-control design, reliance, all limitations, which limit the ability to draw causal conclusions, from being based on serum markers instead of local markers, and from incomplete dose-duration information. Susceptibility did not demonstrate resistance to any of the drugs; were very sensitive to meropenem and amikacin, and moderately resistant to tobramycin and imipenem. These are locally informative and should be interpreted in conjunction with the types of bacteria, previous exposure, and pregnancy susceptibility.[3,19] Larger prospective cohorts. Balanced exposure groups, standardized culture criteria and detailed dose-duration information are available on them. This estrogen profile had to be verified to see if it is repeatable.

CONCLUSION

The use of dydrogesterone was not significantly correlated with the occurrence of UTI, the distribution of bacteria, and the presence of pyuria, and This cohort did not have a change in antimicrobial susceptibility, immune-marker alteration or renal biomarker change. It had the most consistent association with an elevated estrogen profile, especially in UTI-positive. These effects persisted, statistically, after taking into account the age and pregnancy stage of the women. The following should be looked at as an is an exploratory endocrine association and not evidence of causality and should be confirmed through the following steps larger, prospective studies.

Meaning of Abbreviated Words:

- **MMP-1:** (Matrix Metalloproteinase-1) An enzyme also known as Interstitial collagenase and that break down collagen in the body.
- **VITEK-2 System:** An automated microbiology platform made by biomerieux used to quickly identify bacteria and test their resistance to antibiotics.
- **ELISA:** Enzyme linked Immunosorbent Assay) – a blood test used to detect antibodies and antigens.
- **CLSI:** Clinical and Laboratory Standards Institute

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Lina Qays Yaseen, Reyam F.Saleh
Drafting or Revising Critically:	Shaymaa M.Murshed, Firas Faris Rija, Bilal A Alarabi
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Analysis of Caesarean Sections using the Robson 10-Group Classification System in Dow University Hospital, Karachi

Nazia Hakeem[1], Rabia Jamil[2], Kanwal Jan Memon[3], Wajiha Karim[4] and Tehreem Farooqui[5]

ABSTRACT

Objective: To examine the cesarean section rates of the Robson 10-Group Classification System and the significant obstetric groups contributing to cesarean section at Dow Ojha Hospital, Karachi.

Study Design: Retrospective study.

Place and Duration of Study: This study was conducted at the Department of Obstetrics and Gynecology, Dow Ojha Hospital, Karachi, during the period of 15th March 2026 to 15th May 2026.

Methods: The data were obtained by non-probability consecutive sampling of 4527 delivery records of the period from January 2023 to December 2025. Data was analyzed within eight weeks in month of April 2026 after IRB approval on 10/3/2026.

Results: The total cesarean delivery rate was 38.6%. Group 5 had the highest percentage of cesarean deliveries (45.8%), followed by Group 10 (17.7%) and Group 1 (9.1%). The highest cesarean section rate (100%) was observed in Group 9, and Groups 5 and 6 had a CS rate of 98.6%.

Conclusion: The study revealed that previous cesarean section, preterm pregnancy, and nulliparous women with a primary cesarean section were the biggest factors associated with the increasing rate of cesarean section.

Key Words: Cesarean section, Robson, delivery, reproductive health, maternal health.

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INTRODUCTION

Cesarean section (CS) is a surgical procedure used to deliver a baby through incision in the mother's abdomen and uterus[1]. It is the most frequently performed procedure within obstetrics to reduce feto-maternal morbidity and mortality. CS may be life-saving, but unnecessary procedures may lead to unnecessary short- and long-term complications[1]. World Health Organization (WHO) reported ideal CS rate of between 10-15%. However, the cesarean delivery rate has increased enormously from the recommended rate in the past few decades[1]. The prevalence of CS in Latin America and Caribbean is 40%[2]. The increasing prevalence of CS has become an important public health issue in both developed and developing countries. Hospitals and health care providers are now being asked to pinpoint the causes of the increased incidence of CS and to take steps to ensure that cesarean delivery is used appropriately[2].

WHO recommends Robson Ten group Classification System. This system divided women into ten mutually exclusive groups by parity, gestation, presenting part, induction, prior cesarean delivery and the number of fetuses. The system allows health care professionals to determine which groups are the significant contributors to the field of computing rate and to target interventions for those groups[2]. The Robson classification is widely accepted around the world due to its simplicity, repeatability, and applicability to various healthcare systems. The classification system is effective in the study of trends in cesarean deliveries and understanding obstetric practices in low-resource and conflict-affected areas[2].

Another study conducted in Nigeria showed the value of the Robson classification in the identification of preventable causes of cesarean section in tertiary health care facilities[3]. The authors also noted that the continuous auditing using Robson system might be able to contribute to the formulation of policies and institutional quality improvement programs. This study confirms the use of Robson classification as a useful audit tool to monitor interventions for CS reduction[3]. Several actions have been taken in Brazil to reduce

[1]Associate Professor / Assistant Professor / Postgraduate Trainee[3] / Senior Medical Officer[4] / House Officer[5], Department of Obs & Gynae, Dow University of Health Sciences, Karachi.

Correspondence: Dr. Nazia Hakeem, Associate Professor
Dow International Medical College, Karachi.
Contact No: 03332271732
Email: nazia.hakeem@duhs.edu.pk

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unnecessary cesarean sections, including the "Project Appropriate Birth", which aimed to promote vaginal delivery if possible and to optimize the practice of obstetricians[4].

The study found that the multidisciplinary approach, patient education, and standardized labor management procedures adopted in the institutions could have a significant effect on the obstetric outcomes[4].

The findings of the retrospective analysis in the northern Uganda also showed the value of Robson classification in assessing the obstetric trends in tertiary teaching hospitals[5]. The study also showed significant regional and institutional differences in cesarean rates, indicating that socioeconomic factors, institutional policies, and physician preferences could play a role in the decision to have a cesarean section[5]. Regular auditing using the Robson classification was found to be a potential way to identify unnecessary cesarean sections and help implement interventions to improve the quality of maternal and neonatal care [6,7].

The same trend of rising cesarean section rates has also been reported in tertiary care hospitals of Pakistan. Local research showed that Robson Group 5 continues to be one of the highest contributing groups to the overall CS rate. The factors that were shown as important determinants were an increase in repeat cesarean sections, maternal request, delayed referrals, and changes in obstetric practices[8]. The study also highlighted the fact that the Robson classification helps health care workers identify high-priority groups to target interventions. The authors recommended that regular institutional audits can enhance decision-making and safe maternal health services[8,9]. The local data regarding the cesarean deliveries according to Robson Classification System is limited therefore the aim of this study to analyze trends in CS deliveries using Robson 10 Group Classification System in tertiary care hospital, Karachi and to determine the predominant groups which are responsible for the increasing rate of cesarean section.

METHODS

SAMPLE TECHNIQUE: Non-probability consecutive sampling technique.

Inclusion Criteria: All women who delivered at DOW Ojha Hospital, Karachi during the study period were included. The Robson classification criteria were used to select all vaginal and cesarean deliveries with complete obstetric records for analysis.

Exclusion Criteria: Incomplete medical records, women with termination of pregnancy, women with uterine rupture and laparotomy and women without key obstetric data were excluded.

Data was collected from the electronic medical records of the hospital, delivery registers and patient files of the Department of Obstetrics and Gynecology, Dow Ojha Hospital, Karachi in a retrospective manner. The information regarding the maternal age, parity, fetal presentation, onset of labour, previous caesarean section, mode of delivery and number of fetuses was collected using a structured proforma. All women eligible were classified according to Robson 10-Group Classification System. The overall cesarean section rate was determined as the proportion of cesarean sections performed to all births. The size of each Robson group, the CS rate and the contribution of each group to the overall CS rate was examined. Data entry and statistical analysis were done with SPSS version 22, within four weeks in Month of (April2026) after ethical approval was obtained from the Institutional Review Board of Dow University of Health Sciences, Karachi (Reference No. IRB-4467/DUHS/Approval/2026/161, dated 10th March 2026).

RESULTS

During the study period, a total of 4527 deliveries were recorded at DOW Ojha Hospital, Karachi. Of these deliveries, 1747 women had cesarean section, giving an overall cesarean section rate of 38.6% as shown in Table 1.

Table No. 1: Overall Delivery and Cesarean Section Rate

Variable	Frequency	Percentage
Total Deliveries	4527	100%
Total Cesarean Sections	1747	38.6%
Vaginal Deliveries	2780	61.4%

The Robson classification further identified the major obstetric groups contributing to this increasing trend of cesarean deliveries.

In comparison, Group 10 (26.4%) had the highest proportion of women, closely followed by Group 5 (25.2%) of all Robson groups. Groups 1, 7, 8, and 9 had the smallest proportions of the study population. The high percentage of women in Groups 5 and 10 showed significant numbers of previous cesarean sections and preterm pregnancies in the institution. The highest cesarean section rate was seen in Group 9, where all women were delivered by cesarean section. Likewise, Groups 5 and 6 had very high CS rates of 98.6%. The rate of 94.4% was also significantly higher for Group 8. Groups 2, 3, and 4 showed lower cesarean section rates. These data suggest that cesarean-scared women, abnormal fetal presentation, and high-risk pregnancies were key factors in surgical deliveries. Group 5 was determined to be the most significant group in the overall cesarean section rate, responsible for 45.8% of all cesarean deliveries. Group 10 was the second largest with 17.7%, followed by Group 1 with 9.1% as shown in table 2 and figure 1.

Table No. 2: Distribution of Cesarean Sections According to Robson Ten-Group Classification

Robson Group	Obstetric Characteristics	Number of CS (n)	Total women in the group	Group C/S rate (%)	Contribution to Overall CS (%)
Group 1	Nulliparous, singleton, cephalic, ≥37 weeks, spontaneous labor	160	450	35.5%	9.1%
Group 2	Nulliparous, singleton, cephalic, ≥37 weeks, induced/pre-labor CS	92	352	26.1%	5.3%
Group 3	Multiparous (no previous CS), singleton, cephalic, ≥37 weeks, spontaneous labor	51	211	24.1%	2.9%
Group 4	Multiparous (no previous CS), singleton, cephalic, ≥37 weeks, induced/pre-labor CS	52	210	24.8%	3.0%
Group 5	Previous CS, singleton, cephalic, ≥37 weeks	800	811	98.6%	45.8%
Group 6	Nulliparous breech	142	144	98.6%	8.1%
Group 7	Multiparous breech	39	45	86.7%	2.2%
Group 8	Multiple pregnancies	68	72	94.4%	3.9%
Group 9	Transverse/oblique lie	36	36	100%	2.1%
Group 10	Singleton, cephalic, <37 weeks	310	850	36.5%	17.7%

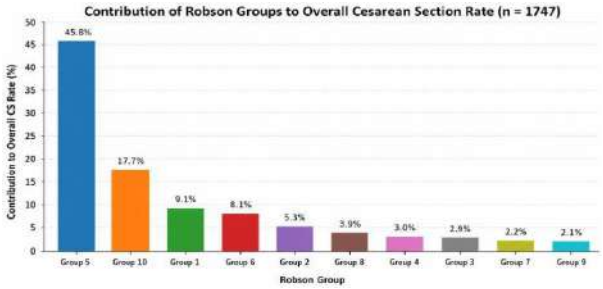

Figure No.1: Contribution of Robon Groups to Overall Cesarean section Rate (n=1747)

DISCUSSION

The present study was designed to find the trend of cesarean section in DOW Ojha Hospital, Karachi according to Robson 10-Group classification. The overall CS rate in this study was 38.6% which is significantly higher than the recommended rate by the WHO of 10-15%. The study conducted by Rizwana et al found the same trend of an increasing cesarean rate in tertiary healthcare facilities[10]. The reason behind high rate of CS in our study is that our hospital is a tertiary care hospital where intensive care and neonatal intensive care facilities are available. Hospital also receive referrals from Primary and secondary care settings. Based on the present finding, it can be concluded that repeated cesarean section is one of the major contributors to the increased rate of surgical delivery in tertiary hospitals. The study conducted by Guner et al and Gilani et al also reported that the highest rate of cesarean section deliveries was in Group 5. The authors emphasized that the rising trend of repeat cesarean section was due to limited implementation of VBAC protocols and growing preference of clinicians for repeat cesarean section [2,11] .

The second largest group was Group10 (preterm). The high cesarean rate among Group 10 pregnancies could be associated with maternal and fetal complications that require urgent obstetric intervention. These results are consistent with other studies[12,13]. The higher number of surgical deliveries in Groups 5 and 10 may be explained by the fact that these groups were attended by tertiary centres, which are more likely to have complicated cases. The findings of the current study also demonstrated that the contribution of the Group 1 to the total CS burden was significantly higher with a cesarean rate of 35.5% in nulliparous women who were in spontaneous labor. This is clinically important as nulliparous women who have undergone a primary cesarean section are at higher risk of having another cesarean section on their next pregnancy. The key indicator of good obstetric practice is the intrapartum care of women with low risk pregnancies. The study in the private hospitals showed that Group 5 continued to be the predominant group, followed by nulliparous women with induced labor or pre-labor cesarean section[14]. Induction of labor without counselling, lower threshold of labor pains and less expertise in instrumental deliveries are responsible for rising trends of CS in nulliparous women.

The extremely high cesarean section rate in the present study was another important observation in groups 6, 7, 8, and 9. Surgical delivery is more commonly performed in breech presentation, multiparity, and abnormal fetal lie due to the risks involved for the mother and fetus in a vaginal delivery. Geze et al. found similar results in Ethiopia, with cesarean section rates significantly higher in women with abnormal presentations and previous cesarean scars. . The study found that the Robson classification was able to identify high-risk groups for targeted clinical attention and intervention [14].

The major contributions of CS in our study was Group 5, 10 and 1. Another study conducted in Pakistan observed that the Group 10(50.9%), 5(14.4%) and

1(11.4%) were the major contributors to the CS[15], while a study from India reported Group 1(33.3%), 5(19.7%) and 2(14.6%) were the commonest group[16]. Group 2, 5 and 10 was found to be the frequently encountered groups[17].

Strengths and limitations: The present study has a number of strengths as it was conducted with a large number of deliveries in a tertiary care setting, with a standardized WHO recommended classification system. The retrospective design limited the information available on detailed clinical data of indication for cesarean section and maternal and perinatal outcomes. It was a single center study. Despite these limitations our study demonstrated that Robson Classification system is a useful tool to find out the reason of high rates of CS. It should be used on a regular basis to analyze the overall trends of CS. It should be implemented in all primary and secondary care facilities. The results of our study provide some clues to the obstetric groups that are responsible for the trend of cesarean section in Karachi, Pakistan.

CONCLUSION

The rate of CS in our study is 38.6%, which is much higher than the WHO recommended rate of CS. Group 5 had the highest contribution to the overall cesarean section rate followed by Group 10 and Group 1. The findings indicate that repeat cesarean section and preterm deliveries are major factors leading to the increased surgical birth rate, while primary cesarean section among nulliparous women is a minor factor.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Nazia Hakeem, Rabia Jamil
Drafting or Revising Critically:	Kanwal Jan Memon, Wajiha Karim, Tehreem Farooqui
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Development and Validation of the Accelerated Inflammatory-Metabolic Index (AIMI): A Composite Biomarker for Biochemical Assessment of Obesity-Associated Cardiometabolic Dysfunction

Hayder Saleh[1], Majid Sirati-Sabet[2] and Zahra Shahsavari[2]

ABSTRACT

Objective: To develop and internally evaluate the accelerated inflammatory-metabolic index, integrating malondialdehyde, total antioxidant capacity, superoxide dismutase, interleukin-6, and homeostatic model assessment of insulin resistance and compare its diagnostic performance with that of its individual components across the obesity spectrum.

Study Design: A cross-sectional study

Place and Duration of Study: This study was conducted at the School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran from 1st March 2026 to 31st May 2026.

Methods: 150 adults in five age-matched groups (n = 30 each): non-obese, overweight, obese, severely obese, and trained athletes were enrolled. Accelerated inflammatory-metabolic index = [malondialdehyde x interleukin-6 x homeostatic model assessment of insulin resistance] ÷ [total antioxidant capacity × superoxide dismutase].

Results: Accelerated inflammatory-metabolic index increased progressively across groups ($F=187.4$, $p<0.0001$, $\eta^2=0.82$), from 0.65±0.19 in athletes to 24.71±8.43 in the severely obese group, and discriminated overweight from non-obese status with area under the curve = 0.976 (95% CI 0.935-1.000), numerically exceeding its strongest component, malondialdehyde (AUC = 0.939), and its weakest, homeostatic model assessment of insulin resistance (area under the curve = 0.693). Athlete accelerated inflammatory-metabolic index scores were significantly lower than non-obese controls (Cohen's d =0.91, $p<0.001$). Body mass index correlated more strongly than waist-to-hip ratio with every accelerated inflammatory-metabolic index component.

Conclusions: Accelerated inflammatory-metabolic index shows promising diagnostic performance for early metabolic deterioration and exercise-associated metabolic protection. External validation in independent, prospective cohorts is warranted before clinical use.

Key Words: Obesity, Oxidative stress, Insulin resistance, Systemic inflammation, Antioxidant capacity, Composite biomarker index, Cardiometabolic risk

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INTRODUCTION

Obesity is a systemic metabolic disorder marked by concurrent oxidative stress, chronic inflammation, impaired antioxidant defense and insulin resistance.[1,2] Although these biological processes interact through interconnected molecular signalling pathways rather than proceeding independently, current clinical assessment relies predominantly on isolated biomarkers that fail to capture their integrated pathophysiology. Body mass index (BMI), the most widely applied anthropometric metric, captures neither adipose distribution nor any biochemical dimension of metabolic dysfunction[3], while single markers such as C-reactive protein (CRP), fasting glucose, or malondialdehyde (MDA) each address only one axis in isolation. The result is that individuals with normal fasting glucose but substantial insulin resistance, elevated oxidative burden, and subclinical inflammation remain undetected by standard clinical panels until disease is advanced.[4,5] Existing composite indices such as the triglyceride-glucose (TyG) index, the Visceral Adiposity Index (VAI), the Lipid Accumulation

[1.] M.Sc. Student / Associate Professor[2], Department of Clinical Biochemistry, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Correspondence: Hayder Saleh, M.Sc. Student, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Contact No: 009647713875448
Email: haider_is@yahoo.co.uk

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Product (LAP), and METS-IR predominantly integrate anthropometric and metabolic variables but do not account for oxidative stress or antioxidant defense, despite substantial evidence implicating these pathways in obesity-related metabolic dysfunction. Experimental evidence suggests that lipid peroxidation products such as MDA may activate NF-κB signalling, thereby promoting interleukin 6 (IL-6) expression and contributing to insulin resistance, and the resulting hyperinsulinaemia may in turn stimulate further peroxidative reactions.[6,7]

To address this gap, we introduce the Accelerated Inflammatory-Metabolic Index (AIMI), integrating oxidative injury (MDA), antioxidant capacity (TAC, SOD), inflammation (IL-6), and insulin resistance (HOMA-IR) - markers selected for biological relevance and strong association with obesity grade in this dataset (all $r \geq 0.80$, $p < 0.001$). A trained-athlete group was included as a physiological reference representing maximal metabolic protection, allowing AIMI to be evaluated across the full spectrum from protection to risk. The purposes were 1) to develop a composite biomarker (AIMI) integrating oxidative, antioxidant, inflammatory, and insulin-resistance axes; 2) to evaluate its diagnostic performance against individual biomarkers; and 3) to examine its association with anthropometric measures and training status.

METHODS

A cross-sectional study was conducted at School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran from 1st March 2026 to 31st May 2026 vide letter IR.SBMU.MSP.REC.1404.775 dated 17th February 2026. A total of 150 adults in five age-matched groups (n = 30 each): non-obese (BMI 18.5-24.9 kg/m²), overweight (25.0-29.9), obese (30.0–39.9), severely obese (≥40.0), and trained athletes (BMI 18.5-24.9; ≥5 training sessions/week for ≥2 years) were enrolled. Demographic and lifestyle characteristics, including smoking status, medication use, comorbidities, and physical activity, were recorded for all individuals screened. Exclusion criteria included diabetes mellitus, cardiovascular disease, chronic inflammatory/autoimmune conditions, renal or hepatic impairment, use of agents affecting oxidative stress, inflammation, or lipid metabolism (statins, metformin, NSAIDs, corticosteroids), pregnancy, and active smoking. Participants fasted 10-12 hours before blood collection. The study was approved by the Institutional Ethics Committee and conducted in accordance with the Declaration of Helsinki (2013 revision); written informed consent was obtained from all participants.

Body mass index was calculated as weight (kg)/height (m)²; waist-hip ratio as waist÷hip circumference. Blood pressure was recorded twice in the seated position after 5 minutes of rest; the mean of two readings was used. Fasting venous blood was assayed in duplicate: MDA and SOD by ELISA; TAC by colorimetric FRAP assay; IL-6 and CRP by ELISA; fasting blood glucose (FBG) by the hexokinase method; insulin by electrochemiluminescence immunoassay; HOMA-IR = [insulin (mIU/mL) × FBG (mg/dL)] ÷ 405; lipid profile by standard enzymatic colorimetric methods. Five markers spanning the oxidative, antioxidant, inflammatory, and metabolic axes were selected for biological relevance and strong correlation with BMI: MDA, TAC (inverse), SOD (inverse), IL-6, and HOMA-IR.

Higher AIMI reflects greater pro-oxidant/inflammatory burden relative to antioxidant/metabolic protection. A multiplicative rather than additive structure was chosen because these axes are considered biologically synergistic. AIMI values were $\log_{10}$-transformed (log-AIMI) before statistical testing to normalise their right-skewed distribution.

The data was analyzed through SPSS-26. One-way ANOVA with LSD post-hoc testing compared groups; Pearson correlation assessed biomarker relationships; effect sizes were Cohen's d and η^2. Univariate logistic regression (BMI/WHR excluded as they define the groups) and ROC analysis (95% CIs and cutoffs via percentile bootstrap, 3,000 resamples) evaluated diagnostic performance. $\alpha = 0.05$, two-tailed.

RESULTS

Groups were age-matched ($F = 0.72$, $p = 0.58$). BMI ranged from 21.94±1.57 (non-obese) to 47.08 ± 8.06 kg/m² (severely obese); WHR rose from 0.803 ± 0.06 to 0.946±0.11, with athlete WHR (0.824±0.06) comparable to controls. All groups recorded normal mean systolic BP (<120 mmHg) [Table 1]. All twelve biomarkers differed significantly across groups (all $p < 0.0001$), generally following a dose-dependent BMI gradient, except IL-6, CRP, and TAC, which showed partial attenuation in the severely obese group (Table 2). HOMA-IR, MDA, CRP, and IL-6 were mutually intercorrelated ($r > 0.74$, $p < 0.001$); TAC–MDA showed the strongest inverse correlation ($r = -0.891$). BMI correlated more strongly than WHR with every AIMI component (advantage 0.05–0.30), contrary to the initial expectation that a central-adiposity measure would outperform a global one (Table 3).

Within-group analyses confirmed that FBG–HOMA-IR (strongest in the obese group, $r = 0.87$) and CRP–IL-6 associations (overweight $r = 0.44$; obese $r = 0.44$) persisted even within narrow BMI bands, indicating these relationships are not simply artifacts of pooling groups with different adiposity (Figs. 1-3). Log-AIMI increased progressively across groups ($F=187.4$, $p<0.0001$, $\eta^2=0.82$), from 0.65±0.19 (athletes) to 24.71±8.43 (severely obese) - a ~38-fold difference. Athlete AIMI was significantly below non-obese controls (Cohen's $d = 0.91$, $p<0.001$) [Table 4].

Table No. 1: Participant characteristics, NS = not significant

Parameter	Non-Obese	Overweight	Obese	Severely Obese	Athletes	p-value
Age (years)	35.47±15.29	37.10±12.20	39.56±15.63	36.37±13.96	32.50±11.19	0.581 NS
BMI (kg/m²)	21.94±1.57	27.37±1.33	34.02±2.56	47.08±8.06	22.12±1.94	<0.0001
WHR	0.803±0.06	0.848±0.07	0.905±0.08	0.946±0.11	0.824±0.06	<0.0001
Systolic BP	109.47±6.18	116.86±4.54	117.43±2.75	114.90±5.29	117.00±3.73	<0.0001

Table No. 2: Biochemical biomarker values across groups, *Partial attenuation in the severely obese group relative to the obese group. Cohen's d between non-obese and severely obese groups; TG = triglycerides

Biomarker	Non-Obese	Overweight	Obese	Severely Obese	Athletes	Cohen's d
FBG (mg/dL)	89.42±2.97	90.34±3.89	103.65±12.47	112.79±14.78	86.39±4.18	2.01
HOMA-IR	1.466±0.20	1.617±0.27	2.913±1.38	7.45±2.29	1.516±0.25	2.89
MDA (nmol/mL)	1.219±0.14	1.630±0.21	3.879±0.57	4.018±1.01	1.280±0.18	4.63
TAC (mmol/L)	1.596±0.08	1.384±0.13	0.943±0.12	1.224±0.21*	1.623±0.12	6.82
SOD (U/mL)	5.35±0.29	4.87±0.38	3.27±0.50	2.01±0.64	5.24±0.37	5.48
CRP (mg/dL)	0.840±0.37	1.838±0.56	10.22±3.17	9.05±3.31*	0.927±0.38	3.71
IL-6 (pg/mL)	2.48±0.71	3.73±1.03	11.81±3.72	7.54±2.59*	2.25±0.87	2.94
Total Chol (mg/dL)	176.41±13.34	184.94±13.11	222.66±25.50	234.01±22.73	178.54±12.38	2.61
TG (mg/dL)	83.98±15.59	113.62±18.08	237.75±60.64	223.10±48.19	81.35±19.61	3.02
HDL-C (mg/dL)	61.87±4.59	56.58±5.69	40.27±5.53	46.66±5.79	61.05±4.02	3.84
LDL-C (mg/dL)	110.36±9.51	110.28±10.77	151.26±20.35	148.89±19.81	105.31±8.54	2.12

Table No. 3: Pearson correlation matrix of AIMI components and BMI (n = 150) ** p < 0.001 (two-tailed)

	HOMA-IR	MDA	TAC	SOD	IL-6	BMI
HOMA-IR	1.000	0.812**	−0.743**	−0.762**	0.741**	0.781**
MDA	0.812**	1.000	−0.891**	−0.814**	0.796**	0.758**
TAC	−0.743**	−0.891**	1.000	0.867**	−0.743**	−0.583**
SOD	−0.762**	−0.814**	0.867**	1.000	−0.718**	−0.865**
IL-6	0.741**	0.796**	−0.743**	−0.718**	1.000	0.508**

Table No. 4: AIMI scores by group (ANOVA p<0.0001; $\eta^2 = 0.82$)

Group	Raw AIMI (Mean±SD)	Log-AIMI (Mean±SD)	Risk Category
Athletes	0.65±0.19	−0.19±0.12	Below reference
Non-Obese (Control)	2.01±0.61	0.30±0.13	Reference
Overweight	3.87±0.94	0.59±0.11	Mild risk
Obese	12.43±3.18	1.09±0.11	High risk
Severely Obese	24.71±8.43	1.39±0.14	Very high risk

Table No. 5: ROC analysis: AIMI vs. individual biomarker components (overweight vs. non-obese, n = 30/group)

Biomarker/Index	AUC	95% CI	Sensitivity (%)	Specificity (%)
AIMI (composite)	0.976	0.936–1.000	90.0	100.0
MDA	0.939	0.869–0.990	80.0	100.0
CRP	0.933	0.863–0.984	86.7	86.7
TAC (inverse)	0.887	0.788–0.963	76.7	96.7
IL-6	0.837	0.722–0.927	73.3	83.3
SOD (inverse)	0.826	0.711–0.918	66.7	90.0
HOMA-IR	0.693	0.554–0.823	66.7	76.7

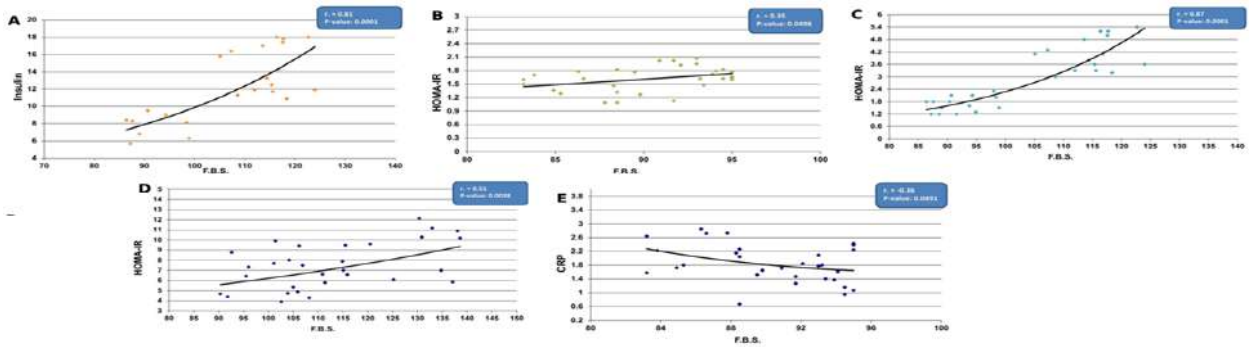

Figure No. 1: Relationship between fasting blood glucose (FBG) and glycaemic/insulin-resistance markers within individual study groups: (A) FBG vs insulin, obese group; (B) FBG vs. HOMA-IR, overweight group; (C) FBG vs. HOMA-IR, obese group; (D) FBG vs HOMA-IR, severely obese group; (E) FBG vs CRP, overweight group

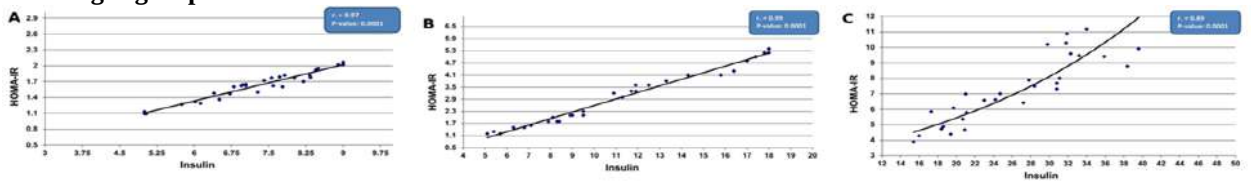

Figure No. 2: Relationship between insulin and HOMA-IR within individual study groups: (A) overweight; (B) obese; (C) severely obese

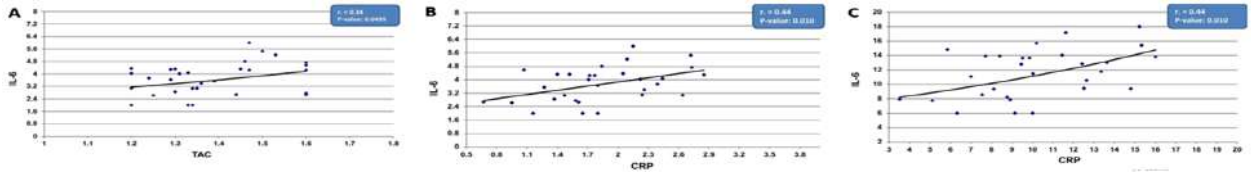

Figure No. 3: Relationship between oxidative/inflammatory markers and IL-6 within individual study groups: (A) TAC vs IL-6, overweight; (B) CRP vs. IL-6, overweight; (C) CRP vs. IL-6, obese

An extreme-phenotype comparison (obese and severely obese vs. non-obese and athletes) was not used because it produced near-ceiling discrimination for most markers and an AUC of 1.000 for BMI, since these groups are themselves defined by BMI thresholds; testing whether BMI predicts a BMI-defined grouping is circular and was dropped rather than reported as a finding, and WHR was excluded for the analogous reason. AIMI achieved AUC=0.976 (95% CI 0.935-1.000; sensitivity 90.0%, specificity 100.0%; Table 5), correctly classifying 57 of 60 participants, and numerically exceeded all individual components; the AIMI-vs-MDA difference was not statistically significant (bootstrap 95% CI: -0.028 to 0.114) [Table 5]. Because BMI and WHR define the groups modelled, neither was entered as a predictor. Each component was independently associated with overweight status per 1-SD increase: MDA (OR = 9.95, 95% CI 5.81-18.19), IL-6 (OR =4.23, 2.48-8.27), HOMA-IR (OR = 1.82, 1.11-3.34), TAC (OR = 0.14, 0.08–0.23), and SOD (OR = 0.25, 0.13-0.41); all 95% CIs excluded 1. A joint multivariable model could not be fitted reliably at this sample size due to quasi-complete separation.

DISCUSSION

The biochemical perturbations of obesity are mechanistically interconnected rather than independent: HOMA-IR, MDA, CRP, and IL-6 were mutually intercorrelated (r>0.74), and TAC–MDA showed a strong inverse relationship (r = −0.891), consistent with MDA-driven NF-κB activation promoting IL-6 expression and insulin resistance while depleting antioxidant reserves.[1] MDA forms adducts with IRS-1 that impair insulin receptor signalling, contributing directly to HOMA-IR elevation[8,9], while the NLRP3 inflammasome links oxidative and inflammatory injury at a single molecular node, amplifying IL-6 production and impairing β-cell function.[7] AIMI's multiplicative ratio structure encodes this synergy directly: as obesity advances, the numerator (MDA×IL-6×HOMA-IR) expands while the denominator (TAC×SOD) contracts, producing a 38-fold AIMI differential between athletes and severely obese participants versus a 3.3-fold difference for MDA alone.

Body mass index predicted all five AIMI components more strongly than WHR (advantage 0.05–0.30), suggesting AIMI reflects total systemic adiposity burden rather than fat distribution specifically. This supports using BMI, already routinely collected, as the primary anthropometric trigger for AIMI screening, though this interpretation requires confirmation since visceral adiposity was not directly measured.

Partial attenuation of IL-6, CRP, and TAC in the severely obese group may reflect chronic IL-6 receptor downregulation under sustained cytokine overexposure, or a compensatory Nrf2-driven antioxidant response as a hormetic reaction to extreme oxidative burden[10,11], though unmeasured confounders such as medication use (particularly statins, which reduce CRP by 25-50%) and dietary antioxidant intake cannot be excluded. Despite this component-level attenuation, mean AIMI remained substantially higher in the severely obese group (24.71±8.43) than in the obese group (12.43±3.18), because continued SOD suppression and HOMA-IR elevation dominate the denominator and numerator respectively.

Athletes recorded AIMI significantly below the non-obese control reference (Cohen's d =0.91, $p<0.001$) while maintaining BMI values comparable to controls, reflecting coordinated physiological adaptations: Nrf2-driven upregulation of Mn-SOD and glutathione peroxidase raises the denominator; reduced basal ROS generation lowers MDA; diminished visceral adiposity attenuates IL-6 secretion; and AMPK-mediated GLUT-4 translocation normalises HOMA-IR. Notably, all five individual AIMI components showed only athlete, control equivalence ($p>0.05$) rather than athlete superiority; AIMI's ratio structure, by multiplying these small individual advantages, converts a pattern of statistical non-difference into a quantifiable index advantage, a mathematical property of composite ratio indices that should be interpreted with appropriate caution in future applications.[12]

Accelerated inflammatory-metabolic index has four potential clinical applications distinct from existing individual markers. For early screening, AIMI was elevated in the overweight group despite near-normal FBG, indicating it detects metabolic deterioration before glucose-based tests become informative. For obesity phenotyping, AIMI integrates the dimensions distinguishing metabolically healthy from unhealthy obese phenotypes. For treatment monitoring, its numerator and denominator components are expected to respond to interventions at different kinetics, allowing serial measurement to track multi-domain recovery. For exercise dose-response research, the athlete group's below-reference AIMI offers a quantitative target for training prescriptions. This rationale for multi-marker profiling extends to other conditions, such as neurochemical panels in methamphetamine use disorder[13,14], reinforcing that composite indices can capture information no single marker provides alone.

Unlike existing composite indices (TyG, LAP, VAI), which rely solely on lipid/glucose variables[15,16], AIMI incorporates the redox dimension, which correlated strongly with insulin resistance (r=0.812) and TAC (r=−0.891) in this dataset. Head-to-head comparison with these established indices in the same cohort is a logical next step for establishing AIMI's incremental value.

CONCLUSION

Accelerated inflammatory-metabolic index detected meaningful metabolic deterioration before FBG or lipid parameters exceeded normal thresholds, maintained directional validity despite partial component attenuation in severely obese participants, and distinguished athletes' exercise-associated metabolic protection (Cohen's d = 0.91), a pattern not detectable from individual markers alone. Before clinical application, AIMI requires prospective validation in larger, diverse cohorts with documented medication use and dietary intake, and direct comparison with TyG, LAP, and VAI. Subject to such validation, AIMI may represent a promising, mechanistically grounded index for cardiometabolic risk assessment, treatment monitoring, and exercise evaluation in obesity.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hayder Saleh, Majid Sirati-Sabet
Drafting or Revising Critically:	Hayder Saleh, Zahra Shahsavari
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Effects of Mentorship Program on the Levels of Resilience and Job Satisfaction among Nurses

Nasir Khan[1], Sarfraz Masih[2] and Madiha Mukhtar[3]

ABSTRACT

Objective: To determine the effectiveness of a nurse-led mentorship program on the improvement of the nurses' resilience in maintaining job satisfaction and the correlation between the two.

Study Design: A quasi-experimental pre-test/post-test study

Place and Duration of Study: This study was conducted at the Bahria International Hospital, Lahore from 1st August 2025 to 31st December 2025.

Methods: Thirty five registered nurses in a private tertiary care hospital in Lahore, Pakistan were enrolled. Convenience sampling was employed and participants filled out the Brief Resilience Scale (BRS-Modified) and the Minnesota Satisfaction Questionnaire (MSQ) pre and post an 8 week nurse-led mentorship program. The intervention involved structured mentor–mentee weekly meetings that were based on professional advice, emotional support, reflection, and professional growth.

Results: Post intervention, the proportion of nurses with normal resilience rose from 20.0% to 97.1%, and that of low-resilient nurses dropped to 2.9% ($p<0.001$). After the intervention, the number of participants with high job satisfaction rose from 20.0% to 88.6% and no participant had low job satisfaction ($p<0.001$). Mean resilience scores improved significantly from 2.78±0.24 to 3.54±0.24 ($t = -12.88$, $p < 0.001$), and mean job satisfaction scores increased from 3.13 ± 0.45 to 4.01±0.35 ($t = -11.42$, $p<0.001$). None of the categorical levels of resilience had a significant correlation with job satisfaction (all $p>0.05$).

Conclusion: The nurse-led mentorship program had a significant effect on the psychological state of nurses, such as increasing their resilience, job satisfaction, and suggesting that it is an effective approach for enhancing psychological well-being and job satisfaction for nurses.

Key Words: Mentorship, Resilience, Job satisfaction, Mentorship program, Nurses

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INTRODUCTION

Nursing is an essential part of health care delivery and an important aspect of safety, quality health care and health promotion. While nurses often perform in challenging settings with high workloads, staff shortages, patient acuity and frequent exposure to stressful situations, unemployment and low job satisfaction are rising. Low job satisfaction and high rates of unemployment among nurses are on the rise, while many continue to work in challenging environments with high patient acuity, staffing shortages, and heavy workloads.

[1.] MSN Scholar / Principal[2] / Associate Professor[3], Lahore School of Nursing, The University of Lahore.

Correspondence: Nasir Khan, MSN Scholar, Lahore School of Nursing, The University of Lahore.
Contact No: 0332-9982839
Email: 70134364@gmail.com

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These workplace issues can be associated with an increased risk of psychological distress, burnout and low job satisfaction among nurses.[1] The capacity to respond positively to challenges and adapt to adverse situations, known as resilience, is gaining greater recognition as an important quality of a nurse in a complex healthcare environment. Resilience has also been related to positive psychological outcomes, such as psychological well-being, coping skills, and less burnout, while low resilience has been linked to psychological distress, including anxiety, depression, compassion fatigue and maladaptation in the workplace.[2] Job satisfaction is also a major factor that influences nurses' performance, retention and psychological health. Absenteeism, employee turnover, and poor quality patient care are risk factors associated with low job satisfaction.[3]

Nurses who experience poor resilience and do not enjoy their work have a direct impact on patients' outcomes. Nurses with high stress and poor coping tend to make clinical errors, have decreased decision-making capacity and decreased attentiveness to patient care.[4] Burnout amongst nurses is a global health concern that creates healthcare instability, higher healthcare costs and poor healthcare outcomes.[5] In this regard, various

interventions have been investigated to improve the resilience among health care professionals and highlighted here is mentorship for nurses' development and psychological health.[6,7] Mentorship is a formalized relationship between experienced and less experienced nurses that includes guidance, emotional support, professional socialization, and career development support. Mentoring programs have been found to enhance nurse resilience, job satisfaction, retention, and job confidence.[8-10]

Although there is emerging evidence internationally, there is a lack of research on the topic of resilience among nurses in Pakistan along with the topic of job satisfaction and mentorship interventions. Furthermore, the relationship between resilience and job satisfaction has not been adequately explored within the local healthcare context. Therefore, this study purpose to assess resilience and job satisfaction among nurses, examine the association between these variables, and evaluate the effectiveness of a nurse-led mentorship program in improving resilience and job satisfaction in a tertiary care hospital setting.

METHODS

A quasi-experimental pre-test/post-test study was conducted at Bahria International Hospital, Lahore from 1st August 2025 to 31st December 2025 vide letter No. UOL/IREB/25/10/0005 dated July 15, 2025. A pre–post test quasi-experimental design was used to assess the impact of a nurse-led mentorship program on registered nurses' level of resilience and job satisfaction in a private tertiary care hospital in Lahore, Pakistan. The sampling method adopted was Non-Probability Conventional sampling that involved selecting participants based on convenience. The final sample size of 35 participants was determined using R statistical software with a 80% power, one sided 5% significance level, 95% confidence interval, an effect size of 0.45 and a 10% allowances for non response. The subject for the study were direct care Registered Nurses between 22 to 38 years of age with a diploma or above in nursing. Nurses in leadership or academic roles, and those who had lost a family member over the past six months were excluded. The intervention consisted of an 8Week structured nurse-led mentorship program based on Bandura's Social Learning Theory and Gibbs' Reflective Cycle.10 Nurse mentors were selected and trained in a mentorship orientation program prior to its implementation. Each mentor was allocated to a mentee and met 1–2 times per week with them to provide professional advice, emotional support, reflective practice, problem solving and professional development. Reflective diaries were kept by mentees and mentorship activities were documented utilizing standard documentation during the intervention period. The baseline (pre-intervention) data were gathered with a structured questionnaire, which included socio-demographic information, Brief Resilience Scale (BRS-Modified), and Minnesota Satisfaction Questionnaire (MSQ). To gather post-intervention data, the same instruments were administered after the mentor training program was completed (8 weeks). The BRS-Modified is composed of six items that are rated on a 5-point Likert scale, four of which are worded negatively which are reverse-scored, and the overall score is the average of all the items, with higher scores associated with higher levels of resilience. The MSQ uses a 5-point Likert scale, which has a higher score as a sign of more job satisfaction. The instruments showed good validity and internal consistency after being validated by experts and pilot tested.

The data was entered and analysed by SPSS-22. Normality of continuous variables was determined by Shapiro–Wilk test. To compare pre- and post-resilience scores and pre- and post-job satisfaction scores, the paired-samples t-test and Wilcoxon signed rank test were used as appropriate. Chi-square test was used for assessing association between job satisfaction level and resilience. Two-tailed p value <0.05 was considered statistically significant.

RESULTS

Most of the participants 24 (68.6%) were aged 20–30 years, males 24 (68.6%) and unmarried 23 (65.7%). Most participants held a Bachelor of Science in Nursing degree 31 (88.6%) and had 1–3 years of clinical experience 24 (68.6%). All participants 100% were permanently employed and worked rotating shifts. Regarding background characteristics, 19 (54.3%) grew up in urban areas, and 25 (71.4%) were residing in hospital hostels (Table 1).

Table No. 1: Demographic characteristics of participants (N = 35)

Variable	No.	%
Age (years)		
20–30	24	68.6
31–40	11	31.4
Gender		
Male	24	68.6
Female	11	31.4
Marital Status		
Unmarried	23	65.7
Married	12	34.3
Religion		
Muslim	22	62.9
Christian	12	34.3
Hindu	1	2.9
Education		
General Nursing	4	11.4
BSc Nursing	31	88.6
Unit		
Surgical Ward	7	20.0
Medical Ward	7	20.0

Surgical ICU	3	8.6
Medical ICU	5	14.3
Special Care Unit	3	8.6
Operating Room	5	14.3
Emergency/Casualty	5	14.3
Work Experience		
< 1 year	8	22.9
1–3 years	24	68.6
>3–5 years	2	5.7
8 years	1	2.9
Working status (single unit)	35	100.0
Type of shifts (all shifts)	35	100.0
Living In		
Hostel	25	71.4
Home	10	28.6
Type of Family		
Single	21	60.0
Joint	14	40.0
Grown Up In		
Rural	16	45.7
Urban	19	54.3

The resilience was improved substantially, with the proportion of nurses demonstrating normal resilience increasing from 7 (20%) at baseline to 34 (97.1%) after the intervention, while the proportion with low resilience decreased from 28 (80%) to 1 (2.9%) [p<0.001]. Similarly, job satisfaction improved significantly after the intervention. The proportion of nurses reporting high job satisfaction increased from 7 (20%) to 31 (88.6%), whereas moderate job satisfaction decreased from 25 (71.4%) to 4 (11.4%), and no participant reported low job satisfaction following the intervention (p<0.001). However, the association between resilience and job satisfaction was not statistically significant at any assessment point (all p> 0.05) [Table 2].

Paired samples t-test results revealed a statistically significant improvement in both resilience and job satisfaction after the mentorship intervention. The mean resilience score increased from 2.78±0.24 pre-intervention to 3.54±0.24 post-intervention (t = −12.88, df=34, p<0.001). Similarly, the mean job satisfaction score rose from 3.13±0.45 to 4.01±0.35 (t = −11.42, df=34, p<.001). These findings indicate that the mentorship program had a highly significant positive effect on both resilience and job satisfaction among nurses (Table 3).

Table No. 2: Distribution of resilience and job satisfaction levels before and after the mentorship intervention and their association (N = 35)

Variable	Category	Pre-intervention	Post-intervention	p-value†
Resilience	Low resilience	28 (80%)	1 (2.9%)	<0.001
	Normal resilience	7 (20%)	34 (97.1%)	
Job satisfaction	Low satisfaction	3 (8.6%)	-	<0.001
	Moderate satisfaction	25 (71.4%)	4 (11.4%)	
	High satisfaction	7 (20%)	31 (88.6%)	

Table No. 3: Paired samples t-test comparing pre- and post-intervention scores (N = 35)

Variable	Pre-Mean±SD	Post Mean±SD	t	df	p-value
Resilience (BRS)	2.78±0.24	3.54±0.24	−12.88	34	< .001
Job Satisfaction (MSQ)	3.13±0.45	4.01±0.35	−11.42	34	< .001

DISCUSSION

The present study explored the relationship between socio-demographic characteristics and the levels of resilience and job satisfaction among nurses. Previous literature suggests that demographic factors such as age, clinical experience, educational level, and work department can influence resilience and job satisfaction. Aburn et al[11] highlighted the need to determine demographic factors which affect nurses' resilience. Some studies have indicated that that the transition to practice may introduce more psychological stress to younger nurses. Shen et al[12] also reported the intensive care unit (ICU) nurses had high stress levels and resulted in anxiety, emotional exhaustion, and sleep disorder. This study however, revealed that only religious affiliation was significantly related to the resilience in pre intervention data. It demonstrates the significant findings that strong religious bonding is important in terms of resilience while the rest of the demographic variables are not associated with resilience. Pakistan has a shortage of nurses, high workload and long working hours which can affect nurses' resilience and job satisfaction.[13]

Waqar and Hamid[14] reported that nurses working in private hospitals demonstrated higher levels of job satisfaction due to better administrative support and organizational benefits. Similarly, Rafiq et al[15] found that psychological empowerment plays an important role in improving job satisfaction and reducing turnover intentions among nurses. This is also opposite to the findings of this study where the level of resilience and job satisfaction was not associated with the working conditions.

Other studies have shown that workplace factors such as organizational support, recognition, and professional autonomy significantly influence nurses' job satisfaction. In addition, workplace incivility and poor organizational climate have been associated with reduced job satisfaction among nurses in Pakistan.[16] However, the present findings, which revealed no significant relationship between resilience and job satisfaction in both pre, and post intervention are inconsistent with existing literature. Previous studies have consistently reported a significant positive relationship between resilience and job satisfaction, with resilience playing a mediating role in reducing the negative impact of role stress on job satisfaction among nursing professionals.[17] The current study's results, along with the lack of association between demographic variables and both constructs, therefore, contradict prior evidence and suggest that the protective role of resilience on job satisfaction may be weaker or absent in this context.

Similarly, Andersen et al[18] suggested that resilience significantly influences job satisfaction, work engagement, and employee retention by reducing workplace stress, role clarity and promoting adaptive coping strategies. Another study conducted among 194 infection control nurses in Korean general hospitals also supports the findings of this study where they found out that role stress was negatively correlated with both resilience and job satisfaction. The major sources of stress identified were lack of job autonomy and heavy job demands.[19]

The findings of the present study support these findings and indicate that resilience serves as a protective factor that helps nurses maintain job satisfaction despite challenging working conditions.[20] This study indicated the requirement for structured mentorship programs and strategies to build resilience among nurses to enhance the quality of healthcare services provided by healthcare organizations.

CONCLUSION

The relationship between resilience and job satisfaction was positive and significant for nurses. The nurse-led mentorship program had a significant effect on enhancing the nurse's resilience and job satisfaction. The mentorship intervention was found to be a good strategy for increasing the coping skills of the nurses and improving their professional wellbeing.

Author's Contribution:

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

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Original Article

Enhancing Activities of Daily Living and Quality of Life among Patients with Post-Operative Hip Replacement Surgery

Kausar Sultana[1], Muhammad Saifullah[2] and Madiha Mukhtar[3]

ABSTRACT

Objective: To determine the effect of clinical practice guidelines on enhancing activities of daily living and quality of life in post-operative hip surgery patients.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the Orthopedic Ward, Kishwar Fazal Teaching Hospital Lahore, Pakistan from 1st May 2025 to 31st December 2025.

Methods: A quasi-experimental study design was employed involving 40 patients undergoing hip replacement surgery. Participants were purposively sampled and divided into experimental and control groups. The experimental group received interventions based on clinical practice guidelines. Data were collected using structured questionnaires assessing activities of daily living and quality of life. Higher scores indicated greater functional limitation for activities of daily living and better perceived quality of life. The reliability of the tools was confirmed with a Cronbach's alpha of 0.912.

Results: The intervention group demonstrated significant improvements in independence in daily activities and overall quality of life compared to the control group. Implementation of clinical practice guidelines facilitated better physical mobility, reduced functional limitations, and enhanced emotional well-being.

Conclusion: Application of evidence-based clinical practice guidelines significantly improves post-operative recovery in hip replacement patients by enhancing activities of daily living and overall quality of life. Incorporating structured rehabilitation protocols into routine post-operative care is recommended to promote patient independence and holistic well-being.

Key Words: Hip replacement, Rehabilitation, Activities of daily living, Quality of life, Post-operative care, Clinical practice guidelines

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INTRODUCTION

Hip joint disorders such as fractures, osteoarthritis, dislocation, and avascular necrosis (AVN) commonly impair mobility and functional independence, often necessitating hip replacement surgery. Hip fractures represent a major global health concern, particularly among older adults. Epidemiological projections estimate that approximately 6.26 million individuals worldwide will experience hip fractures annually by 2050, with a cumulative one-year mortality rate approaching 30%.[1]

[1.] MSN Scholar / Assistant Professor[2] / Associate Professor[3], Lahore School of Nursing, The University of Lahore.

Correspondence: Muhammad Saifullah, Assistant Professor, Lahore School of Nursing, The University of Lahore, Pakistan.
Contact No: 030107889068
Email: muhammad_saifullah@lsn.uol.edu.pk

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Hip replacement surgery is widely performed to restore mobility and alleviate chronic pain associated with degenerative hip diseases such as osteoarthritis and rheumatoid arthritis.[2] Although the procedure is highly effective, patients frequently encounter significant post-operative challenges, including reduced mobility, persistent pain, and diminished quality of life. These difficulties underline the importance of comprehensive post-operative rehabilitation strategies.[3]

Globally, hip disorders rank among the top ten musculoskeletal conditions contributing to disability-adjusted life years.[4] In the United States alone, more than 450,000 hip replacement surgeries are performed annually, and this number continues to rise due to population aging.[5]

In Pakistan, the burden of hip disorders is increasing, particularly among the elderly population. Approximately 8.7% of individuals aged 60 years and above suffer from hip-related mobility problems requiring surgical intervention.[6] Limited access to physiotherapy services and pain management resources in rural regions further compromises recovery outcomes.[7] Although urban centers such as Lahore and Karachi report improved post-operative outcomes, gaps

remain in structured rehabilitation programs nationwide.[8]

Quality of life is a multidimensional concept encompassing physical, emotional, and social well-being. Reduced QoL following hip surgery negatively affects social participation, psychological health, and overall life satisfaction.[9] The World Health Organization emphasizes early mobilization, functional recovery, pain management, and patient education as core components of effective post-operative rehabilitation.[10]

Nurses play a critical role in implementing clinical practice guidelines through patient education, pain control, physical exercises, and continuous monitoring. Nursing-led interventions have demonstrated positive effects on improving ADLs and reducing post-operative discomfort.[11] However, limited research exists in Pakistan regarding the effectiveness of WHO-based clinical practice guidelines on long-term ADL and QoL outcomes following hip replacement surgery. This study addresses this gap.

METHODS

A quasi-experimental study design with control and experimental groups was conducted to evaluate the effect of a nurse-led intervention on activities of daily living (ADL) and quality of life (QOL) among patients undergoing hip replacement surgery. The study was carried out in the Orthopedic Ward of Kishwar Fazal Teaching Hospital, Lahore from 1st May 2025 to 31st December 2025 vide letter UOL/IREB/25/07/0046 dated 17th March 2025. A total of 40 participants selected through purposive sampling and equally divided into experimental and control groups. Data were collected using a demographic questionnaire, an adopted Daily Activity Questionnaire, and the WHOQOL-BREF tool to assess functional ability and quality of life. Baseline (pre-intervention) data were obtained, followed by the implementation of a structured nurse-led intervention program in the experimental group, while the control group received routine care. The intervention included pre-operative education and progressive post-operative rehabilitation sessions focusing on mobility, exercises, and self-care. Post-intervention assessment was conducted to evaluate outcomes. Data were analyzed using SPSS version 25, employing descriptive statistics and non-parametric tests, including Wilcoxon Signed-Rank and Mann-Whitney U tests, with significance set at $p < 0.05$.

RESULTS

The majority of participants in both groups were aged between 31-50 years (45% each), followed by 16-30 years and 51–70 years. In terms of occupation, most participants in the control group were working (70%), whereas the experimental group had an equal distribution of working and non-working individuals (50% each). Regarding gender, males constituted the majority in both groups. Most participants had a height between 5.0-6.0 feet and a weight range of 50-70 kg. Avascular necrosis (AVN) was the most common diagnosis in both groups, followed by fractures. Additionally, caregiving support differed between groups, with wives being the primary caregivers in the control group, while husbands were more commonly reported as caregivers in the experimental group. Overall, the demographic and clinical characteristics were relatively comparable between the two groups (Table 1).

Table No. 1: Demographic and clinical characteristics of participants (N = 40)

Variable	Control Group	Experimental Group
Age (years)		
16-30	5 (25%)	6 (30%)
31-50	9(45%)	9 (45%)
51-70	6(30%)	5 (25%)
Occupation		
Working	14 (70%)	10 (50%)
Non-working	6 (30%)	10 (50%)
Gender		
Male	14 (70%)	13 (65%)
Female	30 (30%)	7 (35%)
Caregiver		
Wife	13 (65%)	4 (20%)
Daughter	3 (15%)	4 (20%)
Others	1 (5%)	-
Height (ft)		
5.0-6.0	17 (85%)	19 (95%)
6.1-7.0	3 (15%)	1 (5%)
Weight (kg)		
50-60	8 (40%)	9 (45%)
61-70	8 (40%)	8 (40%)
71-80	4 (20%)	3 (15%)
Diagnosis		
AVN	14 (70%)	15 (75%)
Fracture	6 (30%)	5 (25%)

Most patients in both groups had severe difficulty in performing activities of daily living, with no patients reporting mild or minimal difficulty. After the intervention, the control group showed no improvement, as all patients remained in the severe category. In contrast, the experimental group demonstrated significant improvement, with patients shifting to mild (60%) and minimal (40%) difficulty levels. Overall, the intervention proved effective in improving patients' functional ability, while routine care showed no impact (Table 2).

Table 3 indicates that before the intervention, there was no significant difference in the level of difficulty in daily living activities between the control and experimental groups (p=0.217). However, after the

intervention, a statistically significant difference was observed (p=0.000), with the experimental group showing greater improvement (lower median score) compared to the control group, demonstrating the effectiveness of the intervention.

Most patients in both groups had poor quality of life. After the intervention, the control group showed slight improvement, with some patients moving to a moderate level, but many still remained in the poor category. In contrast, the experimental group demonstrated marked improvement, with no patients in poor or very poor categories and a majority shifting to good (65%) and moderate (35%) quality of life. This indicates that the intervention was highly effective in improving patients' quality of life compared to the control group (Table 4).

Table 5 indicates that before the intervention, there was no significant difference in quality of life between the control and experimental groups (p=0.152). However, after the intervention, a statistically significant difference was observed (p=0.000), with the experimental group showing greater improvement (higher median score) compared to the control group, highlighting the effectiveness of the intervention in enhancing quality of life. There was no statistically significant association between difficulty in daily living activities and quality of life in both control and experimental groups before and after the intervention (p>0.05). Although a negative correlation was observed in most cases, indicating that higher difficulty may be related to lower quality of life, these relationships were not statistically significant (Table 6).

Table No. 2: Comparison of frequency distribution of Level of activities of daily living of control group and experimental group patients before and after intervention (n=40)

Level of difficulty in daily living activities	Control Group		Experimental Group	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
Minimal difficulty in daily living activities	-	-	-	8 (40%)
Mild difficulty in daily living activities	-	-	-	12 (60%)
Moderate difficulty in daily living activities	3 (15%)	7 (35%)	-	-
Severe difficulty in daily living activities	17 (85%)	13 (65%)	20 (100%)	-

Table No. 3: Comparison of level of difficulty in daily living activities before and after intervention of experimental group and control group nurses (n=40)

Variable	Control Group Median (IQR)	Experimental Group Median (IQR)	p-value
Pre-intervention level of difficulty in daily living activities	61.00(5.75)	62.00(1.00)	0.217
Post-intervention level of difficulty in daily living activities	4.0 (1.00)	2.00(1.00)	0.000

Table No. 4: Comparison of Quality of Life of control group and experimental group patients before and after intervention (n=40)

Level of quality of life	Control Group		Experimental Group	
	Pre-Intervention	Post-Intervention	Pre-Intervention	Post-Intervention
Very Poor Quality of Life	1 (5%)	-	-	-
Poor Quality of Life	18 (90%)	8 (40%)	13 (65%)	-
Moderate Quality of Life	1 (5%)	12 (60%)	7 (35%)	7(35%)
Good Quality of Life	-	-	-	13(65%)

Table No. 5: Comparison of frequency distribution of Quality of Life of control group and experimental group patients before and after intervention (n=40)

Variable	Control Group Median (IQR)	Experimental Group Median (IQR)	p-value
Pre-Intervention Quality of Life	2.00(0.00)	2.00(0.00)	0.152
Post-Intervention Quality of Life	3.0 (1.00)	4.00(1.00)	0.000

Table No. 6: Association between difficulty in daily living activities and quality of life of control group and experimental group patients before and after intervention (n=40)

Variable	Group	Spearman Correlation (ρ)	p-value
Pre-intervention difficulty in daily living activities vs pre-intervention quality of life	Control	–0.289	0.216
	Experimental	-0.306	0.189
Post-intervention difficulty in daily living activities vs post-intervention quality of life	Control	1.000	0.257
	Experimental	-0.385	0.094

DISCUSSION

The present study demonstrated a statistically significant improvement in ADL scores in both control and experimental groups; however, the improvement was substantially greater in the experimental group ($p<0.001$). The experimental group showed a complete shift from severe dependency to mild or minimal difficulty after intervention. These findings are consistent with the study conducted by Zhang[12], which reported that structured nursing interventions significantly improved functional recovery and independence in post-hip replacement patients. Similarly, Rehman et al[7] found that rehabilitation-based nursing care enhances mobility and daily functioning after orthopedic surgery. Smith et al[13] reported significant post-operative improvements in ADL scores among patients undergoing hip replacement following structured nursing and physiotherapy interventions, particularly in mobility and self-care domains. Similarly, Patel and Singh[14] found that targeted rehabilitation programs enhanced not only core physical abilities but also the capacity to perform household and social activities, supporting the notion that comprehensive post-surgical interventions can facilitate holistic recovery.

In contrast, the control group in the present study showed only minimal improvement, which may be attributed to routine care and natural recovery. This finding aligns with Ahmed et al[15], who reported that standard post-operative care alone results in slower functional recovery compared to structured rehabilitation programs.

The study findings revealed a significant improvement in quality of life in both groups; however, the experimental group demonstrated a much higher improvement compared to the control group ($p<0.001$). The majority of patients in the experimental group achieved good quality of life after intervention, whereas the control group remained mostly in the moderate category. These results are supported by Jones et al[9], who found that rehabilitation and nursing education significantly improve quality of life in post-arthroplasty patients. Mohammed et al[16] reported significant post-intervention improvements in both physical and psychosocial aspects of QoL among patients undergoing hip replacement, highlighting enhanced mobility, independence, and social participation. Similarly, Singh et al[17] found that interventions combining physiotherapy, patient education, and functional skill practice resulted in measurable gains in physical functioning and overall well-being, mirroring the present study's results. Taraldsen[18] also emphasized that post-surgical rehabilitation can enhance autonomy and confidence in daily activities, further supporting the current findings.

However, some improvement in the control group was also observed, which is consistent with Mahomed[3], who noted that natural recovery and routine physiotherapy can lead to gradual improvements in QOL even without structured interventions. Furthermore, while family support and resource access were consistently high in the present sample, other studies in less resourced settings have highlighted that limited social support can impede QoL improvements, underscoring the influence of contextual factors on recovery outcomes.[19,20] Overall, the present study confirms that structured intervention is more effective than routine care in improving quality of life.

CONCLUSION

Both groups were similar at baseline, ensuring fair comparison. While the control group showed slight improvement, the experimental group demonstrated significantly greater improvement in activities of daily living and quality of life after the nurse-led intervention. Although both outcomes improved, no significant association was found between them. Overall, the intervention was effective in enhancing patients' functional independence and quality of life.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Kausar Sultana, Muhammad Saifullah
Drafting or Revising Critically:	Kausar Sultana, Madiha Mukhtar
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Determination of Molecular Weight and Partial Purification of Cyclooxygenase-2 Enzyme and its Kinetic Study in Male Colorectal Cancer Patients

Ali Rahman Nama

ABSTRACT

Objective: To purify cyclooxygenase-2 from the serum of male colorectal cancer patients.
Study Design: Combined clinical and experimental in vitro study
Place and Duration of Study: This study was conducted at the College of Science, Kirkuk University, Kirkuk, Iraq from 15th December 2025 to 30th April 2026.
Methods: Sedimentation techniques, hemodialysis, ion-exchange chromatography column separation, and gel filtration were used. The resin material (DEAE cellulose) was positively charged.
Results: Two analogs of the enzyme were obtained. Cyclooxygenase-2 activity was higher in peak A than in peak B, with specific activity values of 4.94 U/mg and 2.17 U/mg respectively. The approximate molecular weight of the cyclooxygenase-2 from (peak A) was estimated by gel filtration using Sefadex G-100 gel and was 71302 dalton. Optimal conditions were estimated after kinetic study of purified cyclooxygenase-2: velocity maximum (Vmax=1 U/mL), Michaelis-Menten constant (Km=2.5 mM, pH=7.5), temperature 38 °C and time period 70 seconds.
Conclusion: The success of the proposed purification protocol in isolating highly purified cyclooxygenase-2 enzyme and maintaining its kinetic stability from complex clinical samples. The inferred kinetic parameters (K m, Vmax) contribute to a deeper understanding of the catalytic behavior of the human enzyme, supporting future research into the mechanisms of pharmacological inhibition.

Key Words: Electrophoresis, Gel filtration, Inhibitors, Ion exchange, Optimum conditions, Risk factors

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INTRODUCTION

Colorectal cancer includes cancer of the colon and rectum. It is the second most malignant cancer leading to death, and the most common type of cancer after lung cancer and breast cancer.[1] Colorectal cancer originates from polyps that develop in the mucous membrane of the colon, which turn into malignant tumors if they are not removed.[2] Adenomatous polyps in the colon begin with a slow, malignant transformation process that takes years. The size of the polyps increases as the tumor grows, and the risk increases when they exceed 1-2 cm.[3] The tumor spreads in the colon wall to distant organs via the lymphatic system or bloodstream.[4]

Lecturer, Department of Biochemistry, General Directorate of Kirkuk Education, Kirkuk, Ministry of Education, Iraq.

Correspondence: Ali Rahman Nama, Lecturer, Department of Biochemistry, General Directorate of Kirkuk Education, Kirkuk, Ministry of Education, Iraq.
Contact No: +9647704043566
Email: alirahman@uokirkuk.edu.iq

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Oxidative stress and chronic inflammation in the intestines, resulting from impaired integrity of the intestinal mucosa, are key factors causing colon damage and colorectal cancer.[5]

Cyclooxygenase-2 (COX-2) enzymes play a vital role in colorectal cancer. COX-2 enzyme levels increase when the cascade of inflammatory reactions begins in the platelets, which activates thromboxanes, leading to tumor initiation, growth, and spread.[6] In chronic inflammatory bowel diseases such as ulcerative colitis and colorectal cancer, COX-2 levels are elevated due to the production of prostaglandin-promoting cytokines. Prostaglandin production promotes tumor growth and development, causing reprogramming of stem cell genes, inhibition of programmed cell death, angiogenesis, and proliferation of cancer cells.[7] Disruption in the metabolism of polyunsaturated fatty acids in the liver leads to an increase in the production of oxylipins. Oxylipins are derived from arachidonic acid, particularly prostaglandins, causing an increase in COX-2 hyperactivity, forming inflammatory oxylipins that are the main cause of the inflammatory response caused by oxidative stress.[8]

In colorectal cancer, oxidative stress contributes through lipid peroxidation, DNA damage, and protein

modification leading to the production of reactive oxygen species (ROS) with weakened antioxidant defenses. High levels of ROS contribute to genetic mutations, alter the tumor microenvironment, and activate inflammatory pathways, thereby promoting the survival and proliferation of cancer cells.[5]

Colorectal cancer is closely associated with two types of risk factors. Modifiable factors related to habits and lifestyles, and these factors can be controlled by individuals, such as (obesity, smoking, alcohol, diet, socioeconomic and environmental lifestyle, and psychological stress). Non-modifiable factors that are beyond the control of individuals include (gender and race, family history, age, chronic diseases, benign tumors in the intestines, intestinal microbiota).[9]

There are two symmetrical forms from cyclooxygenase enzymes, namely cyclooxygenase-2 (COX-2) and cyclooxygenase-1 (COX-1). These two enzymes share overlapping catalytic functions, but they differ significantly in physiological roles, gene expression patterns, and genetic structure.COX-2 is considered a crucial factor in tumor growth and a therapeutic target for many types of cancer in humans and animals.[10]

The protective efficacy of COX-2 in colorectal cancer has been established by taking treatments that reduce or inhibit COX-2 expression. Excessive COX-2 secretion is directly correlated with malignancy and disease progression, making COX-2 a promising therapeutic target. Non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors have shown chemo-preventive results in the treatment of adenomas.[10,11]

COX-2 inhibitors work as preventatives against chronic inflammatory bowel disease and colorectal cancer. Elevated COX-2 levels are diagnostic vital signs and detection of early-stage colorectal cancer. COX-2 is related to inflammation and tumor development, It has a crucial role in initiation, progression, and transformation of colorectal cancer into adenocarcinomas and subsequently into malignant tumors.[10]

The purpose is to characterize both the disease and the enzyme, and their intrinsic relationship. The approximate molecular weight of the purified COX-2 enzyme is determined. The enzyme's kinetics were studied, the values of both the Michaelis-Menten constant (Km) and the maximum velocity (Vmax) were calculated, and the factors affecting the activity of cyclooxygenase-2 and the optimal conditions for its operation were studied.

METHODS

This combined clinical and experimental in vitro study was conducted at College of Science, Kirkuk University, Kirkuk, Iraq from 15th December 2025 to 30th April 2026 vide letter No. 3131/QM/Approval/kdjkjrr dated 1st December 2025. Blood samples were taken from 15 male patients with colorectal cancer attending Azadi Teaching Hospital in Kirkuk Governorate. The patients were aged 50-70 years. Ethical consent was obtained from the patients before blood samples were drawn as part of the work requirements. Initially, it was confirmed that the patients did not have chronic heart disease with high blood pressure, diabetes, etc, and that they did not consume alcohol or smoke. Three milliliters of blood were drawn from a vein and placed in sealed, dedicated tubes (gel tubes). The samples were placed in the water bath and incubated for 15 minutes at 37°C. The blood serums were separated by centrifuge for (20 minutes) at a speed of (3000 rpm). Finally, it was frozen in specially prepared plastic tubes at -20°C until partial purification for cyclooxygenase-2.

Colorimetric methods were used to measure COX-2 activity.[12] Tetramethylphenylenediamine (TMPD) is oxidized by hydrogen peroxide reduction, yielding a blue compound,, and the absorbance is measured at (λ=610 nanometers).Estimating total protein concentration was modified Lowry method was used to estimate the total protein amount at (λ=280 nm).[13]

Highly soluble neutral salts, such as ammonium sulfate were used in the protein precipitation process during purification.[14] Ammonium sulfate is added gradually to (15 mL) of crude blood serum. The mixture is stirred for one hour using a magnetic stirrer, at a temperature of (4°C). The mixture was left in the refrigerator for 24 hours to ensure better precipitation. The separation was done by centrifugation for (25 minutes) at a speed of (6000 xg) to separate the filtrate from the precipitate. Total protein and Cyclooxygenase-2 activity were measured for both the filtrate and the sediment. The COX-2 enzyme activity was highest in the precipitate after the addition of distilled water. The solution was stored at -20°C in preparation for use in subsequent purification steps.

The dialysis process is carried out at (4°C) to remove the remaining ammonium sulfate. The dialysis bag was filled with the proteinaceous precipitate solution. The bag was then completely submerged in a (500 mL) glass flask containing (0.1 M) ammonium bicarbonate solution. The flask was placed on a magnetic stirrer, and the solution was stirred for (24 hours). The solution was replaced every (6 hours). COX-2 activity and total protein content are estimated, and the solution is kept at a temperature of (-20°C) until it is used in subsequent steps.[15]

The purification column with dimensions (2.5 cm × 50 cm) was used with the positively charged resin diethyl aminoethyl cellulose (DEAE cellulose).[16] The column was washed using a buffer solution (trishydroxy-methylaminomethane) with a pH of (pH=8) and a concentration of (0.1M). The flow rate was set to (1 mL/minute). (9 ml) of the sample solution resulting from the dialysis step was injected gently from the top of the column. The samples were collected at the

bottom of the column in test tubes. The total protein amount was measured at the absorbance (280 nm) with COX-2 activity at (610 nm) in all separated parts.

Gel filtration technique was used to estimate the molecular weight of cyclooxygenase-2 approximately.[17] The proteins with known standard molecular weights ranging from 204 to 2,000,000 Daltons were used. A separation column measuring (2 cm in diameter and 100 cm in height) was filled with Sefadex G-100 gel to a height of (90 cm). After filling, the gel (stationary phase) was washed with buffer solution (mobile phase).[18] The protein material was rinsed at a flow rate of (5 mL/5 minutes), or at a rate of (60 ml/hour)The standard calibration curve for the volumes of protein aceous materials was plotted with the logarithm of their molecular weights. The enzyme's approximate molecular weight was determined from the resulting linear equation, from the calibration curve. The purified enzyme is kept until the kinetic study and the factors affecting it are carried out. The data was analyzed through SPSS-25.

RESULTS

The partial separation and purification of COX-2 in the serum of male patients with colorectal cancer was shown in Table 1. After the precipitation process using ammonium sulfate, an increase in the specific activity of COX-2 was observed from (1.20-1.40 U/mg). Fold increased from (1-1.17), and the yield ratio became (87.34%). The remaining ammonium sulfate salt was removed from the precipitation process by dialysis. The specific activity increased after hemodialysis to (2.01 U/mg), the folding increased to (1.67), and the yield ratio became (90.34%). Two bands, (band A) and (band B), of COX-2 enzyme symmetrical were obtained using ion-exchange chromatography (Fig. 1)

The kinetics of COX-2 was studied to determine the optimal conditions and solutions with different pH values 5-8.5 were prepared. The optimal pH was found to be 7.5. The optimal time for the highest peak COX-2 activity at different time intervals (10-110 seconds) was (70 seconds). The optimal temperature at different temperatures 30-46°C was 38°C. Different concentrations of the substrate (hydrogen peroxide) were prepared, ranging from 1-12 mM. COX-2 activity was measured at these concentrations, and it was observed that COX-2 activity increased with increasing substrate concentration. A high substrate concentration leads to an increased rate of enzyme reaction. Until the active sites of the COX-2 enzyme become saturated with the substrate. When the rate of enzyme reaction reaches its maximum speed (Vmax), it remains constant no matter how much the substrate concentration increases (Fig. 2)

The Lineover-Berke plot was used between COX-2 activity and substrate concentration (Fig. 3). The value of velocity maximum was determined (Vmax = 1 U/mL), and the Michaelis-Menten constant was (Km=2.5 mM). Km is defined as the substrate concentration when the COX-2 enzyme activity (velocity) is half the velocity maximum (0.5 Vmax).

The purified COX-2 enzyme was passed through (peak A) on the gel separation column. The highest COX-2 activity was obtained through the highest peak at the elution volumes 171 mL (Fig. 4).

Table No. 1: Steps for purifying cyclooxygenase-2 enzyme in the serum of male colorectal cancer patients

Purification steps	Volume (mL)	Total protein (mg)	Activity of COX-2 (U/mL)	Total activity (U*)	Specific activity (U/mg)	Fold	Yield %
Serum	15	11.1	0.89	13.35	1.20	1	100
Ammonium sulphate (50%)	11	8.31	1.06	11.66	1.40	1.17	87.34
Dialysis	9	6.01	1.34	12.06	2.01	1.67	90.34
Ion Exchange DEAE-Cellulose (A50)							
Pack A	12	2.65	1.09	13.08	4.94	4.10	97.98
Pack B	10	4.65	1.01	10.1	2.17	1.55	86.62

Table No. 2: Estimation of elution volumes from the titration curve of standard proteins with the logarithm of their molecular weights

Materials	Molecular Weight	Elution Volume	Log Molecular Weight
Blue dextrin	2000000	64.3	6.30
Urease	48000	97.4	6.68
Bovine serum albumin	67000	135.9	4.83
Egg albumin	45000	177.7	4.68
Pepsin	36000	200.1	4.56
Insulin	5750	325.3	3.76
Tryptophan	204	372.7	2.31

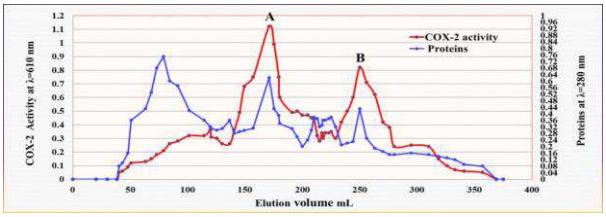

Figure No. 1: Ion-exchange chromatography for purification of cyclooxygenase-2 enzyme in the serum of male colorectal cancer patients

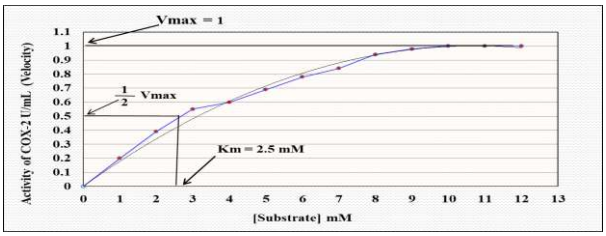

Figure No. 2: Impact of substrate concentration on the reaction rate of purified cyclooxygenase-2 activity

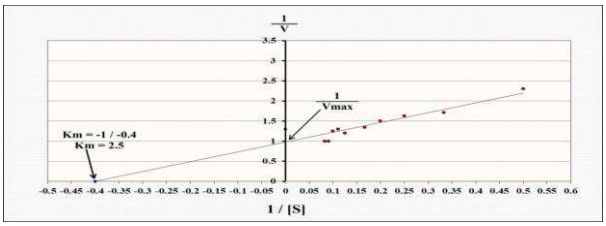

Figure No. 3: Lineover-Berke plot to find Vmax and Km values for cyclooxygenase-2 enzyme activity

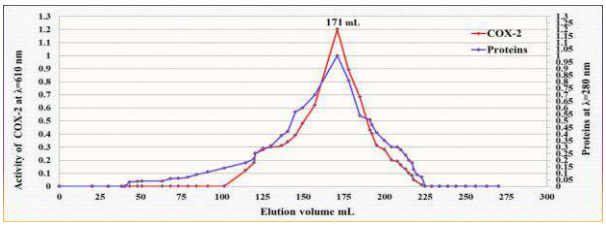

Figure No. 4: Gel filtration chromatography for partial purification of cyclooxygenase-2 enzyme in the serum of male colorectal cancer patients

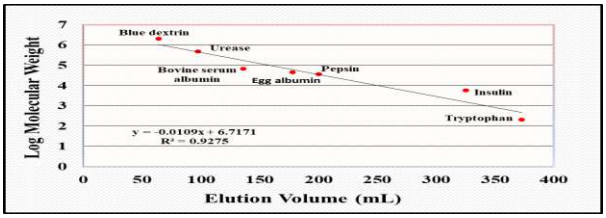

Figure No. 5: Standard calibration curve using gel chromatography for estimating the approximate molecular weight of cyclooxygenase-2

The molecular weight of the purified enzyme was estimated passing proteins of known molecular weights (204–2,000,000 Daltons) through a gel filtration column. In Table 2, the elution volumes were obtained for the known molecular weights.

The relationship between the logarithms of the known molecular weights of proteins and their elution volumes was plotted using a calibration curve. The equation of the straight line shown in figure 5 was obtained.

DISCUSSION

Two bands, band A and band B, of COX-2 enzyme analogs were identified using ion-exchange technique. The specific activity of band A (4.94 U/mg) was higher than that of band B (2.17 U/mg). Their yield rates were (97.98%) and (86.62%) respectively. The COX-2 activity in (band A) was higher than in (band B). The approximate molecular weight was estimated from (band A) with the highest enzyme activity. The specific activity of purified COX-2 in (band A) increased fourfold compared to raw blood before purification. Gel filtration chromatography was used to determine particle sizes and molecular weights in solution.[19] Finally, the molecular weight of COX-2 was estimated using electrophoresis, and it was found to be 71 kDa.[20]

In the gel filtration technique, the volume of pure COX-2 (171 mL) from figure 4 is substituted into the straight-line equation, and we obtain the approximate value of the molecular weight of COX-2, which is (71302 Daltons).In electrophoresis technique, a distinct protein band appeared after travelling a certain distance towards the positive electrode from the starting point. From this, the approximate molecular weight of the COX-2 enzyme was estimated to be (71 kDa).[20] This study is consistent with several previous studies in which the molecular weight of cyclooxygenase-2 enzyme (69-72 kDa).[21-23]

CONCLUSION

Colorectal cancer originates from polyps that develop in the colonic mucosa. It transforms into a malignant tumor if not removed. Cancer is associated with several risk factors, including obesity, smoking, alcohol consumption, diet, family history, and the microbiome. Cyclooxygenase-2enzymes play a vital role in colorectal cancer. Oxidative stress and chronic inflammation in the intestines and liver, triggered by pro-inflammatory cytokines, lead to the production of prostaglandins. The inferred kinetic parameters (K m, Vmax) contribute to a deeper understanding of the catalytic behavior of the human enzyme, supporting future research into the mechanisms of pharmacological inhibition.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ali Rahman Nama
Drafting or Revising Critically:	Ali Rahman Nama

Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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Original Article

Assessment of Beta-Blocker Induced Bradycardia in Post-Myocardial Infarction Patients: A Dose–Response Analysis

Adnan Jehangir

ABSTRACT

Objective: To assess the frequency, severity, and dose–response relationship of beta-blocker–induced bradycardia in post-MI patients and identify associated clinical predictors.

Study Design: Cross-sectional study.

Place and Duration of Study: This study was conducted at the Poonch Medical College, Rawalakot, from June 2024 to June 2025.

Methods: This study included 185 post-MI patients receiving beta-blocker therapy. Patients were grouped by beta-blocker type (metoprolol, bisoprolol, carvedilol) and dosage (low, moderate, high). Heart rate and clinical parameters were monitored during follow-up. Bradycardia was defined as a resting heart rate <60 bpm, with severity categorized as mild (50–59 bpm), moderate (40–49 bpm), or severe (<40 bpm).

Results: The mean age of patients was 59.7 ± 9.8 years, with 136 males (73.5%) and 49 females (26.5%). Hypertension (65.9%) and diabetes mellitus (49.2%) were the most frequent comorbidities. Metoprolol was prescribed in 56.2% of patients, bisoprolol in 28.1%, and carvedilol in 15.7%. Bradycardia occurred in 58 patients (31.4%), including mild in 34 (18.4%), moderate in 19 (10.3%), and severe in 5 (2.7%). The incidence of bradycardia increased significantly with higher doses—12.1% in low-, 36.7% in moderate-, and 62.5% in high-dose groups ($p < 0.001$). Bisoprolol showed the highest rate of bradycardia (40.4%), followed by metoprolol (29.8%) and carvedilol (24.1%). Independent predictors of bradycardia were high-dose therapy (OR 4.62, 95% CI 2.38–8.95, $p < 0.001$) and age >65 years (OR 2.13, 95% CI 1.09–4.15, $p = 0.03$).

Conclusion: Beta-blocker–induced bradycardia is a common, dose-dependent adverse effect in post-MI patients, particularly among elderly individuals and those receiving higher doses.

Key Words: Beta-blockers, myocardial infarction, bradycardia, dose–response, metoprolol, bisoprolol, carvedilol.

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INTRODUCTION

Beta-blockers remain among the most widely prescribed and evidence-backed pharmacologic agents for the secondary prevention of cardiovascular events following myocardial infarction (MI). Their benefits are largely attributed to their ability to mitigate the deleterious effects of sympathetic overactivity on the myocardium, reduce oxygen demand, prevent arrhythmic death, and promote ventricular remodeling stabilization[1,2].

The physiological mechanism centers on competitive antagonism of β1-adrenergic receptors within cardiac tissue, leading to decreased heart rate, myocardial contractility, and conduction velocity all of which collectively reduce the risk of ischemia and infarct expansion[3]. In numerous large-scale clinical trials, beta-blocker therapy has consistently demonstrated reductions in both short-term and long-term mortality among post-MI patients, forming the foundation of their recommendation in major guidelines by the American College of Cardiology (ACC), American Heart Association (AHA), and European Society of Cardiology (ESC)[4]. However, despite these well-established benefits, beta-blocker therapy is not without adverse effects, and bradycardia remains one of the most clinically significant dose-related complications[5]. Beta-blocker–induced bradycardia (BIB) is a common phenomenon resulting from excessive suppression of sinoatrial node automaticity or impaired atrioventricular nodal conduction[6]. While a mild reduction in heart rate is typically desirable to decrease myocardial workload, excessive bradycardia often defined as a resting heart rate below 50 beats per minute can compromise cardiac output and tissue perfusion, leading to fatigue, dizziness, hypotension, syncope, or even cardiac arrest in extreme cases[7]. The development of BIB represents a delicate balance between achieving therapeutic beta-blockade and avoiding undue suppression of

Professor of Pharmacology, Poonch Medical College, Rawalakot, Pakistan.

Correspondence: Adnan Jehangir, Professor of Pharmacology, Poonch Medical College, Rawalakot, Pakistan.
Contact No: +966 53 969 5518
Email: amalik@kfu.edu.sa

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physiological chronotropy[8]. The relationship between beta-blocker dose and bradycardia is complex and influenced by interindividual variability. Factors such as age, baseline heart rate, renal and hepatic function, left ventricular ejection fraction, and concurrent medication use (e.g., calcium channel blockers, digoxin, or antiarrhythmics) can significantly alter drug sensitivity[9]. Moreover, the pharmacologic characteristics of beta-blockers such as β1-selectivity, intrinsic sympathomimetic activity, and lipid solubility further modulate the dose-response relationship. For instance, cardioselective agents like metoprolol and bisoprolol preferentially target β1 receptors, reducing the risk of bronchospasm but not necessarily mitigating the risk of bradycardia at higher doses[10]. Non-selective beta-blockers such as propranolol and carvedilol, while providing broader sympathetic inhibition, may lead to more pronounced chronotropic depression in susceptible patients[11]. Previous studies have shown variable incidence rates of BIB in post-MI populations, ranging from 10% to 30%, depending on dose intensity and clinical context. The Beta-Blocker Heart Attack Trial (BHAT) and subsequent meta-analyses demonstrated a clear mortality benefit of beta-blockers but also reported higher rates of drug discontinuation due to symptomatic bradycardia or hypotension[12].

METHODS

This was a cross-sectional study conducted at Poonch Medical College, Rawalakot, from June 2024 to June 2025. A total of 185 post-myocardial infarction patients were included in the study. The sample size was calculated using the WHO sample size calculator, assuming a confidence level of 95%, a margin of error of 5%, and an anticipated prevalence of beta-blocker–induced bradycardia of 20%. Non-probability consecutive sampling was employed to recruit eligible patients.

Inclusion Criteria

- Patients of either gender aged between 30 and 80 years.
- Confirmed diagnosis of myocardial infarction (STEMI or NSTEMI) based on clinical, ECG, and biochemical findings.
- Patients on oral beta-blocker therapy (metoprolol, bisoprolol, or carvedilol) initiated during post-MI management.
- Patients hemodynamically stable at the time of inclusion.

Exclusion Criteria

- Patients with pre-existing bradyarrhythmias, sinoatrial node dysfunction, or second/third-degree AV block.
- Patients on other negative chronotropic drugs (e.g., digoxin, diltiazem, verapamil, or amiodarone).
- Patients with acute decompensated heart failure, cardiogenic shock, severe hepatic or renal impairment.
- Patients with pacemakers or those who had discontinued beta-blocker therapy before enrollment.

Data Collection Procedure: The study was conducted following approval from the Institutional Review Board (IRB) and the hospital's Ethics Review Committee. Informed written consent was obtained from all participants. Each patient's demographic data, clinical history, and cardiovascular risk factors were recorded using a structured proforma. Beta-blocker type, prescribed dose, and duration of therapy were documented from medical records. Patients were classified according to the specific beta-blocker used metoprolol, bisoprolol, or carvedilol and were further divided into low-, moderate-, and high-dose groups based on standardized daily dose equivalence. Heart rate and blood pressure were measured in a resting state at baseline and at each follow-up interval (day 3, day 7, and day 14 post-initiation). Bradycardia was defined as a resting heart rate <60 beats per minute, while significant bradycardia was defined as heart rate <50 beats per minute or symptomatic bradycardia associated with dizziness, syncope, or hypotension. The severity of bradycardia was categorized as mild (50–59 bpm), moderate (40–49 bpm), or severe (<40 bpm). Other variables including age, sex, diabetes mellitus, hypertension, smoking status, and ejection fraction (EF%) were recorded. Laboratory parameters such as serum creatinine and electrolyte levels were obtained to exclude secondary causes of bradycardia.

Data Analysis: Data were analyzed using SPSS version 26.0. Continuous variables such as age, ejection fraction, and heart rate were expressed as mean ± standard deviation (SD), while categorical variables including gender, comorbidities, type of beta-blocker, and occurrence of bradycardia were presented as frequencies and percentages. Comparisons between dose groups were made using one-way ANOVA for continuous variables and the Chi-square test or Fisher's exact test for categorical data. The relationship between beta-blocker dose and severity of bradycardia was analyzed using linear regression analysis. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Data were collected from 185 patients, mean age of the patients was 59.7 ± 9.8 years, ranging from 34 to 80 years, with a male predominance (73.5%). Hypertension (65.9%) and diabetes mellitus (49.2%) were the most common comorbidities, followed by smoking (36.8%), dyslipidemia (31.9%), and a prior history of ischemic heart disease (22.7%). The mean left ventricular ejection fraction (LVEF) was 46.2 ± 8.7%, indicating mild systolic dysfunction in many

patients. Metoprolol was the most frequently prescribed beta-blocker (56.2%), followed by bisoprolol (28.1%) and carvedilol (15.7%). Regarding dosage, 40.0% of patients were on low-dose, 42.7% on moderate-dose, and 17.3% on high-dose beta-blocker therapy. Beta-blocker–induced bradycardia (heart rate <60 bpm) occurred in 58 patients (31.4%). Most cases were mild (18.4%) or moderate (10.3%), while severe bradycardia (<40 bpm) was seen in only 2.7% of patients. Symptomatic bradycardia, associated with dizziness or hypotension, occurred in 12 patients (6.5%).

Table No. 1: Baseline Demographic and Clinical Characteristics of Post-MI Patients (n = 185)

Variable	Frequency (%) / Mean ± SD
Age (years)	59.7 ± 9.8 (Range: 34–80)
Gender	
Male	136 (73.5%)
Female	49 (26.5%)
Comorbidities	
Hypertension	122 (65.9%)
Diabetes mellitus	91 (49.2%)
Smoking	68 (36.8%)
Dyslipidemia	59 (31.9%)
Prior ischemic heart disease	42 (22.7%)
Mean ejection fraction (%)	46.2 ± 8.7
Type of Beta-Blocker	
Metoprolol	104 (56.2%)
Bisoprolol	52 (28.1%)
Carvedilol	29 (15.7%)
Dose Category	
Low dose	74 (40.0%)
Moderate dose	79 (42.7%)
High dose	32 (17.3%)
Severity of Bradycardia	
Mild (50–59 bpm)	34 (18.4%)
Moderate (40–49 bpm)	19 (10.3%)
Severe (<40 bpm)	5 (2.7%)
Total Bradycardia Cases (<60 bpm)	58 (31.4%)
Symptomatic Bradycardia	12 (6.5%)

Table No. 2: Incidence of Bradycardia According to Beta-Blocker Dose Category

Dose Category	Total (n)	Bradycardia n (%)	Mean HR (bpm) ± SD	p-value
Low dose	74	9 (12.1%)	68.5 ± 6.2	<0.001
Moderate dose	79	29 (36.7%)	61.3 ± 5.7	
High dose	32	20 (62.5%)	52.4 ± 5.3	
Total	**185**	**58 (31.4%)**	—	—

There was a strong dose–response relationship, with a bradycardia incidence of 12.1% in the low-dose group, 36.7% in the moderate-dose group, and 62.5% in the high-dose group ($p < 0.001$). The mean resting heart rate decreased significantly as the dose increased: 68.5 ± 6.2 bpm in the low-dose group, 61.3 ± 5.7 bpm in the moderate-dose group, and 52.4 ± 5.3 bpm in the high-dose group.

Bisoprolol showed the highest rate of bradycardia (40.4%), followed by metoprolol (29.8%) and carvedilol (24.1%). Severe bradycardia occurred most frequently with bisoprolol (5.8%) and least with carvedilol (0%). The mean dose at which bradycardia developed was 95.6 ± 21.4 mg for metoprolol, 5.8 ± 1.1 mg for bisoprolol, and 18.6 ± 3.5 mg for carvedilol.

Patients older than 65 years had a significantly higher incidence of bradycardia (43.2%) compared to those aged ≤65 years (26.4%) ($p = 0.02$). Similarly, patients with LVEF ≤40% had more frequent bradycardia (41.7%) than those with better ejection fraction (27.3%) ($p = 0.04$). Gender, hypertension, and diabetes mellitus were not significantly associated with bradycardia ($p > 0.05$).

High-dose therapy emerged as the strongest predictor, with an odds ratio (OR) of 4.62 (95% CI: 2.38–8.95, $p < 0.001$). Age above 65 years was also an independent predictor (OR 2.13, 95% CI: 1.09–4.15, $p = 0.03$). Low ejection fraction showed a positive trend (OR 1.76, $p = 0.08$) but did not reach statistical significance. Hypertension and diabetes had no significant impact.

Table No. 3: Comparison of Bradycardia Incidence by Beta-Blocker Type

Beta-Blocker	Total (n)	Bradycardia n (%)	Mean Dose ± SD	Severe Bradycardia n (%)
Metoprolol	104	31 (29.8%)	95.6 ± 21.4 mg	2 (1.9%)
Bisoprolol	52	21 (40.4%)	5.8 ± 1.1 mg	3 (5.8%)
Carvedilol	29	7 (24.1%)	18.6 ± 3.5 mg	0 (0%)
Total	**185**	**58 (31.4%)**	—	**5 (2.7%)**

Table No. 4: Association of Clinical Variables with Beta-Blocker–Induced Bradycardia

Variable	Category	Bradycardia n (%)	p-value
Age group	≤65 years	33 (26.4%)	
	>65 years	25 (43.2%)	0.02
Gender	Male	41 (30.1%)	
	Female	17 (34.7%)	0.38
Ejection fraction	≤40%	25 (41.7%)	
	>40%	33 (27.3%)	0.04
Hypertension	Yes	42 (34.4%)	
	No	16 (25.0%)	0.19
Diabetes mellitus	Yes	29 (31.9%)	
	No	29 (30.9%)	0.88

Table No. 5: Logistic Regression Analysis of Predictors of Beta-Blocker–Induced Bradycardia

Variable	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
High-dose therapy	4.62	2.38 – 8.95	<0.001
Age >65 years	2.13	1.09 – 4.15	0.03
Low LVEF (≤40%)	1.76	0.91 – 3.42	0.08
Hypertension	1.21	0.64 – 2.28	0.56
Diabetes mellitus	1.04	0.54 – 2.01	0.91

DISCUSSION

In this study of 185 post-myocardial infarction (MI) patients on beta-blocker therapy, the frequency of drug-induced bradycardia was found to be 31.4%, with a clear dose-dependent relationship between beta-blocker intensity and heart rate reduction. The findings reaffirm that while beta-blockers remain indispensable in post-MI secondary prevention, careful titration is crucial to avoid bradyarrhythmic complications. The dose–response association observed here provides valuable insight for optimizing therapy while maintaining hemodynamic stability. The mean age of patients in this study was 59.7 ± 9.8 years, with a predominant male population (73.5%). Hypertension and diabetes were the most frequent comorbidities, consistent with previous research identifying these as the most common risk factors among post-MI populations. The prevalence of bradycardia was notably higher in older patients (>65 years) and those with reduced left ventricular ejection fraction (≤40%), supporting prior evidence that age-related autonomic dysfunction and ventricular impairment amplify the chronotropic effects of beta-blockers. The observed bradycardia incidence of 31.4% aligns closely with earlier studies that have reported rates between 25% and 35% in post-MI cohorts on chronic beta-blocker therapy. In the Beta-Blocker Heart Attack Trial (BHAT), for instance, approximately one-third of patients experienced heart rate reduction beyond the target range, though the majority were asymptomatic. Similarly, previous research demonstrated that dose escalation beyond moderate therapeutic levels conferred minimal additional cardioprotection but significantly increased the risk of bradycardia and hypotension. The current findings parallel this pattern only 12.1% of patients on low doses developed bradycardia compared to 62.5% on high doses ($p < 0.001$), indicating that excessive dosing does not necessarily translate to improved outcomes[13].

Among the different agents, bisoprolol showed the highest bradycardia incidence (40.4%), followed by metoprolol (29.8%) and carvedilol (24.1%). These variations likely reflect the pharmacologic selectivity and receptor affinity profiles of each agent. Bisoprolol's higher β1-selectivity and longer half-life contribute to sustained chronotropic suppression, explaining the higher bradycardia rate, particularly at doses above 5 mg/day[14]. Conversely, carvedilol's additional α1-blocking properties may confer a more balanced hemodynamic effect, reducing the likelihood of excessive bradycardia despite its nonselective β-blocking action. Previous research has similarly indicated that β1-selective agents tend to cause more pronounced heart rate reduction compared to mixed β- and α-blockers. Age >65 years and high-dose therapy emerged as independent predictors of beta-blocker–induced bradycardia in the multivariate analysis, with odds ratios of 2.13 and 4.62 respectively ($p < 0.05$). These findings underscore the importance of individualizing dose titration, especially in elderly and high-risk patients[15]. The association between low ejection fraction and bradycardia approached significance ($p = 0.08$), suggesting that patients with compromised ventricular function may have reduced compensatory mechanisms to tolerate excessive rate reduction. The majority of bradycardic episodes were mild to moderate and reversible with simple clinical interventions such as dose adjustment or temporary discontinuation. No cases in this study required permanent pacing or resulted in fatal outcomes, which

aligns with earlier evidence indicating that bradycardia from beta-blockers is generally dose-related and self-limiting rather than idiosyncratic. Nevertheless, even transient bradycardia can disrupt optimal therapy adherence, leading to under-dosing and diminished cardioprotective benefit over time[16]. Clinically, the results reinforce a key principle: "start low, go slow." Early initiation of low-dose beta-blockers in stable post-MI patients followed by gradual titration remains the safest approach. Heart rate monitoring during dose escalation is essential, particularly in older adults and those on concurrent rate-limiting drugs. The data also support the growing notion that moderate dosing achieves a therapeutic plateau for mortality reduction while minimizing adverse hemodynamic effects[17]. This is consistent with findings from the meta-analysis of a researcher which reported no incremental survival benefit at higher doses compared to standard ones. Limitations of this study include its single-center design, relatively small sample size, and short duration of follow-up, which limit the ability to assess long-term outcomes such as mortality or recurrent ischemic events. Additionally, serum beta-blocker concentrations were not measured, so interindividual pharmacokinetic variability could not be assessed.

CONCLUSION

It is concluded that beta-blocker–induced bradycardia is a relatively common adverse effect among post-myocardial infarction patients, observed in approximately one-third of the study population. The occurrence of bradycardia demonstrated a clear dose-dependent pattern, with significantly higher rates at increased doses of beta-blockers. Advanced age and reduced left ventricular ejection fraction were additional factors contributing to increased susceptibility. Among the different beta-blockers used, bisoprolol showed the highest frequency of bradycardia, likely due to its higher β1-selectivity and longer half-life, while carvedilol exhibited a comparatively lower incidence, suggesting a more favorable hemodynamic profile.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Adnan Jehangir
Drafting or Revising Critically:	Adnan Jehangir
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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Original Article

Impact of High-Fructose Diet on Hepatic Enzyme Regulation and Insulin Resistance in Young Adults

Farhana Ayub

ABSTRACT

Objective: To evaluate the impact of high-fructose dietary intake on hepatic enzyme regulation, insulin resistance, and metabolic profiles in young adults, and to determine correlations between fructose consumption and key metabolic indicators.

Study Design: Prospective, comparative study

Place and Duration of Study: This study was conducted at the Poonch Medical College Rawalakot, from July 2024 to June 2025.

Methods: This prospective, comparative study include 210 young adults aged 18–30 years.

Results: High-fructose consumers demonstrated significantly higher BMI (27.4 ± 4.3 kg/m²), waist circumference (88.7 ± 10.1 cm), and daily caloric intake (2450 ± 430 kcal) than low-fructose individuals. Hepatic enzymes including ALT (50.7 ± 15.1 vs. 34.9 ± 10.2 U/L), AST (41.8 ± 12.1 vs. 31.0 ± 8.9 U/L), ALP, and GGT were elevated in the high-fructose group (all $p < 0.001$). Hepatic steatosis prevalence was nearly double (39% vs. 20%). Insulin resistance markers were significantly worse, with higher fasting glucose (102.7 ± 11.1 mg/dL), fasting insulin (18.2 ± 7.1 µIU/mL), and HOMA-IR (4.65 ± 1.52 vs. 2.39 ± 0.96). Lipid abnormalities included higher triglycerides (189.4 ± 45.2 mg/dL), higher LDL, and lower HDL. Fructose intake strongly correlated with ALT ($r = 0.62$), HOMA-IR ($r = 0.71$), triglycerides ($r = 0.58$), and waist circumference ($r = 0.49$), all $p < 0.001$.

Conclusion: High fructose intake is associated with significant hepatic stress, insulin resistance, dyslipidemia, and central adiposity in young adults. Early metabolic disruption underscores the need to reduce fructose-rich foods and beverages to prevent long-term metabolic disease.

Key Words: high-fructose diet, hepatic enzymes, insulin resistance, HOMA-IR, young adults, metabolic syndrome, triglycerides, hepatic steatosis, NAFLD

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INTRODUCTION

The rising consumption of fructose-rich foods and beverages has emerged as a major nutritional concern, particularly among young adults who represent the highest consumers of processed sugars worldwide[1]. Fructose intake has increased markedly over the past few decades due to widespread availability of sugar-sweetened beverages, high-fructose corn syrup (HFCS), and ultra-processed foods. Unlike glucose, fructose follows a distinct metabolic pathway that bypasses key regulatory steps of glycolysis, leading to rapid hepatic uptake and unregulated lipogenesis[2].

Professor of Biochemistry, Poonch Medical College Rawalakot, Pakistan.

Correspondence: Farhana Ayub, Professor of Biochemistry, Poonch Medical College Rawalakot, Pakistan
Contact No: +966 53 969 5518
Email: fmalik@kfu.edu.sa

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As a result, high-fructose diets have been implicated in the development of metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), and insulin resistance conditions whose prevalence is alarmingly rising among younger populations. Hepatic metabolism of fructose is unique in that it is primarily driven by fructokinase (ketohexokinase), which converts fructose into fructose-1-phosphate without feedback inhibition by insulin or ATP levels. This unregulated phosphorylation can lead to ATP depletion, elevated uric acid production, formation of reactive oxygen species (ROS), and increased triglyceride synthesis in hepatocytes[3]. These biochemical events contribute to hepatic steatosis, mitochondrial dysfunction, and endoplasmic reticulum stress, which collectively impair insulin signaling pathways. Previous controlled feeding trials have demonstrated that high-fructose diets increase liver fat content even in otherwise healthy young adults within a matter of weeks, highlighting the rapid and detrimental hepatic effects of fructose overconsumption[4]. Fructose-induced insulin resistance is linked to impaired IRS-1 and Akt phosphorylation, increased hepatic gluconeogenesis, and dysregulated lipid metabolism. Young adults are particularly vulnerable to these alterations because their dietary

patterns often include high daily fructose loads and low intake of fiber and antioxidant-rich foods[5]. Epidemiological findings further suggest that excessive fructose intake correlates with elevated fasting insulin levels, increased HOMA-IR scores, and early metabolic derangements even in non-obese individuals[6]. Additionally, fructose stimulates de novo lipogenesis (DNL) to a far greater extent than glucose, contributing to triglyceride accumulation, elevated VLDL secretion, and dyslipidemia factors strongly linked with cardiometabolic risk in young populations[7]. High-fructose diets also affect hepatic enzyme regulation. Studies show increased expression of lipogenic enzymes such as ACC, FAS, and SREBP-1c, and altered activities of ALT and AST biomarkers traditionally used to assess hepatocellular injury[8]. This suggests that chronic fructose exposure may predispose individuals to NAFLD progression, particularly in the early adult years when lifestyle choices strongly shape long-term metabolic trajectories. Furthermore, the inflammatory response triggered by hepatic fructose metabolism including TNF-α, IL-6, and CRP elevation promotes systemic insulin resistance, independent of body weight changes[9]. Given the rising global burden of metabolic diseases and the disproportionately high consumption of fructose among young adults, it is critical to understand how fructose influences hepatic enzyme regulation and insulin sensitivity in this demographic[10].

METHODS

This prospective comparative study was conducted at Poonch Medical College, Rawalakot, from July 2024 to June 2025 and included 210 young adults aged 18–30 years. Participants were divided into a high-fructose intake group (n=105) and a low-fructose intake group (n=105). Individuals with a regular dietary pattern for at least three months, willingness for fasting blood sampling, and informed consent were included. Participants with known liver disease, diabetes or prediabetes, alcohol intake >20 g/day, use of steroids, hepatotoxic or weight-loss medications, inflammatory illnesses, pregnancy, or lactation were excluded.

Data Collection

Demographic and lifestyle data, including age, gender, BMI, physical activity, and beverage consumption, were recorded. Dietary fructose intake was assessed using a validated food frequency questionnaire covering sugar-sweetened beverages, fruit juices, processed snacks, bakery products, and high-fructose corn syrup–containing foods. After an 8–10-hour fast, blood samples were collected for ALT, AST, ALP, GGT, lipid profile, fasting glucose, fasting insulin, and uric acid. Insulin resistance was calculated using HOMA-IR, while liver ultrasound was performed where indicated to assess hepatic steatosis.

Statistical Analysis

Data were analyzed using SPSS version 25.0. Continuous variables were expressed as mean±SD and compared using the independent t-test, while categorical variables were presented as frequencies and percentages and compared using the Chi-square test. Pearson correlation was used to assess associations between fructose intake, hepatic enzymes, and HOMA-IR, while multiple linear regression identified independent predictors of insulin resistance and elevated liver enzymes. A p-value <0.05 was considered statistically significant.

RESULTS

The two groups were similar in age (24.1±3.6 vs. 23.5±3.2 years; p=0.28) and gender distribution (63/42 vs. 55/50; p=0.19). However, the high-fructose group had significantly higher BMI (27.4±4.3 vs. 24.8±3.8 kg/m²), waist circumference (88.7±10.1 vs. 79.7±8.2 cm), and daily caloric intake (2450±430 vs. 1970±350 kcal), all p<0.001. Physical inactivity was also more common in the high-fructose group (57 vs. 41 participants; p=0.03), indicating a less favorable anthropometric and lifestyle profile. (Table 1)

Daily fructose intake was substantially higher in the high-fructose group (72.4±15.3 vs. 36.8±11.4 g/day; p<0.001). These participants also consumed more sugary beverages (12.4±3.9 vs. 4.0±2.1 servings/week) and processed foods (14.3±4.8 vs. 7.3±3.2 servings/week), while consuming less natural fruit (4.8±2.4 vs. 7.4±3.1 servings/week) and dietary fiber (15.6±5.3 vs. 21.4±5.8 g/day), with all differences statistically significant at p<0.001. (Table 2)

Table No. 1: Baseline Demographic and Anthropometric Characteristics (n = 210)

Variable	Total (n = 210)	High-Fructose Group (n = 105)	Low-Fructose Group (n = 105)	p-value
Age (years), mea ± SD	23.8 ± 3.4	24.1 ± 3.6	23.5 ± 3.2	0.28
Gender (Male/Female)	118 / 92	63 / 42	55 / 50	0.19
BMI (kg/m²), mean ± SD	26.1 ± 4.2	27.4 ± 4.3	24.8 ± 3.8	<0.001*
Waist circumference (cm)	84.2 ± 9.6	88.7 ± 10.1	79.7 ± 8.2	<0.001*
Physical activity (Active/Inactive)	112 / 98	48 / 57	64 / 41	0.03*
Daily caloric intake (kcal)	2210 ± 410	2450 ± 430	1970 ± 350	<0.001*

Liver-related parameters were significantly higher among high-fructose consumers. Mean ALT was 50.7±15.1 vs. 34.9±10.2 U/L, AST 41.8±12.1 vs. 31.0±8.9 U/L, ALP 95.1±22.8 vs. 81.3±23.1 U/L, and GGT 47.8±13.0 vs. 35.2±9.8 U/L. All differences were

significant (p≤0.001). Hepatic steatosis was also more frequent in the high-fructose group, affecting 41 (39.0%) compared with 21 (20.0%) participants (p=0.003), suggesting an association between higher fructose intake and poorer hepatic status.

Table No. 2: Dietary Intake Profile and Fructose Consumption

Variable	Total	High-Fructose Group	Low-Fructose Group	p-value
Daily fructose intake (g/day), mean ± SD	54.6 ±18.9	72.4 ± 15.3	36.8 ± 11.4	<0.001*
Sugary beverages (servings/ week)	8.2 ± 4.1	12.4 ± 3.9	4.0 ± 2.1	<0.001*
Processed food intake (servings/ week)	10.8 ± 5.3	14.3 ± 4.8	7.3 ± 3.2	<0.001*
Natural fruit intake (servings/ week)	6.1 ± 3.0	4.8 ± 2.4	7.4 ± 3.1	<0.001*
Fiber intake (g/day)	18.5 ± 6.2	15.6 ± 5.3	21.4 ± 5.8	<0.001*

Table No. 3: Hepatic Enzyme Levels

Variable	Total (n = 210)	High-Fructose (n = 105)	Low-Fructose (n = 105)	p-value
ALT (U/L), mean ± SD	42.8 ± 14.3	50.7 ± 15.1	34.9 ± 10.2	<0.001*
AST (U/L), mean ± SD	36.4 ± 11.8	41.8 ± 12.1	31.0 ± 8.9	<0.001*
ALP (U/L), mean ± SD	88.2 ± 24.7	95.1 ± 22.8	81.3 ± 23.1	0.001*
GGT (U/L), mean ± SD	41.5 ± 12.6	47.8 ± 13.0	35.2 ± 9.8	<0.001*
Hepatic steatosis, n (%)	62 (29.5%)	41 (39.0%)	21 (20.0%)	0.003*

The high-fructose group showed significantly less favorable glycemic parameters. Fasting glucose was 102.7±11.1 vs. 93.7±8.6 mg/dL, fasting insulin 18.2±7.1 vs. 11.4±4.2 µIU/mL, HOMA-IR 4.65±1.52 vs. 2.39±0.96, and HbA1c 5.6±0.5% vs. 5.2±0.3%, with all p<0.001. Impaired fasting glucose was also more frequent among high-fructose consumers, occurring in 31 (29.5%) compared with 11 (10.5%) participants (p=0.001), indicating greater insulin resistance and impaired glucose regulation.

Table No. 4: Glycemic and Insulin Resistance Parameters

Parameter	Total (n = 210)	High-Fructose (n = 105)	Low-Fructose (n = 105)	p-value
Fasting glucose (mg/dL)	98.2 ± 10.4	102.7 ± 11.1	93.7 ± 8.6	<0.001*
Fasting insulin (µIU/mL)	14.8 ± 6.2	18.2 ± 7.1	11.4 ± 4.2	<0.001*
HOMA-IR score	3.52 ± 1.40	4.65 ± 1.52	2.39 ± 0.96	<0.001*
HbA1c (%)	5.4 ± 0.4	5.6 ± 0.5	5.2 ± 0.3	<0.001*
Impaired fasting glucose, n (%)	42 (20.0%)	31 (29.5%)	11 (10.5%)	0.001*

High-fructose intake was associated with a more adverse lipid and metabolic profile. Total cholesterol was 202.3±34.2 vs. 174.5±32.9 mg/dL, triglycerides 189.4±45.2 vs. 136.2±40.7 mg/dL, and LDL-C 113.6±26.9 vs. 93.8±24.7 mg/dL, while HDL-C was lower at 41.3±8.9 vs. 48.3±9.1 mg/dL. Uric acid was also higher in the high-fructose group (6.4±1.2 vs. 5.2±1.0 mg/dL). All differences were statistically significant at p<0.001, reflecting a less favorable cardiometabolic profile among participants with higher fructose intake.

Table No. 5: Lipid Profile and Metabolic Markers

Variable	Total (n = 210)	High-Fructose (n = 105)	Low-Fructose (n = 105)	p-value
Total cholesterol (mg/dL)	188.4 ± 36.1	202.3 ± 34.2	174.5 ± 32.9	<0.001*
Triglycerides (mg/dL)	162.8 ±48.6	189.4 ± 45.2	136.2 ± 40.7	<0.001*
HDL-C (mg/dL)	44.8 ± 9.7	41.3 ± 8.9	48.3 ± 9.1	<0.001*
LDL-C (mg/dL)	103.7 ± 28.2	113.6 ± 26.9	93.8 ± 24.7	<0.001*
Uric acid (mg/dL)	5.8 ± 1.3	6.4 ± 1.2	5.2 ± 1.0	<0.001*

DISCUSSION

In this study, there was a strong correlation between the high fructose intake with early metabolic dysfunction in young adults, such as adiposity, hepatic function,

insulin resistance, and lipid metabolism. Participants who consumed the highest amounts of fructose had significantly higher BMI (27.4±4.3 kg/m²) and waist circumference (88.7±10.1 cm), reflecting higher central and overall adiposity. This was similar to the previous studies that demonstrated de novo lipogenesis and central fat accumulation with fructose[11]. This poor metabolic profile could have been further aided by the increased calorie intake and the decrease in exercise in the high fructose group. Results of the dietary analysis revealed that high fructose consumers consumed almost twice as much fructose per day and significantly more sweetened beverages and processed foods. In line with previous studies, consumption of sugar-sweetened beverages were linked to higher triglycerides, fatty liver, and insulin resistance[12]. Contrary to this, the high fructose group consumed less natural fruit and dietary fibre, which could further increase the metabolic risk as previous research has shown that fibre may have a beneficial effect on some of the adverse effects of the excessive intake of fructose[13]. The findings in the liver were singled out. The levels of ALT and AST were also significantly elevated in the high-fructose group at 50.7±15.1 U/L and 41.8±12.1 U/L, respectively, with elevated ALP and GGT. Hepatic steatosis was more common in high fructose consumers (39.0%) than in low fructose consumers (20.0%). The results are similar to those from other studies that have shown that excess fructose increases lipogenesis and alters fatty acid metabolism, leading to hepatic fat accumulation[14].

High fructose consumers also had significantly higher insulin resistance. Fasting glucose and fasting insulin were significantly elevated, as well as HOMA-IR (4.65±1.52 vs. 2.39±0.96 in the low fructose group). Moreover, impaired fasting glucose occurred more often (29.5% vs. 10.5%). Fructose metabolism has been previously associated with ATP depletion, uric acid production, inflammation and impaired insulin signaling[15]. The results indicate that metabolic dysfunction can occur prior to the onset of clinical diabetes. The same was found for positive associations of fructose consumption with total cholesterol (r= 0.37), LDL-C (r= 0.37), uric acid (r= 0.53) and waist circumference (r= 0.49), which corroborate the previous reports of lipogenic properties of fructose[16]. In addition, fructose consumption was highly associated with ALT (r=0.62) and HOMA-IR (r=0.71), all of which are linked to unregulated lipogenesis, oxidative stress and insulin signaling deficits after excessive fructose consumption, as described previously[17]. The results overall suggest that high fructose consumption is linked to early hepatic and metabolic dysfunction even in young adults. Early dietary changes, more exercise, and reducing the intake of processed sources of fructose, may therefore help to reduce the progression to fatty liver disease, insulin resistance, and future metabolic syndrome.

CONCLUSION

It is concluded that high-fructose intake adversely affects hepatic and metabolic health in young adults. Higher fructose consumption was associated with elevated liver enzymes, greater hepatic steatosis, increased fasting insulin and HOMA-IR, higher triglycerides and LDL-C, and lower HDL-C. These findings suggest that excessive fructose intake may contribute to early hepatic stress, insulin resistance, and metabolic dysfunction even in otherwise healthy young adults.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Farhana Ayub
Drafting or Revising Critically:	Farhana Ayub
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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Original Article

The Role of the Gut Microbiome in Urinary Tract Infections and Recurrent Cystitis

Abdulaziz Saleh Alanazi[1], Ahmed Abu El Gasim Abdelrahman[1], Faisal Saleh Alanazi[5], Rakan Jazi Almokhlef[2], Rakan Mohammed Alhuwaydi[3] and Rakan Nasser W. Alrabea[4]

ABSTRACT

Objective: To investigate the association between gut microbiome composition and recurrent UTI, evaluate changes in gut microbial diversity following treatment, and assess the effectiveness of probiotic supplementation as an adjunct to standard antibiotic therapy.

Study Design: Pre-test/post-test quasi-experimental study.

Place and Duration of Study: This study was conducted at the Outpatient Urology Clinics of Mansoura University Hospital, Egypt, from January 2023 to June 2024 for a period of 18 months.

Methods: A pre-test/post-test quasi-experimental study was conducted involving 60 patients with recurrent UTIs and 60 healthy controls recruited from outpatient urology clinics.

Results: At baseline, patients with recurrent UTIs exhibited significantly lower gut microbial alpha diversity than healthy controls (Shannon index: 2.84 ± 0.43 vs. 3.91 ± 0.38; $p < 0.001$). Intestinal colonization with Escherichia coli was significantly more frequent in patients with recurrent UTIs than in controls (68.3% vs. 18.3%, $p < 0.001$). After 12 weeks, patients receiving probiotic supplementation demonstrated significantly greater restoration of gut microbial diversity compared with those receiving antibiotics alone (Shannon index: 3.62 ± 0.41 vs. 2.91 ± 0.39; $p < 0.001$).

Conclusion: Gut microbiome dysbiosis is strongly associated with susceptibility to recurrent UTIs. Adjunctive probiotic therapy targeting restoration of the gut–bladder axis significantly improves gut microbial diversity and reduces UTI recurrence.

Key Words: Urinary tract infection, recurrent cystitis, gut microbiome, gut–bladder axis, probiotics.

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent bacterial infections seen in clinical practice and it is estimated that more than 150 million UTIs occur worldwide each year[1]. It is estimated that around 50–60% of women will have at least one UTI in their lifetime, and nearly 25–30% of women will have three or more UTIs in a 12-month period (recurrent UTI)[2].

[1.] Consultant / Physician[2] / Resident Doctor[3], Department of Urology / Resident Doctor, Department of Day Surgery[4], King Abdulaziz Specialist Hospital.
[5.] Medical Student, College of Medicine, Qassim University.

Correspondence: Dr. Ahmed Abu El Gasim Abdelrahman
Consultant, Department of Urology, King Abdulaziz Specialist Hospital, Saudi Arabia
Contact No: +966 55 492 6295
Email: dr.ahmedabuelgasim@outlook.com

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Uropathogenic Escherichia coli (UPEC) are the most prevalent uropathogens, accounting for 80–85% of community-acquired UTIs, followed by Klebsiella pneumoniae, Staphylococcus saprophyticus and Enterococcus faecalis[3]. Recurrent cystitis is a considerable burden to patients and the health care system, including direct costs, adverse effects of antibiotics, development of antimicrobial resistance (AMR) and severe impact on quality of life (QoL)[4]. Traditional antibiotic prophylaxis is no longer effective in preventing UTIs due to the increasing frequency of multidrug-resistant (MDR) uropathogens, thus necessitating the search for new strategies in therapy and prevention of UTI[5]. In previous research, the urinary tract was thought to be a sterile environment; however, the development of new culture independent microbiological techniques has proven that a unique urinary microbiome exists[6]. This paradigm shift has created new opportunities to improve the understanding of the microbial ecology of UTIs beyond the one-pathogen model[7]. Additionally, the urinary microbial community is not an isolated ecosystem, but rather is closely intertwined with other mucosal microbial communities, such as the gut and vaginal microbiome[7]. A concept that has arisen is the gut–bladder axis which

suggests that gut colonization with uropathogens can act as a reservoir of ascending urinary tract colonization[8,9]. Although there has been a rise in interest, prospective clinical studies that have looked at the relationship between gut microbiome shifts and recurrence of UTIs are limited, especially in populations from the Middle East and North Africa (MENA) region[10,11].

METHODS

A quasi-experimental controlled pre-test/post-test design was used. It was carried out in the Outpatient Urology Clinics of Mansoura University Hospital, Egypt from January 2023-June 2024 for a period of 18 months. A total of 120 female adult subjects (18-65 years) were recruited. Study group (n = 60) consisted of women who had a confirmed diagnosis of recurrent UTI (three or more UTI episodes with positive culture in the last 12 months), and the control group (n = 60) consisted of age- and BMI-matched women who did not have a history of UTI in the last 24 months.

Inclusion criteria:

- Female gender, age of 18-65 years.
- Recurrent UTI (3 or more episodes per year) and the predominant pathogen is UPEC (bacteria)
- Fecal and urine samples were collected at baseline and follow-up, and willingness to provide these samples was noted.

Exclusion criteria:

- Use of antibiotics or probiotics within 4 weeks prior to enrollment
- Immunocompromised status (HIV, malignancy, corticosteroid use ≥ 3 months)
- Structural abnormalities of the urinary tract, urinary calculi, or permanent urinary catheter.
- Any inflammatory bowel disease, irritable bowel syndrome, or known gastrointestinal disorder.

Intervention Protocol

The participants were randomly allocated in the UTI group (1:1 ratio using computer-generated random allocation). The Probiotic+Antibiotic arm (n = 30) was given: (1) oral nitrofurantoin 100 mg twice daily for 7 days during episodes of acute UTI (according to standard institutional practice); (2) a commercially available probiotic capsule containing Lactobacillus rhamnosus GR-1 (10^9 CFU) and Lactobacillus reuteri RC-14 (10^9 CFU) daily for 12 consecutive weeks. The Antibiotic-only arm (n = 30) received nitrofurantoin only for the treatment of acute episodes, and no probiotic or placebo. No intervention was performed on healthy controls (n = 60) and they were used as the normative reference population for the microbiome.

Sample Collection

Fecal samples were taken at two time points: T0 (pre-test, baseline) and T1 (post-test, week 12). All participants received a standardized sterile collection kit for stool samples (OMNIgene·GUT, DNA Genotek) and were given detailed written instructions on how to collect samples at home. Samples were frozen at −80 C until further processing. Midstream clean-catch urine specimens (10 mL) were collected at both time points for standard urine culture and sensitivity, urinalysis, and urinary 16S rRNA analysis.

Extraction of DNA and Sequencing of 16S rRNA Gene: A total microbial DNA was extracted from the fecal sample by using the QIAamp PowerFecal DNA Kit (QIAGEN) according to the manufacturer's instructions. NanoDrop spectrophotometry (A260/A280 ratio ≥ 1.8) was used to check the quality of DNA. The universal primer pair 341F/806R was used to amplify the V3–V4 hypervariable regions of the 16S rRNA gene. Library preparation was conducted according to Illumina 16S Metagenomic Sequencing Library Preparation protocol. The Illumina MiSeq platform (MiSeq Reagent Kit v3, 600 cycles) was used for paired-end sequencing (2 × 250 bp). The raw sequences were quality filtered, chimeras removed, and taxonomically classified through the QIIME2 bioinformatics pipeline (v2023.2) and SILVA 138 reference database.

Outcome Measures

At T0 and T1 the following outcomes were evaluated:

- Primary outcome: Number of UTI episodes per participant per 12 weeks (confirmed by urine culture)
- Alpha diversity: Shannon entropy index, Simpson's index (1-D) and observed OTU richness.

Pre-Test/Post-Test Assessment Instrument: At T0 and T1, a validated structured questionnaire was used to evaluate the following aspects: (1) UTI symptom severity (Urinary Tract Infection Symptom Assessment - UTISA questionnaire); (2) quality of life (SF-12 Health Survey); (3) diet (24-hour food recall questionnaire adapted for fermented and high-fiber foods); and (4) adherence to probiotic regimen (pill-diary method, adherence ≥ 80% was considered as compliant).

Statistical Analysis: Data analysis was done with R v4.3.1 using the packages: vegan, phyloseq, DEseq2 and ggplot2. Means ± standard deviations (SD) or medians [interquartile ranges] were used for continuous variables as appropriate. Categorical variables are represented as frequencies (%). A level of $p < 0.05$ (two-tailed) was used for statistical significance.

RESULTS

There were 120 participants with 60 in each study group (UTI and healthy control). The mean age was 34.7 ± 9.2 years in the UTI group and 33.9 ± 8.7 years in the control group ($p = 0.621$). BMI was comparable between groups (UTI: 26.1 ± 3.8 kg/m²; controls: 25.7 ± 3.5 kg/m²; $p = 0.547$).

Table No. 1: Baseline Demographic and Clinical Characteristics of Study Participants

Variable	UTI Group (n=60)	Healthy Controls (n=60)	p-value
Age (years), mean ± SD	34.7 ± 9.2	33.9 ± 8.7	0.621
BMI (kg/m²), mean ± SD	26.1 ± 3.8	25.7 ± 3.5	0.547
UTI episodes/year, median [IQR]	4 [3–6]	0 [0–0]	< 0.001
Prior antibiotic courses/year, mean ± SD	3.8 ± 1.2	0.4 ± 0.6	< 0.001
UPEC as primary pathogen, n (%)	51 (85.0%)	N/A	—
Gut UPEC colonization, n (%)	41 (68.3%)	11 (18.3%)	< 0.001
Vaginal Lactobacillus dominance, n (%)	22 (36.7%)	49 (81.7%)	< 0.001
Shannon index (gut), mean ± SD	2.84 ± 0.43	3.91 ± 0.38	< 0.001
Simpson index (gut), mean ± SD	0.81 ± 0.07	0.94 ± 0.04	< 0.001

Table No. 2: Pre-Test Gut Microbiome Diversity Indices and Key Taxonomic Abundances

Parameter	UTI Group (n=60)	Healthy Controls (n=60)	p-value
Shannon index	2.84 ± 0.43	3.91 ± 0.38	< 0.001
Simpson's index (1-D)	0.81 ± 0.07	0.94 ± 0.04	< 0.001
Observed OTU richness	182.4 ± 38.6	294.7 ± 52.1	< 0.001
Firmicutes (% relative abundance)	41.2 ± 9.1	52.8 ± 8.3	< 0.001
Bacteroidetes (% relative abundance)	19.6 ± 6.4	15.5 ± 5.8	0.003
Proteobacteria (% relative abundance)	8.2 ± 3.1	2.9 ± 1.4	< 0.001
Faecalibacterium prausnitzii (%)	3.1 ± 1.4	8.2 ± 2.6	< 0.001
Escherichia-Shigella (%)	6.8 ± 3.2	1.2 ± 0.8	< 0.001
Bifidobacterium (%)	2.4 ± 1.1	5.9 ± 2.0	< 0.001
Lactobacillus (%)	1.8 ± 0.9	4.2 ± 1.8	< 0.001
Klebsiella (%)	3.6 ± 1.9	0.8 ± 0.5	< 0.001

Table No. 3: Pre-Test vs. Post-Test Gut Microbiome Diversity: Intervention Arm Comparison

Parameter	Probiotic+Abx T0	Probiotic+Abx T1	Abx-only T0	Abx-only T1	p*
Shannon index	2.82 ± 0.41	3.62 ± 0.41†	2.86 ± 0.45	2.91 ± 0.39	< 0.001
Simpson's index	0.81 ± 0.07	0.91 ± 0.05†	0.81 ± 0.07	0.82 ± 0.06	< 0.001
Observed OTUs	183.2 ± 39.1	261.4 ± 43.8†	181.6 ± 38.2	186.3 ± 40.1	< 0.001
E. coli colonization (%)	66.7%	26.7%†	70.0%	63.3%	0.003
Faecalibacterium (%)	3.0 ± 1.3	6.1 ± 2.2†	3.2 ± 1.5	3.4 ± 1.6	< 0.001
Escherichia-Shigella (%)	6.9 ± 3.3	3.1 ± 1.8†	6.8 ± 3.1	6.5 ± 2.9	< 0.001

† $p < 0.05$ compared to T0 within the same arm (paired test); * p-value for post-test between-group comparison.

Patients with recurrent UTI had significantly lower microbial alpha diversity in their gut compared with healthy controls, with lower Shannon entropy (2.84 ± 0.43 vs. 3.91 ± 0.38; $p < 0.001$) and Simpson's index (0.81 ± 0.07 vs. 0.94 ± 0.04; $p < 0.001$). The Bray–Curtis PCoA showed that there was a good separation between the UTI patients and controls (PERMANOVA $F = 18.74$, $R2 = 0.14$, $p < 0.001$).

After 12 weeks of treatment, the Probiotic+Antibiotic group had a significant increase in gut microbial alpha diversity in comparison to baseline and the Antibiotic-only group. The Shannon index increased from 2.82 ± 0.41 at T0 to 3.62 ± 0.41 at T1 in the probiotic arm (paired t-test: $t = 8.43$, $p < 0.001$), versus a more modest and non-significant change from 2.86 ± 0.45 to 2.91 ± 0.39 in the antibiotic-only arm ($p = 0.612$).

DISCUSSION

This study offers prospective evidence of gut microbiome dysbiosis as a powerful, biological marker of recurrent UTI in women, and that targeted probiotic supplementation for targeted probiotic supplementation to restore gut microbial diversity correlates with clinically significant decreases in the incidence of recurrent UTI. These results align with and build upon the growing body of literature on the role of the gut–bladder axis as a major contributor to urinary health. Faecalibacterium produces butyrate, which is known to strengthen the tight junctions of the intestinal epithelium, thereby helping to prevent bacterial translocation, thus its absence may allow Enterobacteriaceae overgrowth and bacterial translocation[12]. The observed decrease in abundance of Lactobacillus species in the gut and the vagina of UTI patients is consistent with the findings of Stapleton 2016 that Lactobacillus dominance at mucosal surfaces protects. What is striking about the effectiveness of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 for the reduction of UTI recurrence (RR = 0.38; NNT = 3.8) in the present study is that it is

comparable to the efficacy of antibiotic therapy for RR. The strains have previously been tested in small, randomized trials for vaginal microbiome restoration and UTI prevention with promising results; the current study is one of the first to document their effect specifically in terms of gut microbiome restoration, suggesting that their positive impact may also involve the gut–bladder axis[13]. Importantly, Shannon diversity in the probiotic arm increased substantially, approaching healthy control levels, within 12 weeks, suggesting the gut microbiome is malleable and can be targeted. The inability of antibiotics alone to restore the diversity of the gut microbiome is in accordance with the well-known side effects of antibiotics on the gut microbiome[14]. Furthermore, the antibiotic-only arm had essentially non-changing Shannon indices and continued gut colonization with UPEC at post-test, which was likely a mechanistic explanation for the 43.3% recurrence rate in the antibiotic-only arm and serves as an argument for the importance of using microbiome-protective strategies in addition to antibiotic treatment[15]. A pre-test/post-test comparison of our UTISA questionnaire and SF-12 questionnaires gave patient-reported outcome measures which matched results from microbiological tests, which increases the clinical validity of our results from a diagnostic standpoint[16]. This dramatic improvement in both physical and mental quality of life in the probiotic arm highlights the potential value of probiotics in restoring the microbiome, not just eradicating pathogens[17,18].

CONCLUSION

Here, the study illustrates gut microbiome dysbiosis (α diversity reduction and Enterobacteriaceae enrichment) strongly correlates with recurrent UTI in women, which serves as a proof of the gut-bladder axis as a mechanism involved in UTI pathogenesis. Probiotic supplementation with Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 in addition to antibiotic therapy resulted in significant improvement in microbial diversity, UTI recurrence rate (-62% vs antibiotic alone, NNT = 3.8), and symptom burden and QoL.

The results have significant clinical implications: A microbiome profile could be a biomarker for UTI susceptibility and microbiome-targeted intervention is a rational and evidence-based adjunct treatment to antibiotics for the management of recurrent cystitis.

Limitations: There are some restrictions to this study which should be noted. A placebo effect is possible in the antibiotic only group, and future studies should consider using double-blind placebo-controlled designs. Second, our study population was limited to women in a single centre in Egypt, so the findings may not be representative of other populations and geographic locations, and the MENA region is underrepresented in the field of microbiomes. Third, 16S rRNA sequencing provides taxonomic resolution but lacks functional resolution like that provided by shotgun metagenomics, and future studies should be combined with metatranscriptomics, to gain functional resolution of the microbiome state.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Abdulaziz Saleh Alanazi, Ahmed Abu El Gasim Abdelrahman, Faisal Saleh Alanazi
Drafting or Revising Critically:	Rakan Jazi Almokhlef, Rakan Mohammed Alhuwaydi, Rakan Nasser W. Alrabea
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Risk Factors Associated with Premature Deliveries among Newly Born Babies at Maternity Teaching Hospital in Mosul City, Iraq

Mahmood Hazim Sulaiman, Nada Hani Idrees, Doaa Tareq Suhail and Nasir Muwfaq Younis

ABSTRACT

Objective: The aim of this study was to determine the risk factors associated with premature deliveries among newly born babies at maternity teaching hospital in Mosul city, Iraq.

Study Design: A case-control study

Place and Duration of Study: This study was conducted at maternity teaching hospitals in Mosul city. The study conducted from October 2025 to April 2026.

Methods: Purposive sampling method was used in collection the study sample at maternity teaching hospitals in Mosul city.

Results: The finding of the study mothers with gravida of >= 4 times and the spacing between pregnancy of <2 years are more likely to have preterm deliveries in comparison with mothers who are primigravida or gravida < 4 times and who have spacing of >2 years between pregnancy and the other.

Conclusion: The present study conclude that premature delivery was more common among mothers with urinary tract infection, premature rupture of membrane, bleeding and anemia compared to control groups there was significant difference between case and control groups.

Key Words: Pregnancy, premature, Risk factors, Mother, Infant.

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INTRODUCTION

Preterm birth (PTB), as defined by the World Health Organization (WHO), refers to "a live birth taking place prior to the completion of 37 weeks of gestation[1]. Preterm birth is the largest risk factor for infant morbidity and mortality, not only in the immediate neonatal period but also in infancy, childhood, and even adulthood[2]. Compared to children born at term, preterm babies are more likely to die and experience long-term neurological and developmental problems. Every year, 15 million preterm births occur worldwide, with preterm birth rates ranging from 5 to 13% among nations. It is one of the biggest obstacles to contemporary public health since it can impact behavioral, cognitive, and physical aspects of health[3,4].

College of Nursing, University of Mosul, Iraq.

Correspondence: Nasir Muwfaq Younis, Professor Community Health Nursing, College of Nursing, University of Mosul, Iraq.
Contact No: 009647703000601
Email: nasir.mufaq@uomosul.edu.iq

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Additionally, respiratory distress, interventricular hemorrhage, sepsis, thrombocytopenia, blindness, and gastrointestinal problems have all been linked to multi-organ immaturity[5,6]. Premature birth has been associated with several factors, such as history of preterm birth, anemia, high catecholamine levels in the maternal urine, tobacco consumption, premature rupture of membranes (PROM), high blood pressure (HBP), vaginal bleeding, inter gestational intervals ≤ 1 year, urinary tract infection (UTI), lack of prenatal care, inadequate prenatal care, maternal age less than 20 years, maternal age over 35 years, oligohydramnios, history of induced abortion, preeclampsia, twin pregnancy, advanced maternal age[7]. The prematurity rates can be significantly reduced and neonatal outcomes can be improved with public health interventions to improve access to prenatal care, increase maternal education, and provide social support [8,9]. Strategies aimed at preventing or delaying premature birth and reducing morbidity and death associated with prematurity fall into two categories. Due to the fact that there are very few and almost nonexistent studies on preterm labor in general in our nation, particularly when it comes to the high prevalence of preterm labor among women and the causes of this high prevalence at the republic-wide and Mosul governorate-specific levels[10,11]. Premature

delivery is a major public health issue due to its high contribution to neonatal morbidity and mortality. Babies born prematurely are at increased risk of complications such as respiratory distress syndrome, infections, neurological impairment, and long-term developmental disabilities. Identifying the risk factors associated with premature births at maternity teaching hospital in Mosul city will help healthcare providers and policymakers in planning targeted interventions, improving prenatal care services, and reducing adverse neonatal outcomes.

METHODS

A case control research aimed at evaluating the risk variables linked to premature births in infants; the study conducted from (1st Oct 2025 until 5th Apr 2026).The tool used for collection the data of this study was a questionnaire which had been established by the researcher based on the literature review and previous studies, this questionnaire consists of three parts: Part One: Biographical Data / Obstetric History, Part two: Estimated risk factors related to maternal obstetric history, Part three: Estimated risk factors related to maternal health condition during pregnancy . The study sample was gathered using the purposeful sampling approach between December 5, 2025, and February 5, 2026. The study sample included 169 deliveries condition at maternity teaching hospitals in Mosul city .Inclusion and exclusion criteria was used to determine the study sample. Preterm < 37 weeks and Term infant by a gestational age > 37 wks. and free from congenital malformation were included in the study. Neonates with congenital anomalies were excluded from the study.This study was conducted in delivery and NICU departments at three maternity teaching hospitals in Mosul city "Al Batoul , Ibn al–Atheer , Al-khansa and Al-Salam teaching hospitals" , these hospitals located in the left side of Mosul city and provided different medical services to the patients who attend from inside and outside of Mosul city for management by specialists in these hospitals. Visits were conducted to the hospital, specifically to the neonatal intensive care unit (NICU), pediatric ward, and obstetrics and gynecology ward, in order to collect data from mothers regarding risk factors associated with preterm birth. Data were gathered through direct interviews with the mothers after giving their verbal consent, who were cooperative and willingly responded to all questions beginning by Biographical Data and Obstetric History then estimated risk factors related to maternal obstetric history. We verified the information by reviewing their medical records and examinations. Neonates with congenital anomalies were excluded from the study. The validity of the study tool for data collection (questionnaire) is confirmed by a group of the specialists in different fields regarding the subjects of the proposed study, they are asked to explore their viewpoint whether the questionnaire is clear and adequate enough to achieve the purpose of the study. Reliability was done to measure consistency of tool and to detect any mistake or weak point in it, each item in the study tool was assessed through statistical analysis by alpha Cronbach test (alpha coefficient of reliability), the reliability and consistency of the study tool was high at alpha value (0.75).After the study tool had been established, pilot study is applied on 20 subjects to test the reliability of this tool, the time assigned to carry out the pilot study was 10 days before initiating in collecting of the study sample, the used sample in pilot study was excluded from the original sample. The data of the study were analyzed statistically by the (SPSS) version 26, descriptive statistics was used to demonstrate the variables of the study sample and to explore the mother's and neonatal characteristics. The differences regarding risk factors among preterm and term infant were assessed by the statistical test (Chi-X^2).

RESULTS

Table 1: During the study period 169 deliveries were performed at maternity teaching hospitals in Mosul city, 69(40.828%) of them were born preterm babies and the rest 100(59.171%) of them were term babies."The number of live births prior to 37 weeks of gestation was divided by the total number of live births to determine the preterm birth rate" table 1 show the maternal and neonatal characteristics of the studied population, according to this table the maturity of preterm deliveries were recoded among male babies compared with female and from mothers with age group <18 year (50.7%), (49.1%) , although there was no significant difference between groups (p- value = 0.212 and .111). also preterm deliveries were occurred more frequently among mothers from low educational level (elementary and below =67.5%). There were significant differences between groups (p-value=0.000). At it is demonstrated in table 2. the obstetric characteristics that are associated with preterm delivery are multigravida and interpregnancy interval of <2 years, according to this table mothers with gravida of >= 4 times and the spacing between pregnancy of <2 years are more likely to have preterm deliveries in comparison with mothers who are primigravida or gravida < 4 times and who have spacing of >2 years between pregnancy and the other, there are significant difference between two groups (p-value = 0.010, 0.043), in addition the odd of delivering preterm births in mothers with gravida of >4 times and the interval between the pregnancy and the other is < 2 years was 2.250 and 1.922 times higher than in those with gravida of < 4 times and the interpregnancy spacing of > 2 years. Also the odds of delivering a preterm birth was significantly high among mothers who were deliver twin babies and who have inadequate antenatal carte visits (OR= 10.15,2.89) in comparison with control group. Although mothers who were experience a previous abortion were more likely to have a preterm delivery in comparison with those

how do not have previous abortion there was no significant difference between case and control groups. (P-value= 0.198). According to the table (3) premature delivery was more common among mothers with urinary tract infection, premature rupture of membrane, bleeding and anemia compared to control groups there was significant difference between case and control groups (P-value= 0.005, 0.000, 0.024 and 0.001). (OR=2.457, 3.210, 2.64 and 2.765). Amniotic fluid abnormalities (oligohydramnios and polyhydramnios) and placental abnormalities (placenta previa and abruptio placenta) shown to be risk factors associated with premature delivery (OR= 3.574, 3.959). Mothers with hypertension or gestational DM have odds of delivering a preterm babies (5.009,2.428) times higher than those mothers without these medical conditions.

Table No. 1: Socio-demographic Characteristics of the Study Sample

The study variables	Preterm n.=69(40.828%)		term n.=100(59.171%)		Mode	SD	Chi-square	p-value
maternal age					1,2	.969	4.391	.111
<18	26	49.1%	27	50.9%				
19-35	26	32.5%	54	67.5%				
>35	17	47.2%	19	52.8%				
Educational level					2	.710	21.141	0.000
elementary and below	27	67.5%	13	32.5%				
Secondary	31	41.3%	44	58.7%				
University and above	11	20.4%	43	79.6%				
Gender					1	.504	1.560	0.212
Male	35	50.7%	41	41%				
Female	34	49.3%	59	59%				

Sd: standard deviation, the difference between preterm and term babies regarding demographic characteristics is significant at p-value (p-value < 0.05).

Table No. 2: Risk Factors of Preterm Deliveries Related to the Obstetric Characteristics:

Risk factors	Preterm n.=169 (40.828%)		term n.=100 (59.171%)		Chi-square	p-value	Odd-ratio	CI(lower limit-upper limit	
Gravidity					6.525	.010	2.250	1.204	4.208
>4 times	40	51.3%	38	48.7%					
<4 times	29	31.9%	62	68.1%					
Pregnancy type					42.64	0.000	10.159	4.819	21.416
Single	26	23.2%	86	76.8%					
Multiple	43	75.4%	14	24.6%					
Pregnancy interval					4.098	.043	1.922	1.017	3.629
<2 years interval	46	47.4%	51	52.6%					
>2 years interval	23	31.9%	49	68.1%					
Previous apportion	38	45.8%	45	54.2%	1.657	.198			
Adequate Antenatal care visits	30	30.3%	69	69.7%	10.960	.001	2.894	1.530	5.473

The risk factors are considered significant at p-value <0.05, CL: confidence intervals.

Table No. 3: Risk factors of preterm delivers related to medical illness and heath condition during pregnancy

Risk factors	Preterm		Term		Chi-square	p-value	OR	CI	
UTI	37	53.6%	32	46.4%	7.902	0.005	2.457	1.305	4.626
PMR	43	55.8%	34	44.2%	13.201	0.000	3.210	1.695	6.082
Bleeding	34	51.5%	32	48.5%	5.119	0.024	2.064	1.097	3.338
Amniotic fluid abnormalities	40	58.8%	28	41.2%	15.251	0.000	3.547	1.857	6.776
DM	36	53,7%	31	46.3%	7.650	0.006	2.428	1.287	4.580
Hypertension	44	62.9%	26	37.1%	24.002	0.000	5.009	2.579	9.729
Placental	42	60.3	27	39.7	17.846	0.000	3.959	2.062	7.603
Anemia	42	53.8%	36	46,2%	10,161	0.001	2.765	1.469	5.207

The risk factors are considered significant at p-value <0.05, CL: confidence intervals.

DISCUSSION

One of the leading causes of child death worldwide, particularly in poorer nations, is preterm birth. In order to identify the risk factors associated with premature deliveries among newly born babies, the current investigation was carried out[12]. The study finding show the maternal and neonatal characteristics of the studied population, according to this table the maturity of preterm deliveries were recoded among male babies compared with female and from mothers with age group <18 year (50.7%), (49.1%), concur that the study shows the maternal age of term controls and preterm cases. The risk of preterm birth was 6.63 times higher for women under 20 than for those between (25-29)[13,14]. The odds of delivering a preterm birth was significantly high among mothers who were deliver twin babies and who have inadequate antenatal carte visits in comparison with control group. This conclusion is consistent with a study that found monochromic twins had a significantly greater rate of spontaneous preterm births compared to dichromic twins [15]. The study found that premature delivery was more common among mothers with urinary tract infection, premature rupture of membrane, bleeding and anemia compared to control groups there was significant difference between case and control groups and this result comparable with[16,17]. They found that there was a statistically significant positive correlation between the incidence of PTB and UTIs during pregnancy. A substantial positive correlation between UTIs and the development of PTB was further verified by additional REM analysis on adjusted ORs. Mothers with hypertension or gestational DM have odds of delivering a preterm babies (5.009,2.428) times higher than those mothers without these medical conditions, agree with 18,19 they came to the conclusion that GDM is linked to an increased risk of unfavorable neonatal outcomes in premature babies and a higher risk of unfavorable perinatal outcomes in pregnant mothers.

CONCLUSION

The study conclude that mothers with gravida of >= 4 times and the spacing between pregnancy of <2 years are more likely to have preterm deliveries in comparison with mothers who are primigravida or gravida < 4 times and who have spacing of >2 years between pregnancy and the other.

RECOMMENDATION

The present study Recommend Nineveh health directorate to the necessity of opening special centers for family health education to raise the mother's awareness about causes and consequences of preterm delivery, obstetrician need to focus on health education during their discussion and guide the pregnant women in making their decision.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mahmood Hazim Sulaiman, Nada Hani Idrees
Drafting or Revising Critically:	Doaa Tareq Suhail, Nasir Muwfaq Younis
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Nurses' Proficiency and Preventive Measures for Pressure Ulcers in Unconscious Patients

Ahmed T. Saud[1], Maha Muqdad Idan[1], Saja K. Jassim[1] and Nasir Muwfaq Younis[2]

ABSTRACT

Objective: The study aims to find out the level of proficiency and practice of nurses about pressure ulcer prevention among unconscious patients.

Study Design: A descriptive study

Place and Duration of Study: This study was conducted at the Basrah Teaching Hospitals in Al-Basrah Governorate (Basra Teaching Hospital, Al-Fayhaa Teaching Hospital and Al-Sadr Teaching Hospital). The study started on the 1st of October, 2024, and lasted until the 15th of March, 2025.

Methods: The sample of the study consists of 40 nurses who work in the intensive care units. Data were collected throughout the utilization of the adopted questionnaire by self-administered information and observational checklists. The questionnaire is composed of Ten items related to nurses' knowledge and Ten items for practice toward pressure ulcer prevention. The reliability of the instrument was 0.80 by using Cronbach's alpha, and the data analysis was conducted using statistical methods, which include descriptive and inferential statistics.

Results: The findings of the present study revealed that most nurses (62.5%) know a moderate amount about preventing pressure injuries in unconscious patients, while (37.5%) know very little, and none of them know (MS±SD = 1.77±0.781).

Conclusion: The study concluded that there is no significant relationship between nurses' (age, sex, education level, and years of experience) and their proficiency and practice about Pressure Injury Prevention among Unconscious Patients.

Key Words: Nurses' Proficiency, Preventive Measures, Pressure Ulcers, Unconscious Patients.

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INTRODUCTION

Localized lesions to the skin and underlying soft tissue brought on by prolonged, continuous pressure include bedsores, pressure sores, pressure ulcers, decubitus ulcers, and pressure injuries. Tissue ischemia results from this, which lowers the availability of oxygen and other essential nutrients, ultimately leading to tissue necrosis and potentially fatal consequences[1].Since the 20th century, pressure ulcers have been identified as one of the most expensive and physically incapacitating consequences. In addition to causing mortality and disability, pain and agony from pressure ulcers prolong illness, hinder recovery, and postpone discharge. Because of the need for supplies and nursing hours, these lead to a significant rise in healthcare costs[2].Regardless of a nation's income level, the prevalence of hospital-acquired pressure ulcers is still a major concern worldwide. Recent studies indicate that the worldwide prevalence is (8.4%). The prevalence is much greater among patients in intensive care units (ICUs), with global rates between (6.60 and 36.80%) [3] . The prevalence of PU in Iraq varies by location, with hospital populations ranging from (4.7% to 32.1%). In ICU patients in Europe, the incidence of PU ranges from (8.10 to 31%). According to studies conducted in Middle Eastern countries, (8.90%) of ICU patients in Iran experienced PI during their stays, followed by (17%) in Turkey, (39.30%) in Saudi Arabia, and (33.70%) in Lebanon. ICU patients in China have the highest PI incidence (4.48%), which is significantly greater than the (0.63%) total PU incidence[4].These days, pressure injuries are acknowledged globally as one of the most frequent reasons why patients suffer injury and as an avoidable patient safety issue. Furthermore, it is increasingly being referred to as a gauge of the standard of treatment rendered by healthcare institutions. Patients with pressure ulcers are frequently admitted to acute and long-term care hospitals, and they place a heavy strain on themselves,

[1.] College of Nursing, University of Basrah, Iraq.
[2.] College of Nursing, University of Mosul, Iraq.

Correspondence: Nasir Muwfaq Younis, Professor Community Health Nursing, College of Nursing, University of Mosul, Iraq.
Contact No: 009647703000601
Email: nasir.mufaq@uomosul.edu.iq

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their loved ones, and their caregivers[5] .Stated that pressure ulcers can be painful or even fatal, require time away from work, and be expensive to treat. Although this unconsciousness of patients' consequences is frequent, it can be avoided. People can reduce their chance of acquiring pressure sores and enhance their quality of life by doing routine skin examinations, cushioning bony regions of the skin, keeping a healthy weight, and attempting to reposition the body throughout the day[6,7]. The increased incidence of pressure ulcers was influenced by nurses' poor or insufficient knowledge and practice. In addition to raising nurses' awareness of the issue of pressure ulcers, knowledge, and practice also gave them the framework to develop and sustain their competency in providing high-quality nursing care and the foundation for making well-informed decisions[8]. Therefore, the objective of the study is to ascertain the level of nursing practice and knowledge about preventing pressure injuries in patients who are unconscious as well as the correlation between these factors and the demographics of the nurses.

METHODS

In this study, a descriptive design was used. The intensive care units of Basrah Teaching Hospitals served as the study's location included that (Basra Teaching Hospital, Al-Fayhaa Teaching Hospital and Al-Sadr Teaching Hospital). The study comprised 40 nurses who worked in intensive care units as a convenience sample. Nurses having at least six months of intensive care unit experience were available at the time of the study, and all nurses working in the previously chosen environment who are willing to participate in the study meet the inclusion criteria. The questionnaire utilized in this study was on an international scale, and it was designed to achieve the study's goal. It is composed of three sections that provide information about the following: Part 1: Demographic Characteristics Self-Administered Questionnaire Sheet It is focused on the demographic information of the nurse, including years of experience, education level, sex, and age. The second section is a self-administered questionnaire sheet about nurses' proficiency of pressure ulcer prevention. It is composed of (10) items which are rated according to know (3), not sure (2) score, and I don't know (1) related to the nurses' proficiency. Data were collected by self-reported information. Third part: Observational Checklist Related to Nurse's Practice Concerning Pressure Ulcer Prevention Evaluate the nurse's practice regarding pressure ulcer prevention to observe and check for correct or incorrect practices. The observational checklist consisted of (10) responses and was rated according to Always (3), Sometimes (2), and Never (1). And the practice of doing during observational checklist through three (first, second, third) observations at the same time. The questionnaire comprised two main sections. The first concerned nurses' proficiency of pressure ulcer prevention, using the following scale: (Know = 3, Unsure = 2, Don't Know = 1). The second section assessed nurses' practices regarding pressure ulcers to monitor and verify correct or incorrect practices, using the following scale: (Always = 3, Sometimes = 2, Never = 1). The researchers used the Nurse's Observational Checklist sheet to evaluate nurses' performance while caring for patients. The fact that nurses were being observed was not disclosed to them. A experts of seven adult nursing professionals was tasked with ensuring the authenticity of the questionnaire. Using the SPSS software, Version (31), Cronbach's Alpha was used to evaluate the research instrument's dependability for ten questions. Based on Cronbach's Alpha value of 0.80, the results showed that the instrument is appropriate and sufficient for evaluating the sample.

RESULTS

Table (1): shows socio-demographic information of the nurses involved in the present research.

Table No. 1: Demographic data of the nurses N=40

Variables	Classes	Frequency	**Percent**
Age MS±SD = 29.33±6.099	20-30	29	72.5
	31-40	9	22.5
	41-60	2	5.0
	Total	40	100.0
Sex	Male	23	57.5
	Female	17	42.5
	Total	40	100.0
Education level	Secondary School	8	20.0
	Diploma	16	40.0
	Bachelor's Degree	16	40.0
	Total	40	100.0
Working Experience	5-10	23	57.5
	10-20	6	15.0
	20-30	11	27.5
	Total	40	100.0

In terms of educational attainment, the largest percentage of them (40%) had bachelor's and certificate degrees, and 72.5% of them were in the 20–30 age range. Over half of them (57.5%) were male. Those with 5–10 years have the highest percentage of years of experience (57.5%). According to these results, the Table (2) demonstrated that the most nurses (62.5%) proficiency a moderate amount about preventing pressure injuries in unconscious patients, while 37.5% proficiency very little and none of them proficiency (MS±SD = 1.77±0.781).The table (3) findings show that there is no significant relationship between nurses' years of experience, age, sex, and education level and their understanding of how to prevent pressure injuries

in unconscious patients (P-value > 0.05).While the results of the table(4) appearance that only 2.5% of nurses have satisfactory practice of the mean score and standard level deviation = (1.55+0.647), whereas the majority of nurses (97.5%) have inadequate practice regarding pressure injury prevention among unconscious patients. Finally, the findings in the table (5) indicate that there is no significant correlation (P-value > 0.05) between the practice of nurses preventing pressure injuries among unconscious patients and their years of experience, age, sex, or educational attainment.

Table 2: Nursing staff's proficiency of pressure injury prevention among unconscious patients

Levels	Frequency	Percent	Scale	Total		
				MS	Sd	Assessment
Poor	15	37.5	1 – 1.66	1.77	0.781	Moderate
Moderate	25	62.5	1.67 – 2.32			
Good	0	0	2.33 – 3			
Total	40	100.0				

MS = Mean Score, Sd=Standard Deviation.

Table No. 3: Relationships of demographic data and their nurses' proficiency

Variables	Classes	Proficiency			Significant
		Poor	Moderate	Total	
Age	20-30	10	19	29	Fisher's Exact Test = 3.006 Df= 2 P-Value= 0.400 NS
	31-40	3	6	9	
	41-60	2	0	2	
	Total	15	25	40	
Sex	Male	8	15	23	Chi-Square = 0.171 Df= 1 P-Value= 0.680 NS
	Female	7	10	17	
	Total	15	25	40	
Education Level	Nursing Secondary School	2	6	8	Fisher's Exact Test = 0.795 Df= 2 P-Value= 0.875 NS
	Diploma Degree	7	9	16	
	Bachelor’s Degree	6	10	16	
	Total	15	25	40	
Years of Experience	5-10	8	15	23	Fisher's Exact Test = 0.532 Df= 2 P-Value= 0.925 NS
	10-20	2	4	6	
	20-30	5	6	11	
	Total	15	25	40	

Df: Degree of freedom, P: Probability value, NS: Not Significant

Table No. 4: Nursing staff's practice of pressure injury prevention among unconscious patients

Assessment levels	Frequency	Percent	Scale	Total		
				MS	Sd	Assessment
Unsatisfactory	39	97.5	1 – 2	1.55	0.647	Unsatisfactory
Satisfactory	1	2.5	2.1 – 3			
Total	40	100.0				

MS = Mean Score, Sd=Standard Deviation.

Table No. 5: Relationships of demographic data with their nurses' practice

Variables	Classes	Practice			Significant
		Unsatisfactory	Satisfactory	Total	
Age	20-30	28	1	29	Fisher's Exact Test = 1.827 Df= 2 P-Value= 1.000 NS
	31-40	9	0	9	
	41-60	2	0	2	
	Total	39	1	40	
Sex	Male	22	1	23	Fisher's Exact Test = 0.758 Df= 1 P-Value= 1.000
	Female	17	0	17	
	Total	39	1	40	

					NS
Education Level	Secondary School	8	0	8	Fisher's Exact Test = 1.649
	Diploma Degree	15	1	16	Df= 2
	Bachelor's Degree	16	0	16	P-Value= 1.000
	Total	39	1	40	NS
Years of Experience	5-10	22	1	23	Fisher's Exact Test = 1.223
	10-20	6	0	6	Df= 2
	20-30	11	0	11	P-Value= 1.000
	Total	39	1	40	NS

Df: Degree of freedom, P: Probability value, NS: Not Significant

DISCUSSION

One measure of the quality of care is the prevention of pressure ulcers. The development and prevention of pressure ulcers are significantly impacted by nursing care. Therefore, pressure ulcers are a significant result that nurses should be aware[9]. So this study was aimed to describe the level of proficiency and practice of nurses about pressure ulcer prevention among unconscious patients. The study finding out the showed that the age group was (20-30) years with a percentage of (72.5%), This finding aligns with research conducted by[10], which elucidate that the largest proportion of respondents (55.8%) were between the ages of 25 and 34. According to the sex, the results found that more than half are male (57.5%). These results agreed with study[11]. This study disagrees with another research made by [12]that showed the majority were female (89.1%, n = 90). And showed the highest percentage is seen with a Bachelor's and diploma degrees (40%) for each of them regarding educational levels. This finding is in line with research by [13]which found that the majority of professionals (n = 364, 71.4%) held a bachelor's degree. The majority of participants (57.5%) had 5–10 years of experience, which supports a study by [14] that found the majority of participants had between one and five years (38.8%) of work experience. According to the study's findings in table(2), most nurses (62.5%) Proficiency a considerable amount about how to prevent pressure injuries in patients who are asleep, while 37.5% have poor knowledge and none of the nurses have good Proficiency of the mean score and standard level deviation = (1.77+0.781). Similar findings were made by [15] who discovered that most nurses had a mean Proficiency score of 42.16% (SD 12.09) in pressure injury prevention. These differences in Proficiency between studies may be due to a course of training programs to the nurse that have moderate and good level of Proficiency. This study found in table (3) no significant correlation between nurses' years of experience, age, sex, or educational attainment and their understanding of preventing pressure injuries in unconscious patients (P-value > 0.05). This is in line with a study conducted by [16,17].

The outcome shows what nurses in intensive care units know about preventing pressure ulcers. Furthermore, there were no statistically significant differences between the mean scores of nurses' years of experience education, age, and duration of work in the intensive care unit ($p>0.05$) and their understanding of pressure ulcer prevention strategies, Higher education offers a solid theoretical foundation, while job experience improves practical skills and decision-making abilities. This explains the substantial correlation between nurses' education level, work experience, and training courses with knowledge. Furthermore, continuous training guarantees that nurses remain current with the most recent medical procedures, improving their overall competence. Conversely, the lack of a significant relationship between gender, age, and department suggests that knowledge is more influenced by professional development than by personal or workplace characteristics. The study indicates in table(4) the most of the nurses (97.5%) have unsatisfactory practice about Pressure Injury Prevention among Unconscious Patients, and only (2.5%) have a satisfactory practice of the mean score and standard level deviation= (1.55+0.647) [18]Study, We sought to ascertain nurses' practices and knowledge regarding pressure injuries and to find correlations between these factors and characteristics of professional nurses, is similar to this one. According to the study, this sample of nurses' behaviors addressing pressure injuries was less than anticipated.

Finally, the study found in table (5) no significant relationship between nurses' years of experience, education level, age, or sex and their practice in preventing pressure injuries among unconscious patients (P-value > 0.05). This result runs counter to another study by[19,20], which found a significant relationship between educational attainment and work experience. Therefore, we always encourage and focus on the needs of unconscious patients in intensive care units, as their hospital stays require longer periods. This involves implementing programs, practices, and protocols designed to prevent complications in these patients.

CONCLUSION

The present study has come with the following concluded that the most of the participants have poor and moderate and poor level of proficiency bout pressure ulcer prevention among unconscious patients and most of the nurses have unsatisfactory practice in pressure injury prevention among unconscious patients.

Recommendations

The study recommends that the developing educational programs and posters related to the knowledge and practices for caring for unconscious patients with chronic ulcers that have persisted for extended periods.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ahmed T. Saud, Maha Muqdad Idan
Drafting or Revising Critically:	Saja K. Jassim, Nasir Muwfaq Younis
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.

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Original Article

Biphasic Neuroprotection from Depression-induced Oxidative Stress

Zahraa Qusay Muslim[1], Salman Mohammad Salman[2] and Kaiser N. Madlum[3]

ABSTRACT

Objective: To create a two-hit cellular model of oxidative stress in depression and to assess the neuroprotective effects of fluoxetine and 808 nm photobiomodulation on cell survival, interleukin-6, glutathione and major depressive disorder depending on their doses.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the University of Babylon, Iraq from 15th January 2026 to 30th April 2026.

Methods: The SH-SY5Y cells were subjected to corticosterone (100 μM) and H_2O_2 (100 μM) treatment for 24 hours, after which we used fluoxetine (0.1-50 μM) and photobiomodulation (2.5-60 J/cm^2).

Results: Exposure to dual-hits resulted in decreased viability up to 59.6% ($p<0.0001$). The fluoxetine demonstrated hormesis effect: cell viability reached 102.4% when fluoxetine was used at a concentration of 1 μM. Moreover, there was statistically significant decrease in interleukin-6 (1.42 vs. 3.97 pg/mL), major depressive disorder (8.3 vs. 12.0 mg/dL), and increased levels of glutathione (44.1 vs. 29.2 μM). The photobiomodulation applied at 10 J/cm^2 restored viability to 107.1% ($p<0.0001$) and had similar effect.

Conclusion: Both fluoxetine and 808 nm photobiomodulation confer biphasic neuroprotection in a clinically relevant dual-hit depression model, establishing dose thresholds for future synergy investigations.

Key Words: Major depressive disorder, Corticosterone, Oxidative stress, Fluoxetine, Photobiomodulation, Hormesis, Glutathione, Malondialdehyde, Interleukin-6

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INTRODUCTION

A global prevalence of ~280 million suffers from major depressive disorder (MDD); however, less than 50% succeed in achieving a state of remission under conventional treatment, indicating the failure of the monoamine theory.[1,2] Major depressive disorder is currently viewed as a complex disease associated with HPA axis dysfunction, neuroinflammation, oxidative stress, and mitochondrial malfunctioning.[3]

Chronic stress causes high cortisol levels, induces glucocorticoid resistance, triggers inflammation by activating NLRP3 and releasing pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), whereas GR translocation reduces mitochondrial activity, resulting in superoxide production and perpetuation of the oxidative-inflammatory process.[4,5]

The SH-SY5Y neuroblastoma line featuring GR expression, as well as the expression of SERTs and catecholaminergic phenotypes, was selected for modeling those processes.[6,7] The double-hit approach involving corticosterone and H_2O_2 (100 μM concentration) simulating excessive glucocorticoid secretion and oxidative damage causing 40% reduction in cell viability was used. FLX effects on several signaling pathways (BDNF/TrkB/CREB pathway stimulation and NF-κB inhibition) apart from SERTs[8] and 808 nm laser-induced up-regulation of NRF2 were tested.[9] This study establishes optimal dose thresholds for both interventions as a basis for future synergy testing.[10]

METHODS

This experimental study was conducted at University of Babylon, Iraq from 15th January 2026 to 30th April 2026 vide letter No. 4982 dated January 1, 2026. SH-SY5Y cells were maintained in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco) at 37°C, 5% CO_2 Passages below 15 were used throughout.

Dual-hit stress model: Cells were simultaneously exposed to corticosterone (100 μM; Sigma-Aldrich) and freshly prepared H_2O_2 (100 μM; Sigma-Aldrich) in

[1.] MSc Student / Professor[2], Department of Pharmacology & Toxicology, College of Medicine, University of Babylon, Iraq.

[2.] Professor of Cell Biology and Anatomy, University of Babylon, Iraq.

Correspondence: Zahraa Qusay Muslim, MSc Student, Department of Pharmacology & Toxicology, College of Medicine, University of Babylon, Iraq.
Contact No: 07823859676
Email: zahraaqusay97@gmail.com

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2% FBS medium for 24 h. Vehicle controls received 0.1% DMSO.

Fluoxetine treatment, stress induction, cells were treated with fluoxetine hydrochloride (0.1-50 µM; Macklin) for 24 h (0.1% DMSO). Three biological replicates with 4-5 technical replicates (MTT) or 3 (biochemical assays) were performed.

Photobiomodulation, an 808 nm diode laser (Dragon Lasers, China; 50 mW, 0.25 W/cm^2) was applied transcutaneously from below at fluences of 2.5-60 J/cm^2 (10-240 s). A single session was delivered immediately post-stress; thermal effect was negligible ($\Delta T < 0.5°C$).

Cell viability (MTT assay): MTT (0.5 mg/mL; Carl Roth) was incubated for 4 h; formazanwas dissolved in DMSO and absorbance read at 570/630 nm (BioTek ELx800). Viability was expressed as percentage of control.

Lipid peroxidation, MDA was quantified by the TBARS method (absorbance at 535 nm; $\varepsilon = 1.56 \times 10^5$ $M^{-1}cm^{-1}$) and normalized to protein concentration (Bradford assay).

Reduced glutathione was measured colorimetrically using DTNB (412 nm) against a standard curve and normalized to protein content.

Interleukin-6 was quantified in cell supernatants by sandwich ELISA (Elabscience, E-EL-H6156; 450/570 nm) per manufacturer's instructions and normalized to protein concentration.

The data was analyzed through SPSS-25. One-way ANOVA with Dunnett's post hoc test (viability vs control) or Šidák's test (biochemical markers vs. stressed group) A P<0.05 was considered as significant.

RESULTS

H_2O_2 treatment at 50-300 µM for 24 h decreased viability in a dose-dependent manner (IC_{50} = 150-200 µM; Table 1, Fig. 1). CORT treatment from 100 to 500 µM reduced viability in a similar fashion (Table 2, Fig. 2).

Co-treatment with CORT 100 µM and H_2O_2 100 µM led to a reduction in viability to 59.6% (p<0.0001), significantly higher compared to both conditions tested separately (85.6% and 82.9% respectively (Table 3, Fig. 3).

FLX at 0.1-50 µM demonstrated hormetic protective effects. Doses lower than micromolar were ineffective; the first protective effect was achieved only by FLX concentrations higher than 0.7 µM, where viability reached 55.6% (p=0.0416). The maximal protective effect occurred at a concentration of 1 µM with viability restoration to 102.4% (p<0.0001). Protective effect decreased with 5 µM and 10 µM concentrations, while it became ineffective at 25 µM and higher concentrations (Table 4, Fig 4).

Table No. 1: Dunnett's multiple comparisons test for H_2O_2 cytotoxicity

H_2O_2 (µM)	Mean difference vs control (%)	95% CI (%)	P-value
50	-4.46	-24.29 – 15.37	0.9714 (NS)
100	21.74	1.91 – 41.57	0.0282 (*)
150	31.05	11.22 – 50.88	0.0014 (**)
200	53.82	33.99 – 73.65	<0.0001 (****)
250	76.17	56.34 – 96.00	<0.0001 (****)
300	85.88	66.05 – 105.70	<0.0001 (****)

NS=Not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 2: Dunnett's multiple comparisons test for corticosetrone cytotoxicity

CORT (µM)	Mean difference vs control (%)	95% CI (%)	P-value
100	20.69	3.78 – 37.60	0.0139 (**)
200	26.00	9.09 – 42.91	0.0021 (**)
300	64.13	47.21 – 81.04	<0.0001 (****)
400	69.63	52.71 – 86.54	<0.0001 (****)
500	61.75	44.84 – 78.66	<0.0001 (****)

*p<0.05, **p<0.01, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 3: Dunnett's multiple comparisons test for H_2O_2 and CORT combination model

Comparison	Mean 1 (Control %)	Mean 2 (Treated %)	Mean Difference vs Control (%)	95.00% CI of Difference	P Value
Control vs. CORT 100 µM	100.0	85.59	14.41	1.075 to 27.74	0.0340*
Control vs. H2O2 100 µM	100.0	82.94	17.06	3.731 to 30.39	0.0129*
Control vs. CORT 100 µM + H2O2 100 µM	100.0	59.59	40.41	27.07 to 53.74	<0.0001****

*p<0.05, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 4: Šidák's multiple comparisons test for fluoxetine rescue effects vs. stress

Comparison	Mean Difference vs Stress (%)	95.00% CI of Difference	P Value
Fluoxetine (0.1 μM) vs. stress	9.221	-12.86 to 31.30	0.9083 (ns)
Fluoxetine (0.3 μM) vs. stress	6.982	-15.10 to 29.07	0.9833 (ns)
Fluoxetine (0.7 μM) vs. stress	22.61	0.5266 to 44.69	0.0416 (*)
Fluoxetine (1 μM) vs. stress	69.42	47.33 to 91.50	<0.0001 (****)
Fluoxetine (5 μM) vs. stress	36.86	14.77 to 58.94	0.0001 (***)
Fluoxetine (10 μM) vs. stress	22.14	0.05416 to 44.22	0.0491 (*)
Fluoxetine (25 μM) vs. stress	-12.00	-34.08 to 10.08	0.6919 (ns)
Fluoxetine (50 μM) vs. stress	-18.50	-40.58 to 3.583	0.1597 (ns)
Stress vs. control	-66.99	-89.07 to -44.90	<0.0001 (****)

ns = not significant; *p<0.05, ***p<0.001, ****p<0.0001 vs. stress group (one-way ANOVA, Šidák's test)

Table No. 5: Šidák's multiple comparisons test for laser rescue effects vs stress

Comparison	Mean Difference vs Stress (%)	95.00% CI of Difference	P Value
Laser (10s) vs. stress	-1.516	-28.89 to 25.86	>0.9999 (ns)
Laser (20s) vs. stress	30.68	3.308 to 58.05	0.0208 (*)
Laser (40s) vs. stress	58.35	30.98 to 85.72	<0.0001 (****)
Laser (60s) vs. stress	41.23	13.86 to 68.60	0.0010 (**)
Laser (120s) vs. stress	-13.30	-40.67 to 14.07	0.7366 (ns)
Laser (240s) vs. stress	-34.74	-62.12 to -7.373	0.0067 (**)
Stress vs. control	-51.33	-78.70 to -23.96	<0.0001 (****)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 6: Šidák's multiple comparisons test for IL-6 levels vs. induction control

Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	1.505	0.4229 to 2.586	0.0035 (**)
Fluoxetine (1 μM) vs. induction	-2.545	-3.627 to -1.464	<0.0001 (****)
Fluoxetine (5 μM) vs. induction	-2.939	-4.020 to -1.857	<0.0001 (****)
Fluoxetine (10 μM) vs. induction	-1.435	-2.516 to -0.3529	0.0055 (**)
Laser (10s) vs. induction	-0.9427	-2.024 to 0.1391	0.1141 (ns)
Laser (20s) vs. induction	-0.9505	-2.032 to 0.1313	0.1091 (ns)
Laser (40s) vs. induction	-1.738	-2.819 to -0.6559	0.0008 (***)
Laser (60s) vs. induction	-1.470	-2.552 to -0.3882	0.0044 (**)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 7: Šidák's multiple comparisons test for GSH levels vs. induction

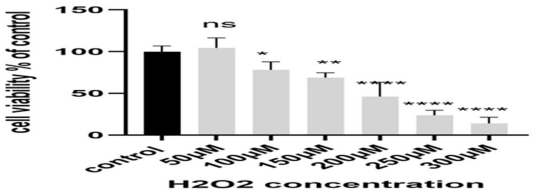
Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	-15.83	-23.06 to -8.606	<0.0001 (****)
Fluoxetine (1 μM) vs. induction	14.93	7.706 to 22.16	<0.0001 (****)
Fluoxetine (5 μM) vs. induction	14.77	7.539 to 21.99	<0.0001 (****)
Fluoxetine (10 μM) vs. induction	8.633	1.406 to 15.86	0.0135 (*)
Laser (10s) vs. induction	0.1333	-7.094 to 7.361	>0.9999 (ns)
Laser (20s) vs. induction	2.433	-4.794 to 9.661	0.9503 (ns)
Laser (40s) vs. induction	9.900	2.672 to 17.13	0.0041 (**)
Laser (60s) vs. induction	4.700	-2.528 to 11.93	0.3912 (ns)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 8: Šidák's multiple comparisons test for MDA levels vs. induction

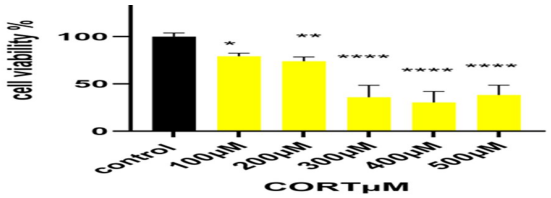
Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	4.804	1.759 to 7.848	0.0010 (**)
Fluoxetine (1 µM) vs. induction	-8.450	-11.49 to -5.406	<0.0001 (****)
Fluoxetine (5 µM) vs. induction	-6.264	-9.308 to -3.220	<0.0001 (****)
Fluoxetine (10 µM) vs. induction	-3.675	-6.719 to -0.6307	0.0124 (*)
Laser (10s) vs. induction	-3.110	-6.154 to -0.06533	0.0434 (*)
Laser (20s) vs. induction	-4.424	-7.469 to -1.380	0.0023 (**)
Laser (40s) vs. induction	-6.325	-9.369 to -3.281	<0.0001 (****)
Laser (60s) vs. induction	-5.667	-8.711 to -2.623	0.0002 (***)

ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 (one-way ANOVA with Šidák's test)



ns = Not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. control

Figure No. 1: Concentration-dependent effect of H_2O_2 (0–300 µM, 24 h) on SH-SY5Y viability (MTT assay)



*p<0.05, **p<0.01, ****p<0.0001 vs. control

Figure No. 2: Concentration-dependent effect of corticosterone (100–500 µM, 24 h) on SH-SY5Y viability (MTT assay)

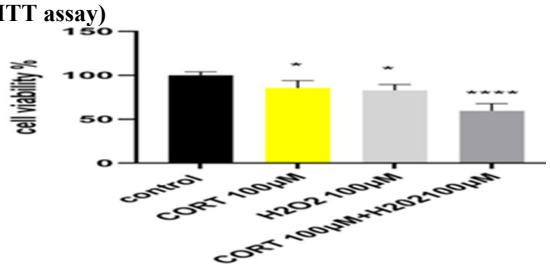


Figure No. 3: Effect of individual and combined corticosterone (100 µM) and H_2O_2 (100 µM) exposure on SH-SY5Y viability (MTT assay, 24 h)
[*p<0.05, ****p<0.0001 vs. control]

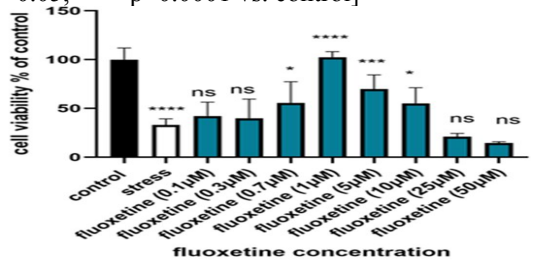


Figure No. 4: Dose-dependent protective effect of fluoxetine (0.1–50 µM, 24 h) against corticosterone/H_2O_2-induced stress in SH-SY5Y cells (MTT assay) [ns = not significant; *p<0.05, ***p<0.001, ****p<0.0001 vs. stress group]

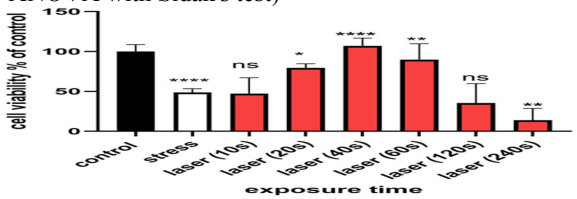


Figure No. 5: Dose-dependent protective effect of 808 nm PBM (10–240 s; 2.5–60 J/cm²) against corticosterone/H_2O_2-induced stress in SH-SY5Y cells (MTT assay, 24 h) [ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 vs. stress group]

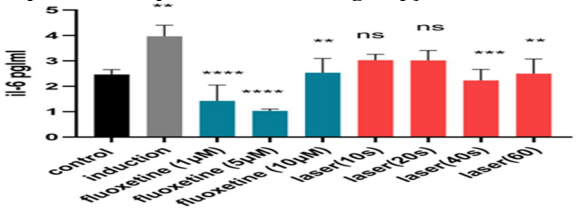


Figure No. 6: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on IL-6 levels in corticosterone/H_2O_2-stressed SH-SY5Y cells (ns = not significant; **p<0.01, ***p<0.001, ****p<0.0001 vs. stress group)

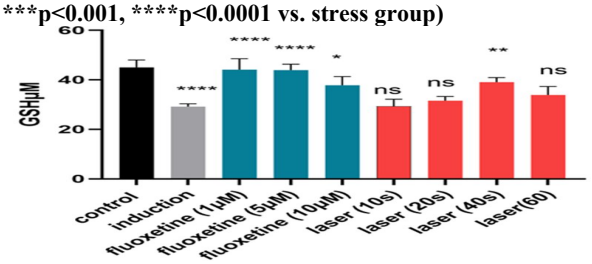


Figure No. 7: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on GSH levels in corticosterone/H_2O_2-stressed SH-SY5Y cells. ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 vs. stress group

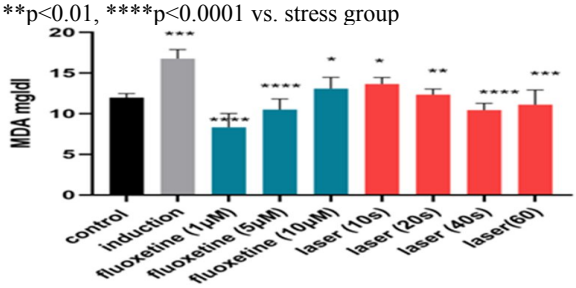


Figure No. 8: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on MDA levels in corticosterone/H_2O_2-stressed SH-SY5Y cells [*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. stress group]

Biphasic viability recovery by PBM follows Arndt-Schulz curve - PBM (10-240 s): 10 s ineffective; 20 s rescued to 79.4% (p=0.0208); 40 s maximum effect (107.1%, p<0.0001); 60 s (89.9%, p=0.0010); 120 s ineffective; 240 s toxic (13.97%, p=0.0067) [Table 5, Fig. 5].

FLX and PBM suppress IL-6: Stress elevated IL-6 to 3.97 pg/mL (control 2.47 pg/mL, p=0.0035). FLX 1 μM reduced IL-6 to 1.42 pg/mL (p<0.0001); 5 μM to 1.03 pg/mL (p<0.0001). PBM 40 s reduced IL-6 to 1.74 pg/mL (p=0.0008); 60 s to 2.50 pg/mL (p=0.0044) (Table 6, Fig. 6).

DISCUSSION

This study presented a double hit CORT/H_2O_2 SH-SY5Y cell model exhibiting features specific for MDD, such as reduced viability, decreased GSH content, increased MDA content, and increased IL-6 production. Effects of the combination of CORT and H_2O_2 are synergistic, however, the precise nature of this phenomenon requires validation since it might differ from the classic glucocorticoid sensitization based on the recent data about neuroprotective effects of CORT in SH-SY5Y cells.[11,12] Effects of both FLX and 808 nm PBM treatment are consistent with biphasic (hormetic) behavior in accordance with Arndt-Schulz law. FLX at 1 μM resulted in close-to-completeness biochemical normalization, including decreased sub-basal level of MDA, indicating its antioxidant effects.[13,14]

Application of PBM with duration of 40 seconds (10 J/cm²) normalized all tested parameters, except for incomplete restoration of GSH level, which can be related to different nature of effects. Toxicity at 240 seconds (60 J/cm²) represents a destructive dose of light, and the possible presence of photothermal effects cannot be excluded.[15] Noteworthy, even sub-therapeutic doses of PBM treatment decreased MDA content, pointing to independent membrane protection.[16]

Overall, both interventions address the oxidative-inflammatory axis through orthogonal mechanisms, justifying future combinatorial studies.[17]

CONCLUSION

The corticosterone/H_2O_2 dual-hit paradigm provides a reliable in vitro model of major depressive disorder-related oxidative and neuroinflammatorystress. Fluoxetine (1 μM) and 808 nm photobiomodulation (10 J/cm^2) each demonstrated hormetic neuroprotection, improving viability, glutathione, and interleukin-6 while reducing major depressive disorder. Their mechanistically distinct profiles support future combinatorial synergy investigations.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa Qusay Muslim, Salman Mohammad Salman
Drafting or Revising Critically:	Zahraa Qusay Muslim, Kaiser N. Madlum
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Investigation the Association Between Parvovirus B19 and Toll-like Receptor 3 Gene Polymorphism in Iraqi Patients with Chronic Myeloid Leukemia

Zahraa Khalid Saleh[1], Shakir H. Mohammed Al-Alwany[2] and Alaa Tariq Shaker[3]

ABSTRACT

Objective: To investigate Parvovirus B19 infection and tool-like receptor-3 gene polymorphism in Iraqi patients with chronic myeloid leukemia.

Study Design: Case control study

Place and Duration of Study: This study was conducted at the College of Science, University of Babylon, Iraq from 1st August 2025 to 31st December 2025.

Methods: One hundred freshly whole bloods were obtained from patients with chronic myeloid leukemia while control group included 100 freshly whole bloods. Viral and total deoxyribonucleic acid genomic extraction were done to detection the B19 by polymerase chain reaction technique and toll-like receptor-3 gene polymorphism by sequencing, respectively

Results: B19 deoxyribonucleic acid was not detected in any patient sample. The results showed that deoxyribonucleic acid polymorphism distribution were deoxyribonucleic acid polymorphism distributions according to major homozygous genotype, heterozygous genotype and minor homozygous genotype were 76.5%, 17.6%, and 5.9%, respectively, in patients with chronic myeloid leukemia and 42.9%; 57.1%and 0.00%, respectively in control group

Conclusion: Toll-like receptor-3 may play a role in the tumor biology of the examined subset of chronic myeloid leukemia and may contributed to their development

Key Words: Chronic myeloid leukemia, Polymerase chain reaction, Toll-like receptor-3, Sequencing

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INTRODUCTION

Chronic myeloid leukemia (CML) is defined as a clonal myeloproliferative neoplasm originating from the hematopoietic stem cell.[1] The disease expresses the Philadelphia chromosome as a result of a reciprocal translocation between chromosomes 9 and 22 that creates the BCR-ABL1 fusion gene, which codes for a tyrosine kinase is always active. Chronic myeloid leukemia is grouped into three phases based on the percentage of blasts in the bone marrow or peripheral blood: chronic, accelerated, and blast crisis.

[1.] MSc Student / Professor[2], Department of Biological Sciences, College of Science, University of Babylon, Iraq.

Correspondence: Zahraa Khalid Saleh, Department of Biology, College of Science, University of Babylon, Iraq.
Contact No: +964 783 173 2293
Email: zahraak353351@gmail.com

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Among other environmental factors that have been linked to the development of leukemia are smoking, exposure to electromagnetic fields, use of hair dye, exposure to organic solvents, and viral infections. Chronic myeloid leukemia is generally not considered a hereditary disease, and no clear genetic predisposition has been identified. Parvovirus B19 (PVB19) belongs to the family Parvoviridae, genus Erythrovirus.[2,3] It is a small, single-stranded DNA virus. Modes of transmission include respiratory secretions, blood transfusion, hand-to-mouth contact, and transplacental route. Infection is largely asymptomatic but can present with mild symptoms such as erythema infectiosum and arthralgia. The reported prevalence ranges from 4-72% in patients with hematological malignancies, reflecting the tropism of the virus for erythroid progenitor cells.[3,4] Toll-like receptor 3 (TLR3) is a receptor of innate immunity that detects viral double-stranded RNA. It is expressed in endocytic compartments and in several immune and epithelial cells.[5,6] Toll-like receptor-3 signaling has been associated with tumor progression, carcinogenesis, and regulation of inflammatory pathways through NF-κB and related mediators; it also has dual regulatory effects in the p53 pathway.[7,8]

Therefore, the present study purpose is to investigate B19 infection and TLR3 gene polymorphism in Iraqi patients with chronic myeloid leukemia.

METHODS

This case control study was conducted at College of Science, University of Babylon, Iraq from 1st August 2025 to 31st December 2025 vide letter No. M250213/QM/Approval/JSDJNEHU dated 19th July 2025. The study was conducted on two hundred specimens, 100 specimens were collected from patients with CML from Heamatological centers in the Middle Euphrates region of Iraq, and 100 specimens from apparently healthy controls (AHC) from the Babylon population. The age of patients and AHC groups ranged from 20 to 65 years.

The viral genome was extracted from blood samples using a commercial DNA/RNA extraction kit (Intron Biotechnology, Korea) according to the manufacturer's instructions. Extracted DNA was stored at −20°C until PCR analysis. The primer sequences used for B19 detection were 5′-AATGCAGATGCCCTCCACG-3′ and 5′-ATGATTCTCCTGAACTGGTCCA-3′, generating a 493 bp product.

Genomic DNA was extracted from whole blood samples using the G-Spin™ Total DNA Extraction Mini Kit (Intron, Korea) following the manufacturer's protocol. Purified DNA was used directly for PCR amplification and stored at −20°C until use. TLR3 primers were: Forward GCCACACACTTCCCTGATGA and Reverse TGTCTCCCCTCCAGCCAATA, producing a 498 bp amplicon at an annealing temperature of 59°C.

Polymerase chain reaction products were subjected to Sanger sequencing. Obtained sequences were analyzed using chromatograms to identify nucleotide variations and determine TLR3 polymorphisms.

Data were analyzed using SPSS-25. Chi-square test was applied and $p<0.05$ was considered significant.

RESULTS

The age of patients' groups were matching with control group (p=0.57). The sex ratio among new diagnosis group and treated group was 1.5 and 1.08 respectively which was match with control group ratio 1.5(p=0.59) [Table 1). Most biochemical parameters showed no significant differences among groups, except creatinine levels between newly diagnosed and relapse patients (Table 2).

The TLR3 genetic sequences located on chromosome 16 were targeted. The 463 bp amplicon analyzed in this research represents part of the exon region of the TLR3molecule, which plays a key role in B cell development and activation. When analyzed using the NCBI BLASTn engine, the obtained sequence showed approximately 99% similarity to the human TLR3 reference sequence (GenBank acc. NC-000016.10) [Fig. 1].

Table No. 1: Age and sex distribution between patients with CML and AHC group (n=200)

Parameters	Patients (n=100)	Controls (n=100)	P value
Age (years)	49±12.43	46±12.9	0.57
Gender			
Male	52 (52%)	56 (56%)	0.59
Female	48 (48%)	44 (44%)	

Table No. 2: Estimation the levels of some biochemical parameters for new diagnosis CML, treated patients respond to treatment and relapse groups

Parameters	New diagnosis (n=20)	Treated (n=100)		P value
		Response to treatment (n=69)	Relapse (n=31)	
Urea (mg/dl)	31.5±7.8	33.3±18.2	30.3±12.1	*0.615 **0.69 ***0.399
Creatinine(mg/dl)	0.71±0.51	1.0±0.73	0.9±0.33	*0.059 **0.01 ***0.329
ALT (IU/L)	29.5±10.9	30.5±10.79	28.3±12.3	*0.73 **742 ***418
AST (IU/L)	33.6±12.6	32.2±12.7	33.2±15.6	*0.671 **931 ***733
LDH (IU/L)	478±201	485±278	438±20.4	*0.909 **0.49 ***0.39

*Comparison between new diagnosis CML and response to treatment patients. **Comparison between new diagnosis CML patients and relapse patients. ***Response to treatment patients and relapse patients

Table 3: Genotyping of TLR3 gene in CML patients and AHC groups

Genotype/ allele	Patients No. (% of called)	Controls No. (% of called)	Chi-square	p-value	Odds ratio (95% CI)	SNP type
G/G	13 (76.5%)	3 (42.9%)	0.0	1.0	1.0	Intron variant
G/A	3 (17.6%)	4 (57.1%)	3.39	0.066	0.17 (0.02-1.22)	Intron variant
A/A	1 (5.9%)	0 (0.0%)	0.23	0.633	0.78 (0.03-23.53)	Intron variant
Allele G	29	10	0.0	1.0	1.0	Intron variant
Allele A	5	4	1.25	0.263	0.43 (0.10-1.93)	Intron variant
Unconfirmed/ excluded	0	0	Not counted	Not counted	Not counted	Weak/conflicting/ no-call trace

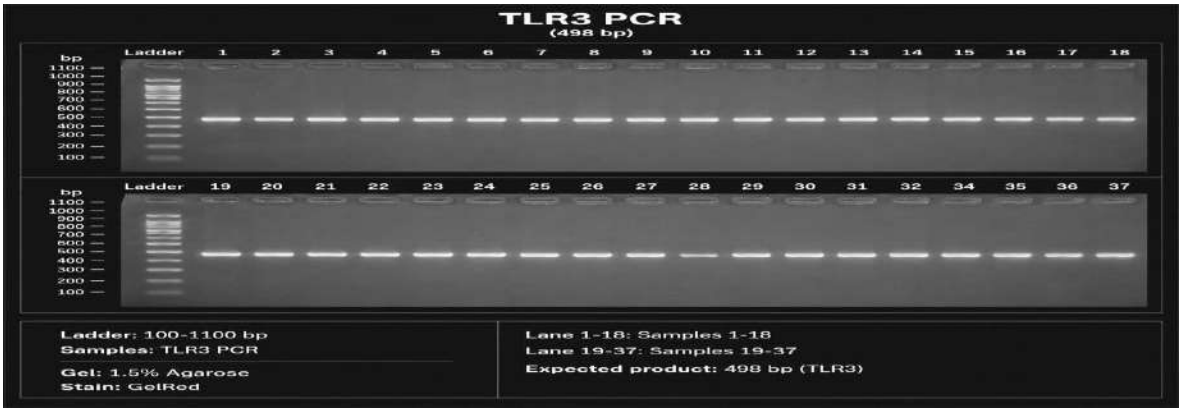

Figure No. 1: Agarose gel electrophoresis of PCR-amplified TLR3 fragments

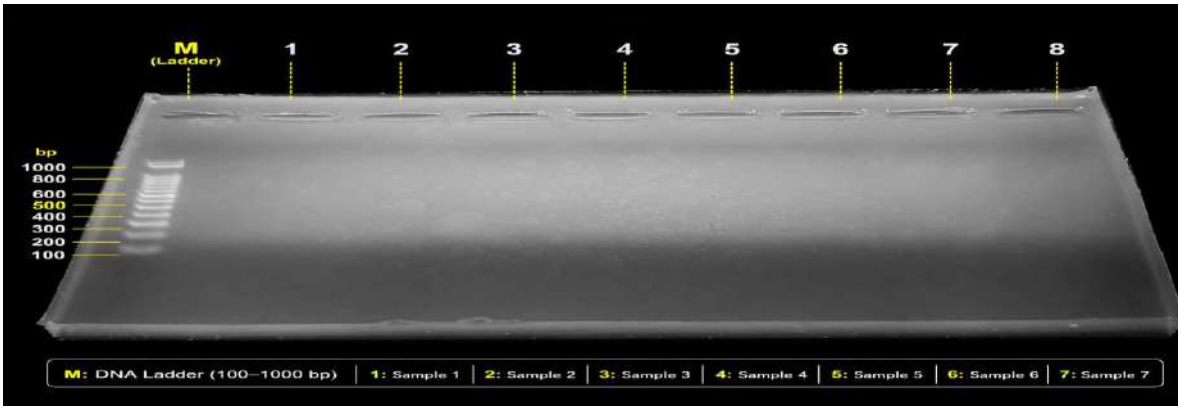

Figure No. 2: Agarose gel electrophoresis of PCR products for B19 DNA in CML patients. M: 100–1100 bp DNA ladder. Samples were separated on 2% agarose gel at 75 V and 20 mA for 120 min, showing no detectable B19 amplification

Table No. 4: The results of PCR for B19-DNA-infection in patients with CML

B19	No.	%
Positive	-	-
Negative	100	100.0

TLR3 genotype frequencies were GG 76.5%, GA 17.6%, and AA 5.9% in patients and 42.9%, 57.1%, and 0.0% in controls. Allele frequencies showed no significant difference between groups (Table 3).

Amplification detection of B19 by PCR technique in samples from patients with CML, B19 DNA was not detected in patients or controls (Table 4, Fig. 2). Spearman's Rho statistical testing of age, sex, B19; SNPs of TLR3in the Studied Population Groups, a significant correlation was observed between TLR3 polymorphism and age (r=0.722, p=0.03), whereas no correlation was found with B19 DNA (Table 5).

Table 5: Spearman's Rho statistical testing of age, sex, B19-DNA-PCR andTLR3 SNP to evaluate the studied markers in patients with CML

Spearman's rho		Age	TLR3 polymorphism	Sex
TLR3 Polymorphism	r	0.722		0.342
	p	0.03		0.6
B19-DNA	r	0.399	0.151	
	p	0.8	0.7	

DISCUSSION

Chronic myeloid leukemia (CML) is a condition in which the bone marrow makes too many white blood cells. It is one type of cancer of the blood and bone marrow. In this study, the age and sex distributions of the patients and the control group were not significantly different (p=0.57 and p=0.59, respectively). These results are in line with other studies that have shown chronic myeloid leukemia to commonly afflict adults with median ages ranging between 35 and 64 years and a slight male predominance.[9,10]

Berger et al[11] reported gender-related differences in CML characteristics: higher platelet counts and smaller spleen size in females; a greater frequency of additional chromosomal abnormalities and poorer survival in males. These differences were not observed in the current study. Most parameters showed no significant differences among the patient groups. Only serum creatinine showed a difference between newly diagnosed and relapse patients. Liver and kidney function markers might be related to disease progression or adverse effects of tyrosine kinase inhibitors (TKIs), including imatinib, dasatinib, and nilotinib. No sex-related differences were observed in this study. A similar trend was not found in the present study. Serum creatinine showed significant difference between newly diagnosed and relapse patients. Liver and kidney function markers might be related to disease progression or adverse effects of tyrosine kinase inhibitors (TKIs), including imatinib, dasatinib, and nilotinib.

Toll-like receptor-3 signaling pathways regulate the survival and proliferation of myeloid leukemia cells. TLR3 shares little in common with other TLRs in terms of downstream signaling, but components of the IRAK1/4–NF-κB pathway are critical for leukemic cell survival. In previous research, it has been shown that myeloid malignancies' growth could be suppressed and apoptosis induced upon IRAK1/4 inhibition, hence supporting these pathways as potential therapeutic targets.[12-14] Results of the current study showed the prevalence of the GG genotype in CML patients. Although allele A was more frequent in patients, no significant association was found. Similar results provide evidence that the dysregulation of TLR3, through inflammatory and immune-related pathways, may contribute to leukemogenesis and disease progression.[15] The prevalence of parvovirus B19 varies depending on geographical region and study population, with reported rates ranging between 20% and 60% in published papers.[16,17] In stark contrast, B19 DNA was not found in any of the patient samples in our study. This difference may be due to variations in population characteristics, sample size, viral circulation, or timing of detection. A significant correlation was seen between TLR3 polymorphism and patient age. No significant association was found between TLR3 polymorphism and B19 infection. Previous studies have demonstrated increasing B19 seroprevalence with age, probably reflecting cumulative exposure over time.[18] Overall, the findings suggest that TLR3 polymorphism may contribute to the biology of CML. Parvovirus B19 does not seem to play a significant role in the studied population.

CONCLUSION

Toll-like receptor-3 may play a role in the tumor biology of the examined subset of chronic myeloid leukemia and may contributed to their development.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa Khalid Saleh, Shakir H. Mohammed Al-Alwany
Drafting or Revising Critically:	Zahraa Khalid Saleh, Alaa Tariq Shaker
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.

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Ethical Approval: No. M250213/QM/Approval/ JSDJNEHU Dated 19.07.2025

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Original Article

Evaluation of Kidney Injury Molecule -1(KIM-1) Quantitative Expression in Renal Tubule following Dose Dependent Chromium Administration

Sajjad Jubair Kadhim[1] and May Fadhil Majid AL-Habib[2]

ABSTRACT

Objective: To evaluate the histopathological alterations in renal tissue following chromium exposure, in different doses with particular emphasis on kidney injury molecule -1as a biomarker of renal damage.
Study Design: experimental study
Place and Duration of Study: This study was conducted at the College of Medicine, Al-Nahrain University, Baghdad, Iraq from 1st September 2025 to 30th November 2025.
Methods: This experimental group was exposed to different doses of chromium and compared with the control group. Histological analysis was performed and Kim-1 was assessed to evaluate tubular injury.
Results: Chromium exposure resulted in noticeable histological changes, treated groups showed enlargement of the urinary space and degenerative changes in proximal convoluted tubules. In addition, kim-1 expression was markedly elevated indicating early tubular injury based on dose dependent effect.
Conclusion: Chromium induces significant dose-dependent renal damage at both structural and cellular levels and the nephrotoxic potential of Chromium and support the utility of kim-1 as a sensitive biomarker for early detection of kidney injury.

Key Words: Chromium, Nephrotoxicity, Kim-1, Kidney, Oxidative stress, Histopathology

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INTRODUCTION

The kidneys are very important organs which control the regulation overflowed balance levels and elimination of metabolic wastes and the basic structural and functional unit of the kidney is called the nephron which is composed of renal corpuscle and a tubular system including the following: proximal convoluted tubule, loops of Henle, distal convolute tubule and collecting duct.[1]

The renal corpuscle contains the glomerulus surrounded by Bowman's capsule and is responsible for the filtration of a blood plasmas, filtrate then enters the proximal convolute tubule (PCT) which represents the most metabolic active segment of the nephron. The high metabolic activity and the filtrated substances make the proximal tubule more vulnerable to toxic injury from drugs, heavy metals and environmental toxins.[2]

Chromium supplement are widely used worldwide as a nutritional aids to support metabolic health despite it is a proposed beneficial effect, increasing evidence of prolonged intake of Chromium supplements may have toxic effect on different organs including the kidneys, since the kidneys are primarily responsible for the filtration and excretion of metal and xenobiotics, renal tissues may accumulate chromium following systemic exposure, This accumulation may induce oxidative stress, inflammatory responses, and cellular damage within renal structure.[3]

KIM-1, a type 1 transmembrane glycoprotein, is poorly expressed in normal kidney but is highly expressed in injured PT epithelial cells.[4] Due to its high sensitivity on the specificity of proximal tubular injury kim-1 immunohistochemical staining has been widely used in experimental nephrotoxicity studies to evaluate renal damage induced by drugs, environmental toxins and heavy metals.[5,6]

METHODS

This experimental group was exposed to different doses of Chromium and compared with the control group at College of Medicine, Al-Nahrain University, Baghdad, Iraq from 1st September 2025 to 30th November 2025 vide letter No. 341/Approval/SFGH dated July 12,

[1.] Dentist / Prefessor[2], Department of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq.

Correspondence: Sajjad Jubair Kadhim, Department of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq.
Contact No: +9647800563081
Email: sajjad.j.kadhim.med24@ced.nahrainuniv.edu.iq

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2024. A total of 30 albino rats (males) were used in the present study and obtained from the animal house of the collage of science in Kufa University, which were already in experimental use. The rats have a body weight ranging between 250- 370 gm and age 8-12 weeks. The rats were divided into three groups: Control group, low dose group and high dose group (10 rats in each group). Oral Gavage Procedure: Homogenous powder was obtained by grinding Chromium tablets 200mcg with an electric grinding machine. The animals were anaesthetized with 80-100 mg/kg body weight of a combination of ketamine and xylazine (8:1). After 5 weeks underwent right kidney nephrectomy. Paraffin blocking and sectioning were done. The data was analyzed through SPSS-25.

RESULTS

The antibody- antigen reaction appeared in PCT cells were assessed in all groups using Aperio software. Only strong positive staining was included in this statistical evaluation of the test, while positive and weak positive reactivity were deliberately excluded from this analysis to obtain the methodological precision and to minimize the background reactivity variability. Analysis of these measurements revealed highly statically significant differences between all groups. Mean of (kim-1) expression level in control group was 11941.3000±3761.37533 pixel/ μ^2 reflecting a base line protein expression and physiological expression while, the low dose group showed substantially elevated mean value of 63112.1000±5701.42797 pixel /μ^2, high dose group showed a striking rise of the mean which recorded 138773.1667±18679.70755 pixel/ μ^2(table 1).Statistical analysis evaluation confirm that this increase with the high significant p -value of (0.05) indicating that the differences is unlikely to be a due to random variation (Table 1).

General examination of the renal parenchyma in control group by light microscope showed that two distinct areas of the kidney were clearly demarcated. Notable, the proximal convoluted tubules were seen to be in greater number than the distal convoluted tubules near the renal corpuscles. The medullary area was less pigmented than the cortex and had fewer cells, with the presence of tubular structures consisting of parallel rays, which were composed mainly of collecting ducts and different parts of the loop of Henle. These medullary rays meet at certain points creating renal papillae which project into the lumen of the minor calyces (Fig. 1). Some variations were recorded in the experimental groups such as enlargement of renal corpuscle, decrease in the height of the cuboidal cells lining the lumen of the PCT with corresponding decrease in the number of microvilli in the lumen of the PCT in the experimental groups. The picture in high dose group is the same as that in low dose but more severe and deconfiguration of some PCT. At higher magnifications the experimental groups show more signs of vascular congestion, especially in the tuft (Fig. 2).

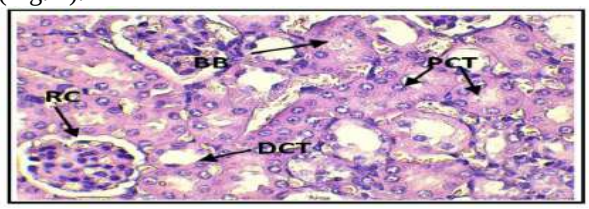

Figure No. 1: Section in the kidney of control group showing renal corpuscle (RC), proximal convoluted tubule (PCT) with high cuboidal epithelium and brush border (BB), distal convoluted tubule (DCT). control group, H&E,X40

Table No. 1: Difference in kim-1 expression between all groups

Groups		Mean Pixel μ^2	Standard Error	P value
Control group	Low dose group	63112.1000	5701.42797	0.006*
	High dose group	138773.1667	18679.70755	0.000*
Low dose group	Control group	11941.3000	3761.37533	0.006*
	High dose group	138773.1667	18679.70755	0.000*
High dose group	Control group	11941.3000	3761.37533	0.000*
	Low dose group	63112.1000	5701.42797	0.000*

Significant (P<0.05)

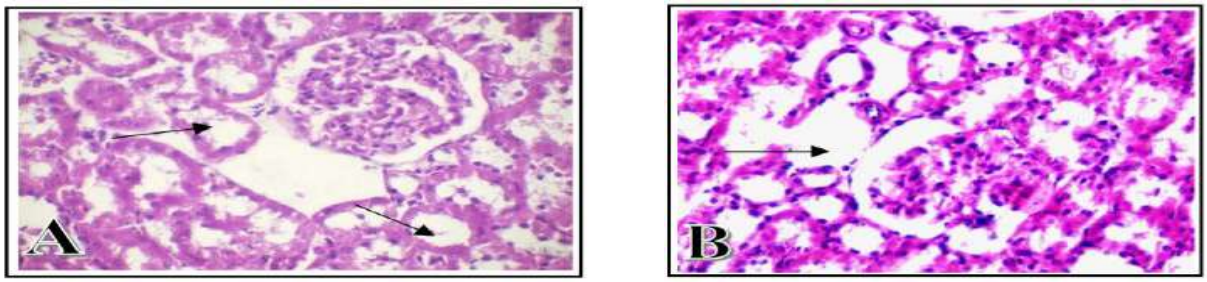

Figure No. 2: section in the kidney A: in low dose group increase in the size of renal corpuscles, loss of microvilli in lumen of PCT associated with decrease of the height of cuboidal cells lining it and increase in the size of the lumen (→) B: high dose group showing deconfiguration of some PCT (→). Experimental groups, H&E, X40

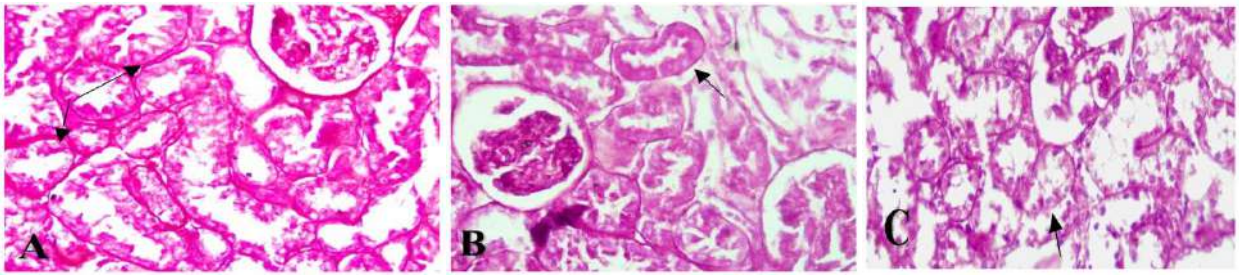

Figure No. 3: section in the kidney A: in control group showing thick basement membrane (⟶) B: with decrease thickness of B.M (⟶). C: high dose group showing with decrease thickness of B.M with some tubules showing loss of continuity of B.M (⟶). Experimental groups, PAS stain, X40.

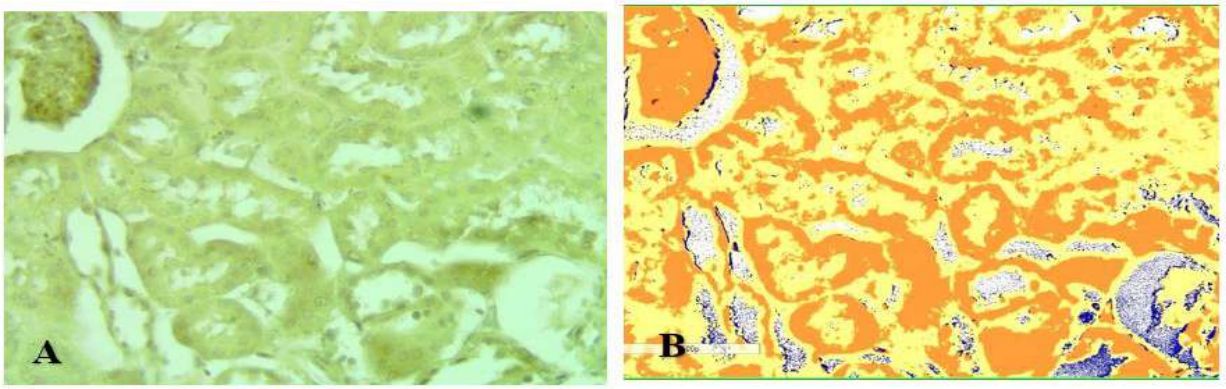

Figure No. 4: Cross-section in the kidney of control group showing A: distribution of DAP with no strong reactivity for KIM1 Ab reactivity B:, snapshot picture seen with aperio-software , (control group , KIM1 Ab, X40)

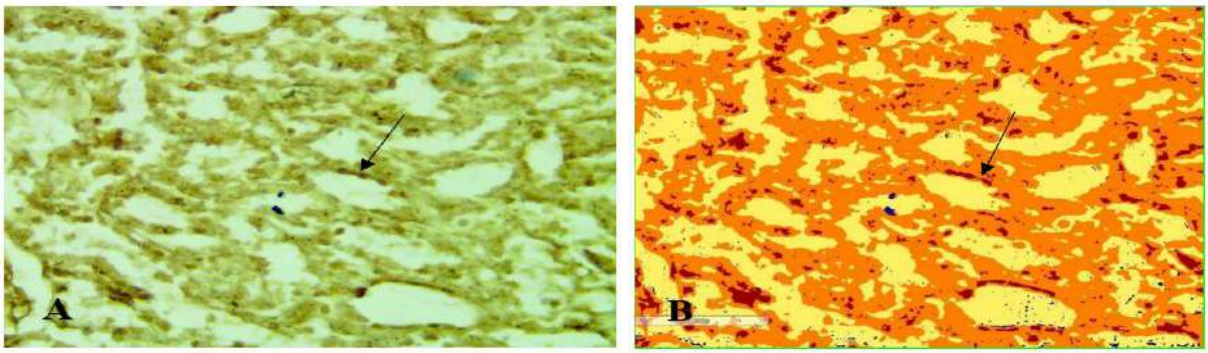

Figure No. 5: Section in the kidney in lower dose group showing A: positive reactivity of KIM1 in the apical cytoplasm of some PCT (⟶). B: snapshot of the same figure showing the reactivity as small reddish brown granules in the apical surface of PCT (⟶). Low dose group, KIM1 Ab, X40)

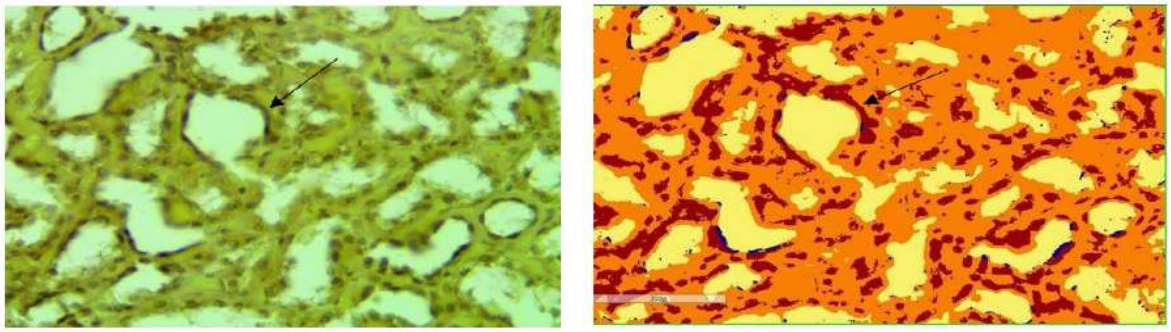

Figure No. 6: Section in the kidney in lower dose group showing A: increased positive reactivity of KIM1 in the apical cytoplasm of some PCT (⟶). B: snapshot of the same figure showing the reactivity as small reddish brown granules in the apical surface of PCT (⟶). Low dose group, KIM1 Ab, X40).

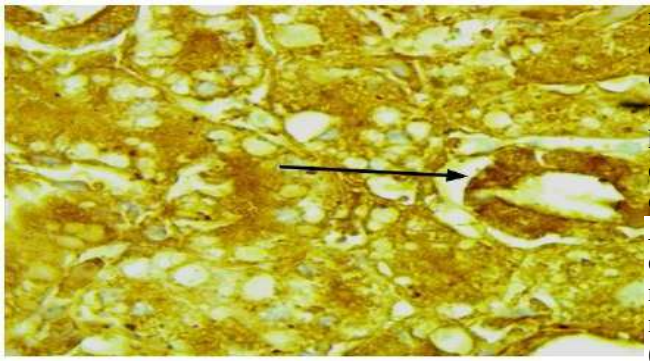

Figure No. 7: Kidney section in experimental group at high magnification indicating dark stained brown granules (⟶) in the cytoplasm of the PCT. (high dose group, KIM1, X100)

Basement membrane is a critical barrier that's found in all parts of the nephron assessment of renal corpuscle and proximal convoluted tubule, basement membrane was done using PAS stain. The overall picture showed differences in the thickness of basement membrane both in glomeruli and PCT. Basement membrane was thick and dense continuous in control group, thin and delicate fine line around the corpuscle and PCT in low dose group and it was also continuous. High dose group basement membrane was not easily detectable with a striking feature of lost continuity of the basement membrane which was seen mainly in some PCT more than renal corpuscles. Glycocalyx was easily seen in control and low dose groups, but was not seen in high dose groups, which could be because of the loss of brush border. Using PAS stain enables easy distinguishing between PCT and DCT by showing densely eosinophilic Glycocalyx inside the lumen (Fig. 3).

The quantitative and qualitative expression of the histochemical product in renal cortex was examined in detail in all the groups by using polyclonal antibody (Kim-1); it was observed as multiple structural chromogenic expression, dark brown color tiny granules and extended in different ranges between light pale yellow, light orang to deep dark brown redish. The chromogenic expression signals were mainly observed around the renal corpuscles, and the site of reactivity was only observed in proximal convoluted tubules; the pattern of distribution of this expression was observed as deep intense brown-red colour in the cell cytoplasm in all three groups, the reaction of PCT to (kim-1) was not observed in other tubules. Evaluation of this chromogenic signal objectively was mainly qualitative including the distribution and localization of Ab-Ag reaction. From an objective point of view, the intensity of this reaction of PCT cytoplasm had different degrees in the three groups (Fig. 4).

In both experimental groups (low and high) Kim-1 reactivity showed significant upregulation of expression as the immune reaction was found as strong brown reddish cytoplasmic granules in the apical portion of the cytoplasm cells in the PCT. This upregulation of the cortical granules in the apical cytoplasm resulted in thicker granules as well as increased intensity in the high dose groups, where the intensely appeared as thick dark brown large granules occupying most of the cytoplasm PCT cells (Figs. 5-6).

At higher magnification the intensity of reaction was so deep that obscuring the nuclei, intensity was seen mainly in the high dose group the obscuring of the nuclei appeared partially and sometimes completely (Fig. 7).

DISCUSSION

The human kidney is the bilaterally located excretory organ. Histologically, the renal cortex is the site of the larger number of renal corpuscles and the proximal and distal convoluted tubules while the renal medulla is composed primarily of loops of Henle and collecting ducts contained within renal pyramids.[7] The renal corpuscle is made up of the glomerulus and Bowman's capsule, which constitute the filtration unit of the nephron.[8] The sample is present on the proximal convoluted tubule, which has a cuboidal epithelium with a prominent brush border to increase the reabsorption surface area.[9]

Exposure to chromium showed some significant structural damage in renal tissue as revealed by the data in the present study which was a reflection of toxic and antioxidant activity of trivalent chromium and has a toxic effect on renal cells and tissues. Chromium compounds have been linked to the development of toxicity in the cells by the mechanism of causing oxidative stress. Reactive oxygen species (ROS) are formed during intracellular reduction of hexavalent and trivalent chromium, which can attack cellular proteins, lipids and DNA.[6] The results obtained by Soudani[10] who reported that rats fed potassium dichromate exhibited histopathological changes such as tubular degeneration, necrosis and vascular congestion in renal tissues were similar. In this present study, Heavy metal like chromium causes a glomerular injury by causing oxidative damage to the glomerular cells and results in a distortion of the glomerular tuft. Glomerular changes were also noted by Goodarzi et al[11] who reported glomerular degeneration and disruption of the renal architecture in rats treated with chromium.

This experimental group showed that degenerative changes in the proximal convoluted tubules. Hasan et al[12] found necrosis damage in the renal tubules of rats after chromium exposure. The progressive renal change noted with the higher doses of chromium in the present study indicates a dose-dependent toxicity. Experimental studies have also shown dose-dependent nephrotoxic effects of chromium, as renal damage was found to be increasingly severe with increasing doses of chromium.[13]

In the present study, high doses of Chromium compounds may cause morphological changes in renal tissues, especially in tubular epithelial cells, the proximal convoluted tubule is regarded as one of the most susceptible parts of the nephron to toxic damage. As it is actively transported and has high metabolic rate, the proximal tubular cells are good targets of toxicity and when damaged by nephrotoxic agents, they can undergo tubular degeneration, cellular swelling, loss of the brush border integrity and impair the absorptive function.[14]

The changes are probably the result of oxidative stress–mediated degradation of proteins at the basement membrane, such as type IV collagen and laminin. These findings were corroborated by experimental nephrotoxicity studies that demonstrated that disruption of the integrity of the basement membrane was linked to tubular injury.[15]

In the present study, changes in the basement membrane were clearly observed in the renal corpuscles and PCT using the PAS stain. In control group, the basement membrane was seen to be thick, dense, continuous and strongly PAS positive, indicating that its structural integrity and glycoproteins and proteoglycans level were normal. The basement membrane is positive for the presence of carbohydrate containing components like type IV collagen, laminin and proteoglycans, hence its positivity.[16]

The basement membrane seemed to be noticeably thinned in the low dose group, but was continuous forming a fine linear border around both glomeruli and proximal tubules. This implies that physical changes occur, but the trucks are not completely destroyed and is similar to early nephrotoxicity, where components of the extracellular matrix are partially degraded or remodelled.[17]

High dose group has well preserved glycocalyx see by pasabsent, while control and low dose groups have well preserved glycocalyx. At the apical surface, the glycocalyx is important and its loss could contribute to damage of the brush border of the proximal convoluted tubular cells. Recently impaired function of the glycocalyx has also been linked to impaired epithelial barrier function and susceptibility to injury.[18]

The kidney injury molecule 1 (KIM-1) is a type I membrane protein that has two domains; an extracellular domain and a cytoplasmic domain. Also known as HAVCR1 (hepatitis A virus cellular receptor 1), also known as TIM1 (T-cell immunoglobulin mucin receptor 1), it is expressed in kidney, liver and spleen. KIM-1 acts via several molecular targets in immunological diseases and renal damage. In HAV infections, KIM-1 is involved, in autoimmunity, in immunological tolerance and in atopic disorders. The level of urine KIM-1 is closely correlated with its tissue level and, like renal tissue injury, is linked to renal tissue injury. KIM-1 is a sensitive biomarker for acute kidney injury (AKI), and might even be a predictive marker for long-term renal outcomes.[19]

The present investigation showed that there was a significant induction of the expression of kim-1 upon chromium exposure which was largely localized in the proximal convoluted tubules of the kidney cortex. Different degrees of staining reactivity were observed, from weak staining reaction in the control group to the highest staining reaction in the treatment group and most intense in the high dose group, staining being cytoplasmic in these groups. No staining was seen in the distal tubular segment or glomeruli. The selective localization is consistent with its known role as a marker of damage in the proximal tubule.[20] The immunoreactivity was seen as brown granules in the apical part of the cytoplasm and increased with the chromium exposure. The same type of staining has been seen in tubular injury models.[21]

The treatment groups report a significant increase in expression when it's compared with the minimal expression reported in the control group. Initial injury is reflected by mild staining in LDO and significant tubular damage is reflected by severe and diffuse staining in HDO. The level of kim-1 expression has been demonstrated to be linked to how bad the damage.[22]

There was a significant dose-dependent increase in expression. This suggests that there is a direct relationship between the severity of renal injury and exposure to chromium. Kim-1 over-expression has been reported with a wide variety of dose-response relationships.[23] These results were confirmed by quantitative analysis showing a significant difference and confirming the reliability of kim-1 as a marker for nephrotoxicity.[21] This agreement between the qualitative and quantitative results increases the validity of the current results.

CONCLUSION

Structural defects of the architecture of the kidneys have been shown by histological and macroscopic examination may be functional because of exposure to chromium. These changes including cortical thinning, discoloration was involved with underlying pathological processes (edema vascular disturbance and cellular degeneration). Histopathological analysis revealed renal damage in the form of both glomerular and tubular damage, particularly in the proximal convoluted tubules, the most sensitive part of the kidneys to toxic injury. The effects were dose-dependent, showing the greatest effects at high doses. The increase in kim-1 expression support it is role as reliable and early biomarker of the tubular damage. Importantly, these effects were dose-dependent with greater alterations seen as exposure levels increased.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sajjad Jubair Kadhim, May Fadhil Majid AL-Habib
Drafting or Revising Critically:	Sajjad Jubair Kadhim, May Fadhil Majid AL-Habib
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. 341/Approval/SFGH Dated 12.07.2024

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Original Article

Stereoacuity Changes following Photorefractive Keratectomy in Myopic Eyes

Mohammed Qasim Alnuwaini[1] and Alyaa Abood Kareem[2]

ABSTRACT

Objective: To find out the stereoscopic changes after photorefractive keratectomy in myopia.
Study Design: Prospective study
Place and Duration of Study: This study was conducted at the Alhaitham Teaching Eye Center, Baghdad, Iraq from 15th April 2025 to 14th October 2025.
Methods: 44 cases of 88 eyes had photorefractive keratectomy achieving emmetropia. The patients had preoperative assessment, their stereoacuity was evaluated using TNO test charts positioned at a distance of 40 cm, this assessment took place 1 week before the scheduled surgery. Subsequently, follow-up evaluation was conducted at 4 week following the operation. Patients who had amblyopia were excluded from the study.
Results: The average age was 25.98±5.4 years, with 19 (43.2%) males and 25 (56.8%) females. Photorefractive keratectomy was uneventful in enrolled individuals. The mean preoperative stereoscopic vision was 220.4±142.2″. After the surgical procedure, the measurement of mean stereoacuity yielded value of 165.17±144.3″ at 4 weeks. Statistical significance between preoperative and 4 weeks (P=0.013) and notable improvement in postoperative stereoacuity among anisometropic participants.
Conclusion: Photorefractive keratectomy has the potential to improve visual acuity and stereoacuity in a substantial proportion of patients who undergo uneventful operation. The individuals diagnosed with an isometropia owned the most improvement in terms of heightened stereoacuity.
Key Words: Stereoacuity, Stereoscopic vision, Stereopsis, Photorefractive, Keratectomy, Refractive surgery, Myopia

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INTRODUCTION

In contrast to other visual capabilities, 3-D vision requires eye coordination, and thus creates its own unique category of visual capability. 3-D vision can be considered the ultimate example of eye cooperation and takes place whenever there is a certain amount of difference between the retinal images created by both eyes, without creating double vision. [1] Stereopsis as an aspect of vision can be defined as the ability to distinguish between what each eye sees differently because of the differences in the position of each eye on the head.[2]

[1.] Assistant Professor of Ophthalmology, Medical College, Jabber Ibn Hayyen Medical University, Iraq.
[2.] Professor, Department of Ophthalmology, Medical College, Kufa University, Iraq.

Correspondence: Dr. Mohammed Qasim Alnuwaini, Assistant Professor of Ophthalmology, Medical College, Jabber Ibn Hayyen Medical University, Iraq.
Contact No: 00964782 898 4370
Email: m.qasim@jmu.edu.iq

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Factors that degrade stereopsis include visual acuity, aniseikonia, pupil size, refractive error, eye dominance, accommodation, contrast sensitivity, heterophoria, and age.[3] Interpupillary distance (IPD) is another important component; SA increases with higher IPD.[4] It is unclear in the healthy population whether distance has a substantial impact on stereopsis outcomes or has no impact on the measurement of SA.[5] Changes in target shape and size and disparity types had no discernible impact on SA.[6]

There are several ways by which stereopsis is measured, but the results vary depending on the type of use, resulting in a lack of correlation between various stereopsis tests. The differences could be due to the size, shape, and degree of familiarity of symbols used for testing, differences in viewing distances, or the wearing of glasses.[7] Stereopsis tests are classified into two types: contour and random dot. The contour tests include Titmus circle test, while random dot tests include the TNO test. Since disparities are different for each test, neural processing would vary based on these disparities.[8] According to Fawcett, stereopsis measurements of healthy people were not significantly different using the Titmus circle test, Randot circle test, and Preschool Randot (random dot).[9]

According to World Health Organization, uncorrected refractive error is the most prevalent source of moderate-to-severe vision loss, responsible for 43% of such occurrences in the world (equivalent to 122 million people).[10] Three methods can be used to correct refractive error problems, including eyeglasses, contact lenses and surgery, more and more patients are now inclined toward surgeries, which include laser eye surgeries and corneal transplant surgeries.[11] Refractive surgeries can be considered not only for refractive purposes, but also as an option for patients with preoperative binocular vision disorders and normal subjects could alter their preoperative normal binocularity.[12] The success or failure rate of a refractive procedure is determined by its stability, safety, and predictability.[13] Surgical interventions involving refraction may greatly lower aniseikonia, and research has shown that changes in aniseikonia above a particular point may have negative impacts on stereopsis. Moreover, research has revealed that wearing sunglasses affects stereopsis more negatively than visual acuity. All refractive procedures have the potential to affect stereoacuity.[14,15]

The patients' satisfaction after a refractive surgery depends on their postoperative uncorrected visual acuity, preoperative expectations, and psychological factors.[16] Despite the excellent postoperative anatomical and optical outcomes, some patients complain of visual symptoms, suc has decreased contrast sensitivity, color vision and generally poor vision.[17]

Photorefractive keratectomy (PRK), a technique involving photoablation of the cornea by an excimer laser, was applied in myopia correction in the 1980.[18] PRK treatment is done at the corneal level, whereby the superficial corneal epithelium up to Bowman's membrane is removed completely, after which the surface of the cornea is remodeled using the excimer laser.[19] PRK is safe and effective in myopia treatment, with patients exhibiting relatively higher levels of satisfaction.[20] Moreover, PRK is less likely to cause ectasia compared to LASIK, and there is no threat of vision loss because there is no flap formation.[21]

Today, the main parameter for assessing the results of refractive surgeries is visual acuity. However, visual acuity is an aspect of vision that provides only are lative view of visual quality.[17,18] On the other hand, stereopsis, as the highest level of visual function, is not routinely measured before and after refractive surgeries, while its measurement is not strenuous.[22]

METHODS

This prospective study was conducted at Ibn Alhaitham Teaching Eye Center, Baghdad, Iraq from 15th April 2025 to 14th October 2025 vide letter No. 124/QM/Approval/dfdfd Date April 3, 2025 and 44 cases of 88 eyes had photorefractive keratectomy with the goal of achieving emmetropia. The pre-operative spherical equivalent (SE) refraction varied from -0.5 to -6.5 dpt, whereas the pre-operative best corrected visual acuity (BCVA) was 6/9 or better. Monocular amblyopia, heterotropia identified through clinical examinations, ocular pathology alters visual function, prior ocular surgery, CCT less than 470 µm, hyperopia, BCVA worse than 6/9 were excluded.

Before undergoing refractive surgery, all participants had their medical history taken in a detailed manner in addition to an ophthalmic examination that was thorough and complete. The procedures included a visual acuity or a refraction, intraocular pressure, ocular alignment, and motility, stereoacuity, slit-lamp examination, and effective corneal topography that was performed with the aid of the Sirius Scheimpflug-Placido device.

An experienced optometrist carried out the stereopsis assessment according to manufacturer's instructions using the TNO test in standardized photopic conditions at 40 cm. Measurement of x-ray was recorded before surgery and again at one and four weeks after surgery.

A single surgeon used the MEL® 90 excimer laser system (Carl Zeiss Meditec) to perform all procedures that permit real-time eye tracking, ablation, and adjustment.

Prior to surgery, topical anesthesia and povidone-iodine antisepsis were administered, followed by debridement of corneal epithelium and photoablation with excimer laser. For all the patients, the optical zone was set at 6.5 mm with 0.02% Mitomycin C. This was followed by saline irrigation and placing of bandage contact lens. The patient was given topical moxifloxacin, and prednisolone, and preservative-free artificial tears in the postoperative period. The medications were gradually tapered as per the healing.

The data have subsequently analyzed using SPSS Version 23. The categorical variables have compared using Chi-square test/Fisher's exact test and numerical variables were analyzed using ANOVA with Tukey post hoc test. The Mann-Whitney U test and Wilcoxon Signed Rank test were used to analyse non-normal data. A p-value of less than 0.05 showed significance.

RESULTS

The average age was 25.98±5.4 years. There were 19 (43.2%) male and 25 (56.8%) female. The preoperative mean spherical equivalent was-2.92±1.42 dpt, Prior to the surgical procedure, 22 (50%) patients exhibited stereoacuity ranging from 240 to 480 arc sec, 19 patients (43.2%) between 60 and 120 arc sec and 3 patients (6.8%) within the range of 15 to 30 arc sec. At four weeks postoperatively, none of the patients exhibited lack of stereopsis, 15 (34.1%) had 240-480″, 18 (40.9%) had 60-120″ and 11 (25%) had 15-30″ (Table 1).

When examining the alteration in stereoacuity relative to baseline, it was seen that after a duration of four weeks, only 3 individuals accounting for 4.5% of the 8 sample, had a deterioration in SA, conversely, 16 representing (36.4%) of the samples shown an improvement in SA (Table 2).

There was statistically significant alteration in mean SA before operation and 4 weeks postoperatively with better SA of 165.17″±144.3″ (Table 3). There was no statistically significant difference in mean ages of patients and the change in SA at 4 weeks, while patients with higher CCT was at higher risk for having their stereoacuity deteriorated (Table 4).

There were no statistically significant differences between cases with SE <-3.0 dpt or ≥-3.0 dpt in regards to SA at any examination time, in addition, there was no statistically significant differences of preoperative SA in comparison to 4 weeks postoperatively (Table 5).

Cases with preoperative anisometropia of atleast 2 dpt was 6 patients, 5 of them had significantly improved SA at 4weeks postoperatively in comparison to both cases with no preoperative anisometropia, and also in comparison to preoperative SA (Table 6). There was no statistically significant difference in SA change and sexes (Table 7).

Table No. 1: Stereoacuity of the study group before and after photorefractive keratectomy

Stereoacuity	Preoperative		4 weeks postop	
	No.	%	No.	%
Nostereopsis	-	-	-	-
240-480″	22	50.0	15	34.1
60-120″	19	43.2	18	40.9
15-30″	3	6.8	11	25.0
Total	44	100.0	44	100.0

Table No. 2: Changes in stereoacuity postoperatively in comparison to baseline

Stereoacuity	4 weeks postoperative	
	No.	%
Deteriorated	3	6.8
Unchanged	25	56.8
Improved	16	36.4

Table No. 3: Pairwise comparison between preoperative SA with four4 weeks postoperative

Time of examination	Stereoacuity in arc sec	P value
Preoperative	220.40±142.2″	0.013
4 weeks postop	165.17±144.3″	

Table No. 4: Distribution of age, CCT and SE according to change of stereoacuity at 4 weeks post PRK

4-weeks postoperative stereoacuity change	Age		CCT		SE	
	Mean	SD	Mean	SD	Mean	SD
Deteriorated	28.00	8.0	568.33	34.7	-2.54	0.94
Unchanged	26.44	5.03	528.98	35.80	-3.09	1.15
Improved	24.88	5.73	525.16	20.18	-2.73	1.81
P value	0.543		0.009		0.434	

Table No. 5: Comparison of stereoacuity according to spherical equivalent and time of examination

Time of examination	Stereoacuity in arc sec				P value*
	SE < -3.0 dpt		SE ≥ -3.0 dpt		
	Mean	SD	Mean	SD	
Preoperative	237.98	146.67	195.00	135.44	0.461
4 weeks postoperative	178.27	148.83	146.25	139.40	0.507
P-value compared to preoperative**	0.051		0.105		

*Mann Whitney U test for two independent samples,**Related-Samples Wilcoxon Signed Rank Test

Table 6: Comparison of stereoacuity according to anisometropia ≥2.0 dpt and time of examination

Time of examination	Stereoacuity in arc sec				P value*
	No anisometropia		Anisometropia		
	Mean	SD	Mean	SD	
Preoperative	212.57	142.65	270.00	139.43	0.461
4 weeks postoperative	182.37	145.57	56.25	36.97	0.507
P-value compared to preoperative**	0.110		0.041		

*Mann Whitney U test for two independent samples,**Related-Samples Wilcoxon Signed Rank Test

Table No. 7: Distribution of sexes according to change of stereoacuity at 1 and 4 weeks postoperative PRK

Time of examination	Sex	Deteriorated	Unchanged	Improved	P value
4 weeks postoperative steroacuity change	Male	-	10 (52.6%)	8 (42.1%)	0.891
	Female	2 (%)	15 (60%)	5 (20%)	

DISCUSSION

Despite exhibiting a visual acuity of 6/6 with a high contrast and the absence of any remaining refractive errors, a number of patients have expressed dissatisfaction with the quality of their vision after refractive procedures. Stereopsis is a crucial element of binocular vision. Stereoacuity is a fundamental prerequisite to certain occupations, like as ophthalmic surgery.[23] The presence of impaired stereopsis has been shown to be correlated with worse microsurgical ability, suggesting potential implications for the field of surgical training.[24] A recently was explored the relevance and significance of stereoscopic vision in the visualization of anatomy among medical students.[25]

This was seen in the present study where 36.36% cases improved, 6.81% cases worsened and 56.81% cases remained stable in stereoacutiy over 4 weeks after photorefractive keratectomy. The mean stereoacuity rose from 220.4±142.8 to 165.17±144.27arc seconds. According to Maden et al[26], Jabbarvand et al[27], Gyldenkerne et al[28] and Marvivic et al[29], stereoacuity or binocular vision improved after surgery in each of these studies.

Anisometropia can cause an imbalance in the vision of both eyes. The present study showed that patients with this condition had a remarkable improvement in stereoacuity after surgery. In fact, mean value improved from 270±139.43 to 56.25±36.97 arc seconds as five out of six patients became better stereoscopic vision. The findings indicate agreement with other studies.[29,30] Liu et al[31] also found stereopsis to improve primarily in patients with aniso-metropia greater than or equal to 2.5 D, probably to inhibit foveal suppression and improvement in retinal image quality.[32] After the treatment of anisometropia monocular blur and a binocular imbalance, the stereopsis improved. Myopic anisometropia shows worse stereoacuity according to Nabie et al.[33] In the current study, patients with preoperative myopia of ≥3 diopters did not demonstrate a significant impact on stereoacuity, similar to Singh et al.[34] However, Jabbarvand et al[27] reported a greater improvement in stereoacuity in patients with higher degrees of myopia.

In comparison, Godt et al[35] and Zarei-Ghanavati et al[36] found a decrease in stereoacuity after the surgery, meaning not everyone achieved a normal stereoacuity. The causative factors may be decentred ablation, anisometropia, alteration of the location of the nodal point, prismatic changes.[31]

In highly myopic patients, binocular visual function was evaluated after SMILE surgery by Gyldenkerne et al[28] Earlier research shows that enhanced contrast sensitivity following wave front-guided refractive surgery improves stereoacuity, while dry eye disease post operation may temporarily reduce visual performance.

CONCLUSION

An overall improvement in stereoscopic vision 4-weeks following photorefractive keratotomy in myopic patients, particularly persons with anisometropia.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mohammed Qasim Alnuwaini, Alyaa Abood Kareem
Drafting or Revising Critically:	Mohammed Qasim Alnuwaini, Alyaa Abood Kareem
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Role of Oxidative Stress Biomarkers and Antioxidant Enzyme Activity in the Pathophysiology of Maternal Anemia During Pregnancy

Manal Nasih Ahmed Hamdan

ABSTRACT

Objective: To compare oxidative stress markers and antioxidant levels in pregnant women with and without anemia, and to look at how these markers relate to hemoglobin concentrations.

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Antenatal Clinics of Kerbala Obstetrics & Gynecology Teaching Hospital, Iraq from 1st June 2025 to 30th November 2025.

Methods: Forty-nine pregnant women with anemia (hemoglobin under 11 g/dL) were enrolled alongside 50 non-anemic pregnant women who served as controls. Serum levels of malondialdehyde, superoxide dismutase, catalase, and reduced glutathione were determined using standard spectrophotometric techniques.

Results: Antioxidant capacity was lower across the board in anemic women compared with controls: superoxide dismutase (0.051±0.055 vs 0.073±0.047; p=0.029), catalase (2.62±1.93 vs 8.48±4.43; p<0.001) and glutathione (49.05±17.5 7 vs 187.10±56.07; p<0.001). Malondialdehyde, on the other hand, was markedly elevated in the anemic group (7.16±5.01 vs 2.91±2.66 µmol/mL; p<0.001). Hemoglobin showed positive correlations with glutathione (r = 0.614), CAT (r = 0.521), and superoxide dismutase (r = 0.386), and a negative correlation with malondialdehyde (r = −0.547), all reaching p<0.001. In the multiple linear regression model, glutathione (β = 0.391) and malondialdehyde (β = −0.315) emerged as the strongest predictors of hemoglobin, and together the model accounted for 48% of the variance ($R^2 = 0.48$; $p < 0.001$).

Conclusion: Pregnant women with anemia showed both heightened lipid peroxidation and weakened antioxidant defenses. The strong links of glutathione and malondialdehyde with hemoglobin point to a role for oxidative stress in maternal anemia and suggest that improving antioxidant status may be worth exploring as a therapeutic avenue.

Key Words: Maternal anemia, Pregnancy, Oxidative stress, Malondialdehyde, Glutathione, Antioxidant enzymees

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INTRODUCTION

Among the complications that arise during pregnancy, anemia stands out as one of the most widespread. A woman's body undergoes substantial shifts in metabolism, hormonal output, and blood physiology as it works to sustain the developing fetus, and these adaptations place real strain on the hematological system.

Assistant Professor of Obstetrics & Gynecology, College of Medicine, Kerbala University, Kerbala, Iraq.

Correspondence: Manal Nasih Ahmed Hamdan, Assistant Professor, Department of Obstetrics & Gynecology, College of Medicine, Kerbala University, Kerbala, Iraq.
Contact No: +964 773 440 1800
Email: manal.naseh@uokerbala.edu.iq

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According to the World Health Organization, a hemoglobin concentration below 11 g/dL constitutes anemia in pregnancy.[1] Roughly four in every ten pregnant women worldwide fall below this threshold, with prevalence climbing much higher in low- and middle-income countries.[2] The clinical fallout is significant: anemia during pregnancy has been associated with preterm delivery, low birth weight, postpartum hemorrhage, and elevated maternal and perinatal mortality.[3]

While shortages of iron, folate and vitamin B12 continue to be recognized as the leading drivers of anemia in pregnancy[4], particularly the severity of certain cases and the inconsistent response to treatment and looked beyond nutritional factors. One mechanism drawing growing interest is oxidative stress, which arises when reactive oxygen species (ROS) outpace the antioxidant systems meant to keep them in check.[5] Pregnancy itself tilts the balance toward a pro-oxidant state. Both the placenta, with its high metabolic activity, and the elevated iron load that comes with gestation, encourage ROS production.[5] As pregnancy

advances normally, several oxidative stress markers have been observed to rise.[7] The trouble starts when antioxidant defenses can no longer match this increase leaving an excess of ROS free to inflict damage on maternal tissues.

Superoxide, hydrogen peroxide and the hydroxyl radical are generated continuously during normal metabolism and are ordinarily neutralized by enzymatic and non-enzymatic antioxidants.[5] Once production exceeds this capacity, ROS attack lipids, proteins, and DNA.[8] Erythrocytes are especially vulnerable. They carry a high oxygen load and abundant heme iron, their membranes are rich in polyunsaturated lipids, and, lacking a nucleus, they cannot replace oxidized proteins.[9] Sustained oxidative injury therefore shortens red-cell survival and can trigger hemolysis and eryptosis, the regulated death of erythrocytes, which provides a direct route from a pro-oxidant state to anemia.[10]

Stable biomarkers offer a practical way to gauge the extent of oxidative damage. The most commonly used of these is malondialdehyde e (MDA), an end product generated during lipid peroxidation.[8] Damage to circulating proteins can be tracked through advanced oxidation protein products (AOPP)[11], while 8- hydroxy-2′-deoxyguanosine (8-OHdG) serves as an indicator of oxidative injury to DNA.[12] Elevated readings of these markers have been documented in conditions such as preeclampsia, gestational diabetes, and fetal growth restriction.[13] How they behave in the context of maternal anemia, however, has not been examined as closely, and the small body of work that does exist tends to be constrained by limited sample sizes and methodological shortcomings.

Counterbalancing ROS is the job of the antioxidant network. Superoxide dismutase (SOD) takes superoxide converts it into hydrogen peroxide, which catalase then breaks down further into water and oxygen. Glutathione peroxidase, originally described in erythrocytes, clears hydrogen peroxide as well as lipid hydroperoxides, drawing on reduced glutathione (GSH) as its substrate.[14] Measuring the activity of these enzymes gives a sense of how effectively the maternal system is holding oxidative stress at bay. Reduced levels of SOD, catalase, and glutathione have been documented in iron-deficiency anemia in non-pregnant individuals[15], yet evidence connecting these defenses to the severity of anemia during pregnancy is still in short supply.

METHODS

The analytical case- control study was done at the antenatal care clinics of Obstetric & Gynecology Teaching Hospital in Kerbala, Iraq from 1st June 2025 to 30th November 2025 vide letter No. 293 dated May 8, 2025. These clinics serve as a referral and follow-up centre for pregnant women across Kerbala and its surrounding districts, which gave access to a broad patient population. The study was carried out in accordance with the principles set out in the Declaration of Helsinki[16] and its reporting follows the STROBE recommendations for observational research.[17]

Ninety-nine pregnant women were enrolled and classified into two groups. The case group comprised 49 women with anemia, defined as a hemoglobin concentration below 11 g/dL according to WHO criteria.[1] The control group comprised 50 non-anemic women with hemoglobin of 11 g/dL or above, matched to cases for age (within 2 years) and gestational age (within 2 weeks). Participants were enrolled consecutively, with eligible women approached in order of attendance until the target for each group was reached.

The women who confirmed singleton pregnancy with gestational age established by last period of menstration orultrasound in first-trimester, were aged 18 to 40 years, attended the clinic for routine follow-up, and gave written informed consent were included. Women were excluded for multiple gestation; pre-existing diabetes mellitus, chronic hypertension, renal, hepatic, or autoimmune disease; hemoglobinopathies such as sickle cell disease or thalassemia; use of antioxidant supplements (vitamin C, vitamin E, or N-acetylcysteine) within the preceding four weeks; active infection or inflammation; preeclampsia or other hypertensive disorders of pregnancy; blood transfusion within the preceding three months; or inability to give consent.

After enrolment, a structured interview recorded sociodemographic and obstetric data, including age, education, parity, gravidity, gestational age, inter-pregnancy interval, and dietary habits. Each participant's weight and height were recorded and body mass index was derived by dividing weight (in kilograms) by the square of height (in metres). Clinical examination note signs of anemia, including conjunctival, nail-bed and palmar pallor, and obstetric history were taken from the antenatal records. Blood pressure, heart rate and gestational ages were recorded at enrolment. A 10 mL venous blood sample was obtained from the antecubital vein under aseptic conditions between 08:00 a nd 10:00 following an overnight fast of no less than eight hours to minimise the effects of diurnal variation. Of the total volume drawn, 3 mL was transferred to EDTA tubes for the complete blood count while the remaining 7 mL went into plain gel- separator tubes. After allowing the latter to clot for 30 minutes, they were spun at 3,000 rpm for 10 minutes. The serum was then separated, divided into aliquots and kept at −80°C until analysis. Every sample was processed within two hours of collection and freeze-thaw cycles were kept to a minimum.

The complete blood count was run on an automated analyzer (Sysmex XN-550 or its equivalent) and covered hemoglobin, hematocrit, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count and white blood cell count.

To classify the type of anemia present, serum ferritin, iron, total iron-binding capacity and folate were also measured.

Serum MDA, used here as an indicator of lipid peroxidation, was quantified using the thiobarbituricacid reactive substances (TBARS) method. Serum samples were mixed with thiobarbituric acid and trichloroacetic acid, heated at 95°C for 60 minutes, and the resulting chromogen measured at 532 nm . Concentrations were determined from a standard curve prepared with 1, 3, 3-tetramethoxypropane and reported as µmol/mL.

Antioxidant assays: Superoxide dismutase activity was estimated by measuring the degree to which the enzyme inhibited the reduction ofnitroblue tetrazolium by superoxide produced through the xanthine-xanthine oxidase system, with absorbance recorded at 505 nm. One unit was taken as the amount of enzyme needed to bring about 50% inhibition and the activity was normalised to sample protein, which was determined by the Lowry method. Catalase activity was assessed by tracking the decomposition of hydrogen peroxide at 240 nm in a phosphate buffer (pH=7.0) containing 30 mmol/L hydrogen peroxide and was likewise expressed in relation to sample protein. Reduced glutathione was measured following the procedure, which relies on the reaction between thiol groups and 5,5′-dithiobis (2-nitrobenzoic acid) at 412 nm, with results given in µmol/mL.

Quality control: All assays were carried out in duplicate and the mean of the two readings was used for analysis. Intra-assay and inter-assay coefficients of variation were maintained below 5% and 8%, respectively. Each batch included both positive and negative controls and laboratory staff remained blinded to the group assignment of every sample.

Data were analysed using SPSS-26. Distribution was checked with the Shapiro–Wilk test. Comparisons between groups were made using the independent-samples t- test. Pearson correlation was applied to look at the associations between biomarkers and hematological parameters and multiple linear regression was used to identify which biomarkers were independently linked to hemoglobin, with adjustments made for age, body mass index, gestational age and parity. A two-sided p value of less than 0.05 was treated as statistically significant.

RESULTS

Ninety nine had anemia and 50 were non-anemic controls. Antioxidant defenses were lower in the anemia group across all measures. SOD, catalase, and reduced glutathione were each significantly reduced relative to controls, while MDA was more than twice as high, indicating greater lipid peroxidation in anemic women (Table 1).

Hemoglobin was low in the anemia group compared to control (109.22 vs 115.92 g/L; p=0.005), in keeping with the case definition. The red blood cell count did not significantly differ between groups, whereas the count of white blood cell was higher among anemic women (Table 2).

Table No 1: Oxidative stress biomarker and antioxidant levels in the anemia and control groups

Variable	Maternal anemia (n = 49)	Control (n = 50)	t value	P value
SOD (µmol/mL/min/mg protein)	0.051±0.055	0.073±0.047	2.211	0.029
CAT (µmol/mL/min/mg protein)	2.623±1.933	8.483±4.427	8.503	< 0.001
MDA (µmol/mL)	7.158±5.005	2.913±2.662	−5.284	< 0.001
GSH (µmol/mL)	49.05±17.57	187.10±56.07	16.459	< 0.001

Table 2: Hematological parameters in the anemia and control groups

Variable	Maternal anemia (n = 49)	Control (n = 50)	t value	P value
RBC ($\times 10^{12}$/L)	3.87±0.44	3.73±0.45	−1.47	0.144
HGB (g/L)	109.22±11.85	115.92±11.33	2.87	0.005
WBC ($\times 10^{9}$/L)	7.35±5.54	5.09±2.07	−2.69	0.008

Table No 3: Correlations among oxidative stress biomarkers and antioxidants

Variable	Pearson correlation (r)	P value
GSH – MDA	−0.277	0.054
SOD – MDA	−0.527	< 0.001
CAT – SOD	0.270	0.061
CAT – MDA	−0.469	0.001

Table No. 4: Hemoglobin level Correlation and oxidative stress biomarkers

Variable	Pearson correlation(r)	P value
SOD (µmol/mL/min/mg protein)	0.386	< 0.001
CAT (µmol/mL/min/mg protein)	0.521	< 0.001
GSH (µmol/mL)	0.614	< 0.001
MDA (µmol/mL)	−0.547	< 0.001

Table No. 5: Multiple linear regression for factors associated with hemoglobin level

Predictors	β coefficients	SE	t test value	P value
SOD	0.182	0.073	2.49	0.015
CAT	0.267	0.088	3.03	0.003
GSH	0.391	0.095	4.12	< 0.001
MDA	−0.315	0.081	−3.89	< 0.001
Constant	8.742	1.654	5.29	< 0.001

Among the oxidative markers, MDA correlated inversely with SOD ($r = -0.527$, $p<0.001$) and with catalase ($r = -0.469$, $p = 0.001$). The SOD–catalase and GSH–MDA correlations were in the expected direction but did not reach significance (Table 3).

Hemoglobin positively correlated with glutathione ($r = 0.614$), catalase ($r = 0.521$), and SOD ($r = 0.386$) and negative with MDA ($r = -0.547$); all correlations were significant at $p<0.001$ (Table 4).

In the multiple linear regression model, glutathione and MDA were the strongest independent predictors of hemoglobin, with glutathione the leading positive predictor and MDA the leading negative predictor. The explained 48% by the model of the hemoglobin variance ($R^2 = 0.48$; adjusted $R^2 = 0.46$; $F = 22.11$; $p<0.001$) (Table 5).

DISCUSSION

In the present study, serum MDA, a measure of lipid peroxidation, was more than twice as high in anemic women as in controls. The antioxidants SOD, catalase and reduced glutathione were all lower in the anemia group and across the sample hemoglobin fell as MDA rose and increased with each antioxidantwith glutathione and MDA emerging as its strongest independent predictors. The pattern is consistent with real oxidative component to maternal anemia rather than incidental association.[18]

The higher MDA in anemic women fits a broad literature that links iron deficiency and anemia to lipid peroxidation. Sundaram et al[19] found raised plasma MDA in iron-deficiency anemia that fell after treatment and Tiwari et al[18] reported increased lipid peroxides alongside depleted antioxidants in pregnant anemic women. Yoo et al[20] found higher oxidant activity and lower total antioxidant capacity in women with iron-deficiency anemia, with values returning to normal after iron therapy and Sharif Usman et al[21] reported raised MDA in iron-deficient and anemic schoolchildren in northern Nigeria. The consistency of higher lipid peroxidation across pregnant women, non-pregnant women, and children suggests that it is a general feature of iron-deficient and anemic states rather than finding peculiar to one population. The susceptibility of erythrocytes helps to explain it: a high oxygen load, abundant heme iron, and polyunsaturated membranes favour radical formation, and the mature red cell cannot renew the proteins that oxidation destroys.[9] Peroxidation of membrane lipids lowers deformability and raises permeability, which ends in hemolysis and a shorter cell lifespan.[10] The elevated MDA in our anemic group is therefore likely to mark accelerated red-cell loss that adds to any nutritional deficit.

The parallel fall in SOD, catalase, and glutathione points to antioxidant defenses that have been outstrip ed. SOD produces hydrogen peroxide from superoxide, and catalase and the glutathione system then remove it[5], so depletion at several steps leaves erythrocytes poorly protected. Our SOD and catalase results agree with Tiwari et al[18], who found these enzymes reduced across grades of anemia, Yoo et al[20] reported lower catalase in iron-deficiency anemia, and Isler et al[22], who described reduced erythrocyte SOD and glutathione peroxidase that improved with treatment. The literature is not uniform, however. Khalid et al[15], working in pregnant women, found reduced SOD but a small, non-significant rise in glutathione peroxidase, which they read as a compensatory response under hypoxia. The earlier work of Macdougall[23] showed that glutathione peroxidase and catalase fall in iron-deficient red cells and that this loss raises their susceptibility to oxidative hemolysis. Such differences are not surprising. Studies vary in the tissue examined (serum or erythrocyte), the assay used, the cause and severity of anemia, and whether patients had already started treatment, and any of these can move enzyme activity in either direction. Set against that background, the lower SOD, catalase, and glutathione in our anemic group place this study with the larger body of work that reports weakened antioxidant defenses in anemia.

Reduced glutathione showed the largest difference between groups, close to a fourfold reduction. This fits its role as the main thiol antioxidant of the red cell and its heavy use under oxidative load.[24] Glutathione maintains protein thiols and supplies glutathione peroxidase, so its loss both reflects and worsens oxidative injury, and a fall in glutathione has long been tied to the oxidative hemolysis of iron-deficient cells.[23] Because iron is also a cofactor for catalase and supports glutathione-dependent reactions, iron deficiency may lower these defenses in its own right.[15]

The correlation and regression results add weight to the group comparisons. Hemoglobin rose with antioxidant capacity and fell with lipid peroxidation, and the regression model accounted for almost half of its variance with glutathione and MDA the leading predictors. Anemia brings tissue hypoxia and disturbed iron handling that promote ROS formation while the resulting oxidative damage impairs erythropoiesis and

shortens red-cell survival.[10] Redox balance also has a regulatory role in red-cell production: the transcription factor Foxo3 controls antioxidant gene expression in maturing erythroid cells, and its loss leads to ROS accumulation, oxidative damage, and a shorter erythrocyte lifespan.[25] Oxidative stress can in addition drive eryptosis, giving a further path from a pro-oxidant state to anemia.[10]

The findings have practical implications. If oxidative stress aggravates anemia, antioxidant status could serve as a marker of severity and potentially as a target for treatment. several studies report that iron therapy corrects hematological values and at the same time restores antioxidant defenses and lowers lipid peroxidation[15,20], which suggests that part of its benefit comes from easing oxidative stress. the picture is not one-sided. iron is itself redox-active, and Tiwari et al[26] found that oral iron supplementation raised markers of oxidative imbalance in anemic women, at least in the short term. This tension means that the dose and form of iron, and perhaps the addition of antioxidants, deserve careful study rather than assumption. Whether antioxidant supplementation improves outcomes in anemic pregnancy is not yet established and would need testing in controlled trials.[14]

This study assessed lipid peroxidation and thiol antioxidants but not protein or DNA oxidation. AOPP and 8-OHdG, which capture those forms of damage are raised in other complications of pregnancy[13], and adding them would give fuller view of the oxidative profile of maternal anemia. Their inclusion in future works help to define which forms of oxidative damage matter most in the disorder.

CONCLUSION

Maternal anemia was accompanied by greater lipid peroxidation and lower antioxidant defenses, with higher MDA and reduced SOD, catalase and glutathione in anemic women. Hemoglobin was closely related to these markers, and glutathione and MDA were its strongest independent predictors and the role for oxidative stress in the biology of maternal anemia and point to antioxidant status as a measure worth following and a possible target for treatment.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Manal Nasih Ahmed Hamdan
Drafting or Revising Critically:	Manal Nasih Ahmed Hamdan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Role of Histology in Lymphoma Classification

Histology in Lymphoma Classification

Hussain Mohammad Tahir Noorwali

ABSTRACT

Objective: To determine the usefulness of histopathology and immunohistochemistry in the precise identification of lymphomas in a retrospective cohort.

Study Design: Retrospective observational study

Place and Duration of Study: This study was conducted at the Department of Histopathology at King Fahad Specialist Hospital in Dammam, Saudi Arabia from 15.03.2024 to 30.05.2024.

Methods: One hundred and fifty lymphoma cases 2020-2023 were analyzed using histomorphology and immunohistochemistry per World Health Organization classification.

Results: There were 52 (35%) Hodgkin lymphoma and 98 (65%) non-Hodgkin lymphoma. The most common type of non-Hodgkin lymphoma was diffuse large B-cell lymphoma (40%), and follicular lymphoma (18%). In 87%, immunohistochemistry was required to make a definitive classification.

Conclusion: The use of histology and immunohistochemistry cannot be replaced in the correct classification of lymphoma and plays a crucial role in the choice of therapy and prognosis.

Key Words: Lymphoma, Histopathology, Immunohistochemistry, Word Health Organization classification

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INTRODUCTION

Lymphomas are a heterogeneous group of malignant neoplasms arising from B lymphocytes, T lymphocytes or natural killer cells at different stages of maturation. They include biologically distinct diseases that vary considerably in morphology, clinical presentation, molecular abnormalities, response to treatment and prognosis. Contemporary classification therefore does not depend on a single diagnostic feature. The fifth edition of the World Health Organization Classification of Haematolymphoid Tumours and the International Consensus Classification define lymphoma entities through the integrated assessment of tissue architecture, cytological features, immunophenotype, genetic alterations and clinical findings.[1] Despite advances in molecular pathology, conventional histological examination remains the essential first step because it demonstrates the growth pattern, cellular composition, degree of atypia, stromal reaction and relationship between neoplastic cells and their microenvironment.

Ph.D Scholar, Department of Basic Medical Sciences, Pathology Division, College of Medicine, University of Jeddah, Jeddah 23218, Saudi Arabia.

Correspondence: Hussain Mohammad Tahir Noorwali, Ph.D Scholar, Department of Basic Medical Sciences, Pathology Division, College of Medicine, University of Jeddah, Jeddah 23218, Saudi Arabia.
Contact No: 00970599239760
Email: hmnoorwali@uj.edu.sa

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Lymphoma represents an important component of the global cancer burden, although its incidence and subtype distribution vary according to age, sex, ethnicity, geographical region, immune status and exposure to infectious agents.[2] Non-Hodgkin lymphoma is generally more frequent than Hodgkin lymphoma, and diffuse large B-cell lymphoma is the most common aggressive subtype in many populations.[3] Saudi cancer-registry data have also identified non-Hodgkin lymphoma as one of the leading malignancies among men, with variations in cancer incidence across different regions of the Kingdom.[4] These epidemiological differences emphasize the importance of institution- and region-specific pathological data because the distribution observed in Western populations may not fully represent patients treated in Saudi Arabian centres.

Adequately processed tissue is fundamental to lymphoma diagnosis. Examination of haematoxylin-and-eosin-stained sections allows the pathologist to assess whether the lymph-node architecture is preserved, follicular, nodular, diffuse or partially effaced. It also helps determine the size and appearance of neoplastic cells and identify diagnostic backgrounds such as granulomas, necrosis, eosinophilia, vascular proliferation or mixed inflammatory infiltrates. Nevertheless, reactive lymphoid hyperplasia, metastatic malignancy and different lymphoma subtypes may show overlapping morphological appearances. Diagnostic-review studies have demonstrated that disagreement frequently involves differentiation between benign and malignant proliferations, determination of lymphoid lineage and assignment of the correct lymphoma subtype.[5]

Immunohistochemistry complements morphology demonstrating lineage, cellular differentiation and abnormal antigen-expression patterns. Pan-leukocyte and lineage-associated markers, including CD45, CD20, PAX5, CD79a and CD3, help distinguish lymphoid neoplasms from non-haematolymphoid malignancies and separate B-cell from T-cell proliferations. CD30 and CD15 support the diagnosis of classical Hodgkin lymphoma when interpreted with characteristic Reed–Sternberg-cell morphology, whereas PAX5 usually shows weak nuclear expression in the neoplastic cells.[6] In follicular lymphoma, the combination of a neoplastic follicular architecture with CD10, BCL6 and abnormal BCL2 expression supports germinal-centre origin and helps distinguish the disease from reactive follicular hyperplasia.[7] In diffuse large B-cell lymphoma, immunohistochemical algorithms employing CD10, BCL6 and MUM1 can provide an accessible approximation of cell-of-origin classification, particularly where gene-expression profiling is unavailable.[8] MYC and BCL2 immunostaining may additionally identify double-expressor disease associated with adverse clinical outcomes, although protein expression should not be considered equivalent to MYC and BCL2 gene rearrangements.[9]

The diagnostic value of immunohistochemistry depends on appropriate antibody selection, technical quality and interpretation within the histological and clinical context. Excessive use of broad panels may increase cost and produce conflicting or nonspecific findings, whereas an insufficient panel can result in incomplete or incorrect classification. A stepwise approach based on the initial morphological differential diagnosis is therefore preferred. Molecular techniques, including fluorescence in situ hybridization, clonality analysis, cytogenetics and next-generation sequencing, are increasingly important for resolving difficult cases and identifying genetically defined entities.[1]

The purposes of this study are to determine the frequency distribution of other subtypes of lymphoma, to determine the diagnostic accuracy of first histological diagnosis, to identify the value of immunohistochemistry in classifying lymphoma and to match histological subtypes with demographic parameters.

METHODS

This retrospective observational study was conducted at the Department of Histopathology at King Fahad Specialist Hospital in Dammam, a major tertiary care and oncology referral center in the Eastern Province of Saudi Arabia vide letter No. 3224 dated February 24, 2024. One hundred and fifty lymphoma cases 2020-2023 were analyzed using histomorphology and immunohistochemistry per World Health Organization classification. Those cases were diagnosed with lymphoma, sufficient tissue to be evaluated histologically, had full work up on immunohistochemistry and full clinical and demographic information were included. The cases where there is a lack of adequate tissue to do the diagnosis, did not have immunohistochemical study, incomplete clinical data cases and cases diagnosed during out of study were excluded.

The following medical records and histopathology reports were analyzed to extract the information, histomorphology characteristics, immunohistochemical panel findings and final diagnosis in WHO classification.

Histopathological assessment of all tissue samples had been fixed in 10% of neutral buffered formalin, routinely processed and embedded in paraffin. Slices of 4-5 μm thickness were cut and stained with Hematoxylin and Eosin (H&E). The histological examination involved examination of Xie et al.[10]

Immunohistochemistry was done by the method of avidin-biotin-peroxidase complex. The list of the antibody panel was chosen according to the primary morphological examination and comprised.[11] A standard antibody panel including B-cell, T-cell, Hodgkin, and proliferation markers was used. Data were evaluated with the help of SPSS-25. Categorical variables were in Chi-square test. A p-value of below 0.05 was taken as statistically significant.

RESULTS

The mean age of the patients was 42.5±16.8 years. The largest age group was 20-40 years, comprising 57 patients (38.0%), followed by those aged 41-60 years, comprising 52 patients (34.7%). Eighteen patients (12.0%) were younger than 20 years, whereas 23 (15.3%) were older than 60 years. The study population included 94 males (62.7%) and 56 females (37.3%), giving a male-to-female ratio of approximately 1.7:1 (Table 1).

There were 98 (65.3%) classified as non-Hodgkin lymphoma, while 52 (34.7%) were classified as Hodgkin lymphoma. Among the non-Hodgkin lymphoma cases, diffuse large B-cell lymphoma was the most frequently reported subtype, accounting for 40.0%, followed by follicular lymphoma, which accounted for 18.0%. The remaining 42.0% comprised other non-Hodgkin lymphoma subtypes (Table 2). Immunohistochemistry was used to confirm B-cell or T-cell lineage in all cases. Among non-Hodgkin lymphomas, immunohistochemistry was required for definitive subtype assignment in 87.0%, whereas histomorphological findings alone were considered sufficient in 13.0% of cases. Immunohistochemistry also contributed to distinguishing reactive from neoplastic lymphoid proliferations in 15.0% of the overall cases and assisted in identifying specific lymphoma entities

Histological examination of classical Hodgkin lymphoma showed scattered large atypical cells within a mixed inflammatory background. The atypical cells demonstrated the characteristic morphology of Reed-Sternberg cells, including abundant cytoplasm, bilobed or multilobated nuclei and prominent eosinophilic nucleoli (Fig. 1).

Table No. 1: Demographic characteristics of patients diagnosed with lymphoma (N = 150)

Variable	No.	%
Age (years)		
<20	18	12.0
20–40	57	38.0
41–60	52	34.7
>60	23	15.3
Gender		
Male	94	62.7
Female	56	37.3

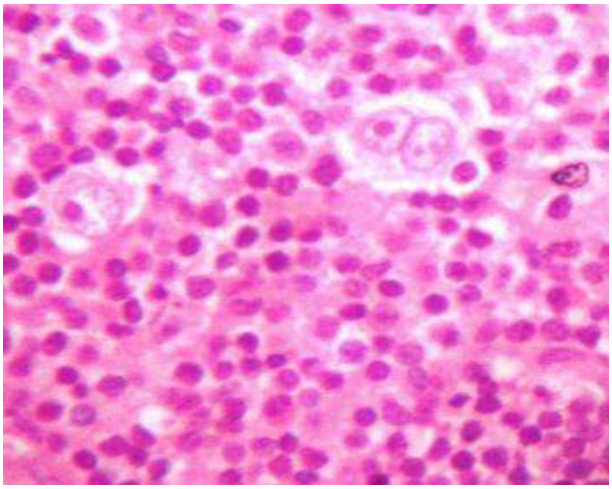

Figure No. 1: High-magnification H&E staining showing the Reed-Sternberg (RS) cells in a mixed inflammatory background. RS cells are characterized by large size, bilobed or multilobated nuclei, prominent eosinophilic nucleoli, and abundant cytoplasm

Immunohistochemical staining demonstrated strong CD30 expression in Reed–Sternberg cells and their variants. The staining showed membranous positivity with accentuation in the paranuclear Golgi region, supporting the diagnosis of classical Hodgkin lymphoma (Fig. 2). The combined morphological and immunohistochemical findings illustrated in Figures 1 and 2 demonstrate the complementary roles of routine histology and CD30 immunostaining in confirming classical Hodgkin lymphoma.

Diffuse large B-cell lymphoma showed diffuse effacement of the normal lymph-node architecture by sheets of large atypical lymphoid cells. These cells had vesicular nuclei, conspicuous nucleoli and a moderate amount of cytoplasm (Fig. 3).

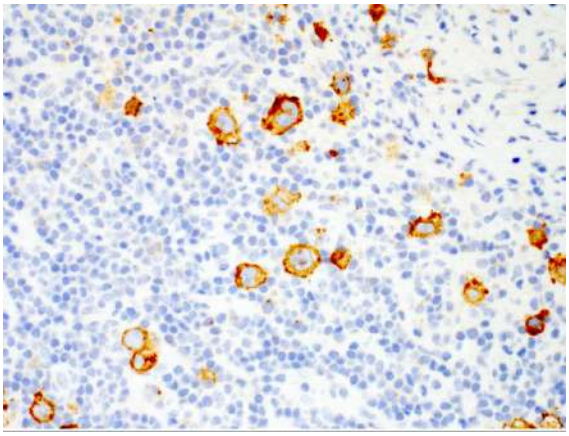

Figure No. 2: CD30 immunohistochemistry showing strong membranous and Golgi-zone positivity in Reed-Sternberg cells and variants, confirming Hodgkin lymphoma

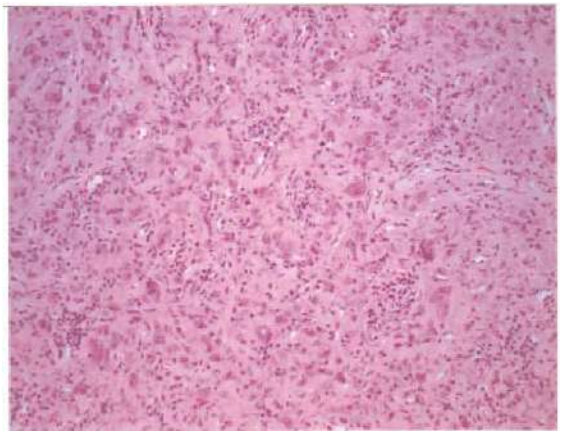

Figure No. 3: H&E staining (40x) demonstrating diffuse proliferation of large lymphoid cells with vesicular nuclei, prominent nucleoli, and moderate cytoplasm, effacing normal nodal architecture

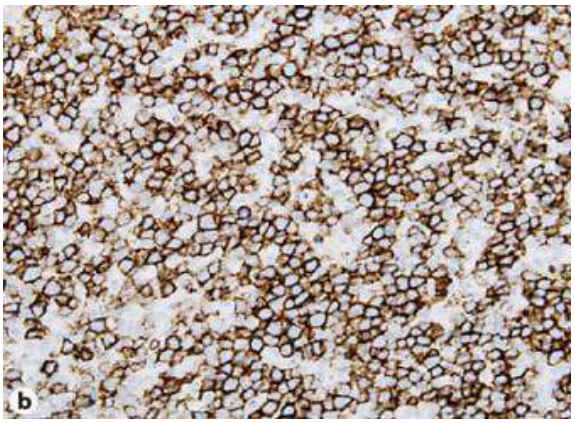

Figure No. 4: Immunohistochemistry (IHC) stain for CD20. The image shows a diffuse infiltrate of large lymphoid cells exhibiting strong, dark-brown membranous positivity for the pan-B-cell marker CD20. This staining pattern is characteristic of a B-cell lineage malignancy, confirming the diagnosis of Diffuse Large B-cell Lymphoma (DLBCL). The background cells are negative, providing an internal control

Table No. 2: Distribution of lymphoma categories and diagnostic contribution of immunohistochemistry

Diagnostic characteristic	No.	%	Denominator
Principal lymphoma classification			
Hodgkin lymphoma	52	34.7	All cases (n=150)
Non-Hodgkin lymphoma	98	65.3	All cases (n=150)
Leading non-Hodgkin lymphoma subtypes			
Diffuse large B-cell lymphoma	Not reported*	40.0	NHL cases (n=98)
Follicular lymphoma	Not reported*	18.0	NHL cases (n=98)
Other NHL subtypes	Not reported*	42.0†	NHL cases (n=98)
Contribution of immunohistochemistry			
Confirmation of lymphoid lineage	150	100.0	All cases (n=150)
Definitive subclassification of NHL	Not reported*	87.0	NHL cases (n=98)
Histomorphology alone sufficient	Not reported*	13.0	NHL cases (n=98)
Differentiation of reactive and neoplastic processes	Not reported*	15.0	All cases (n=150)
Identification of specific lymphoma entities	Not reported*	12.0	All cases (n=150)

*Calculated as the remaining proportion after diffuse large B-cell lymphoma and follicular lymphoma

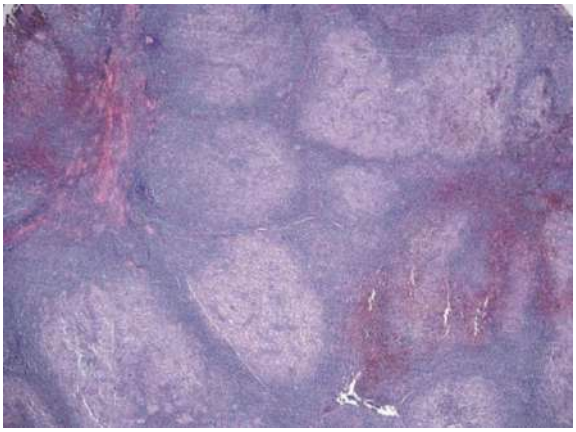

Figure No. 5: H&E staining (10x) showing a nodular growth pattern with closely packed neoplastic follicles lacking normal polarization and tingible body macrophages

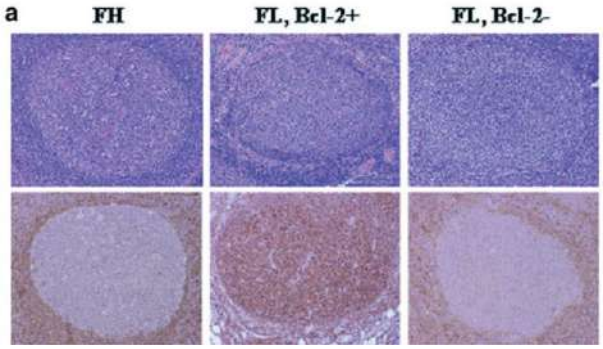

Figure No. 6: BCL2 immunohistochemistry demonstrating strong cytoplasmic positivity in neoplastic follicular centers (abnormal), while reactive follicles would be negative

Immunohistochemical examination showed strong and diffuse membranous CD20 expression in the neoplastic cells, confirming their B-cell lineage. Non-neoplastic background cells showed no comparable staining and served as an internal control (Fig. 4). The morphological features in Figure 3 established the presence of an aggressive large-cell lymphoma, while the CD20 staining shown in Figure 4 confirmed B-cell lineage and supported classification as diffuse large B-cell lymphoma.

Follicular lymphoma demonstrated a predominantly nodular growth pattern composed of closely packed neoplastic follicles. The follicles lacked normal polarization and contained few or no tingible-body macrophages, differentiating them from reactive lymphoid follicles (Fig. 5). BCL2 immunohistochemistry showed cytoplasmic positivity within the neoplastic follicular centres. This abnormal staining pattern supported follicular lymphoma because reactive germinal centres usually show absent or substantially weaker BCL2 expression (Figure 6). Figures 5 and 6 demonstrate that the architectural pattern on routine histology and the abnormal expression of BCL2 provide complementary evidence for diagnosing follicular lymphoma.

DISCUSSION

In the present study, non-Hodgkin lymphoma (NHL) was 65% of all lymphomas and HL 35%. This distribution is consistent with the epidemiological data about NHL in the world, which demonstrates that compared to HL, NHL is more prevalent. Diffuse large B-cell lymphoma (DLBCL) was the most common subtype in NHL (40% of all NHL), as it has been identified as the most common lymphoid malignancy globally.[12,13]

This study showed that immunohistochemistry was critical in the conclusive diagnosis in 87% of the instances. IHC plays several very important roles, CD20/CD79a of B-cells, CD3 of T-cells and CD15/CD30 of Hodgkin lymphoma. This is the most basic step on the classification of lymphoma. Markers specific to the subtypes of lymphoma are used to

identify lymphoma subtypes.[14,15] The subtype markers are cyclin D1 against mantle cell lymphoma, DLBCL subclassification CD10, BCL6, MUM1, BCL2 for follicular lymphoma and CD10 and Ki-67 in Burkitt lymphoma.

In addition to PTCL-NOS and ALCL, immunohistochemistry is indispensable for diagnosing AITL (CD10, CXCL13), ATLL (CD25), MF (CD4, loss of CD7), NK/T-cell lymphoma (CD56, EBER), and EATL (CD8, cytotoxic markers).[16] Proliferation indices such as Ki-67 are prognosticators associated with prognosis. The Hans algorithm (CD10, BCL6, MUM1) further classifies DLBCL into germinal center and non-germinal center with various outcome.

Immunohistochemistry (IHC) panels are used in the case of ambiguous morphology. Indicatively, in differentiating between reactive lymphoid hyperplasia and low-grade lymphoma, it is frequently necessary to show light chain restriction, or aberrant antigen expression.[17,18]

The diagnosis of histology should never be understood out of context. The clinical appearances can be similar histologically observed in various clinical settings. In this study, we have placed special stress on the necessity of comprehensive clinical data to be used in the process of proper classification. The percentage of the DLBCL (40% of NHL) is equal to the international data (30-40%). Distribution of Hodgkin lymphoma (35% of all lymphomas) is greater than that of the Western population (10-15%), but similar to data of developing countries. The male domination and age structure are in line with published epidemiology.

Recommendations

1. Standardized IHC Panels: Establishment of institutional guidelines on the lymphoma work-up according to morphological differentiation diagnosis.
2. Multidisciplinary approach: periodic clinicopathological meetings to compare the findings.
3. Continuous Education: Periodic information about the changes in WHO classification and new entities.
4. Quality assurance: Application of internal and external quality control.
5. Molecular integration: Incremental use of molecular tests in complicated cases.
6. Documentation: Extensive reporting (morphology, IHC, and combined diagnosis).

CONCLUSION

The combination of the molecular methods and the classical histopathology will allow further fine-tuning of the lymphoma types and allow offering personalized medicine options. Nevertheless, a skilled histopathological analysis in conjunction with prudent immunohistochemistry will continue to be imperative in the predictable future.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hussain Mohammad Tahir Noorwali
Drafting or Revising Critically:	Hussain Mohammad Tahir Noorwali
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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

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Original Article

The Role of Leptin and Insulin Resistance in the Onset of Precocious Puberty among Obese Pediatric Patients

Ghada Abd Arhman Taqa[1], Abeer Mansour Abdel Rasool[2] and Zeyad Khalaf Maded[3]

ABSTRACT

Objective: To determine the relationship between insulin resistance, leptin, and CPP in obese children and to clarify their diagnostic role.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Pediatric Endocrinology Outpatient Clinic Iraq from 15th May 2025 to 14th October 2025.

Methods: 90 children ages 5–10 years were enrolled. They were divided into three groups of healthy (Group A), obese without puberty (Group B), and obese with central precocious puberty (Group C). Each group consisted of 30 children. Pubertal signs, fasting insulin, homeostatic model assessment for insulin resistance, leptin, and hormonal profile were assessed. Central precocious puberty predictors were determined using logistic regression and receiver operating characteristic curves.

Results: The degree of leptin actually escalated: 3.22, 1.8, with the groups (A), 12.4, 4.7 (B), 18.9, 6.3 (C). The same was true of homeostatic model assessment for insulin resistance: (1.5 + 0 7), (4.4 + 1.8), and (5.9±2.3) respectively. luteinizing hormone was very high in group C. Homeostatic model assessment for insulin resistance (r=0.78) and body mass index Z-score (r=0.82) were significantly related to leptin. Leptin 14.2 ng/mL or greater predicted central precocious puberty was definite sensitivity (90 %) and specificity (87%).

Conclusion: The evidence confirms the bi-functional location of leptin in the metabolic/reproductive endocrine system, which solidly attaches this molecule to insulin resistance and the emergence of central precocious puberty. Leptin and homeostatic model assessment for insulin resistance as independent predictors were validated using multivariate analysis.

Key Words: Leptin, Insulin resistance, Precocious puberty, Body mass index

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INTRODUCTION

Childhood obesity is rising worldwide and is closely linked to accelerated pubertal development, especially central precocious puberty (CPP) in girls.[1] In obese children, persistently high leptin can produce a leptin-resistant paradox: normal metabolic responses are blunted, yet premature pubertal signals are still switched on. This has turned the interplay between adiposity, hormonal signaling, and reproductive maturation into an important clinical question.[2] The process is multifactorial but is driven mainly by leptin and insulin resistance.

Leptin stimulates the hypothalamic-pituitary-gonadal (HPG) axis through kisspeptin and nitric oxide pathways, while insulin resistance and hyperinsulinemia disturb both metabolic and reproductive signalling.[3] Because raised leptin in obese children often coexists with leptin resistance, it is still unclear whether leptin and insulin resistance are only markers or true independent predictors of CPP.[4] Epidemiological data report wide shifts in the timing of puberty across populations, giving this problem a paradoxical, multi-faceted impact on both short-term child health and long-term adult outcomes.[5,6] The relationship between obesity and precocious puberty is therefore bidirectional and depends on hormone networks that regulate energy balance, growth, and reproduction. Excess adiposity disturbs many of these pathways, most notably through insulin resistance,

[1.] Professor, Department of Basic Dental Sciences, College of Dentistry, University of Mosul, Iraq.
[2.] Lecturer, Department of Pharmacology & Toxicology, College of Pharmacy, Nineveh University, Mosul, Iraq
[3.] Lecturer, Department of Pharmacy, College of Pharmacy, Al-Kitab University, Kirkuk 36015, Iraq

Correspondence: Ghada Abd Arhman Taqa, Dental Basic Sciences Department, College of Dentistry, University of Mosul, Iraq.
Contact No: 07703930157
Email: ghadataqa@uomosul.edu.iq

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hyperinsulinemia, and the inflammatory state that accompanies obesity and diabetes.[7,8]

Obese children with precocious puberty also carry higher risks of metabolic syndrome, type 2 diabetes, cardiovascular disease, and psychological problems that persist into adult life.[9,10] Leptin seems to be the key metabolic gateway that links obesity to pubertal onset. Its leptin-rich state disrupts normal metabolic control and lets pubertal pathways be triggered early[11], and leptin levels are further influenced by the oxidant–antioxidant balance, which is altered in obesity, insulin resistance, and diabetes.[12,13]

Therefore, this study examined leptin and insulin resistance as major mediators of obesity-related CPP and assessed their diagnostic capacity, aiming to clarify their role in the neuroendocrine control of puberty and to support earlier detection and treatment of vulnerable children.

METHODS

This was a cross-sectional study was conducted at Pediatric Endocrinology Outpatient Clinic Iraq from 15th May 2025 to 14th October 2025 vide letter No. UoM.Dent. 25/1083 dated 11th May 2025. The sample comprised 90 children aged 5-10 years recruited from community settings and clinic attendees. They were divided into three groups and each group comprised 30 children according to body mass index percentiles and pubertal status:

Group A: Healthy-weight control subjects, BMI 5th to 85th percentile, prepubertal.

Group B: Obese without puberty, BMI ≥95th percentile.

Group C: Obese with central precocious puberty (CPP), BMI ≥95th percentile, before age 8 in girls or 9 in boys.

G Power software was used to calculate the minimum required sample size of 30 participants (n=90 in total) according to medium effect size (Cohen d = 0.5), 80% power and alpha = 0.05.

Comprehensive clinical assessment followed standardized protocols conducted by qualified pediatric endocrinologists. Anthropometry used a Harpenden Stadiometer for height and a SECA electronic scale for weight, with BMI calculated.

After a 12-hour fast, 15–20 mL blood samples were processed within 2 hours and serum was stored at -80°C. Serum leptin (ELISA), fasting insulin (CMIA), and glucose (hexokinase) were measured to calculate HOMA-IR (resistance > 2.5). LC-MS/MS tested reproductive hormones (LH, FSH, estradiol, testosterone). Thyroid (TSH, FT4) and growth axis parameters (IGF-1, IGFBP-3) were also analyzed.

The data was analyzed through SPSS-28. Group comparisons used ANOVA or the Kruskal-Wallis test with suitable post-tests, and Pearson or Spearman coefficients assessed correlations among leptin, insulin resistance parameters, and pubertal markers. Independent predictors of precocious puberty were identified by multivariate logistic regression of biologically plausible and univariate associations ($p<0.20$). ROC curve analysis and the Hosmer-Lemeshow goodness-of-fit test evaluated model performance.

RESULTS

The three groups were comparable in age and sex, whereas weight and BMI were significantly higher in both obese groups (B and C) than in controls (Table 1). Children of Group C presented a significant increase in LH, FSH, LH/FSH ratio, estradiol (girls) and testosterone (boys) than the rest of the groups. Group C had significantly higher IGF-1 and IGFBP-3 levels than Groups A and B, indicating increased growth factor activity at puberty onset. Group C had highly elevated serum leptin, fasting insulin, and HOMA-IR, indicating severe metabolic dysregulation. Fasting glucose was higher in Groups B and C than in controls, but the smaller effect size points to early, subclinical glycemic changes (Table 2).

Insulin resistance (IR) was highly prevalent in obese children, particularly those with CPP (Group C). Across all HOMA-IR cutoffs, IR was significantly more frequent in Groups B and C than in Group A, with Group C showing the highest odds up to 72-fold above controls (Table 3). Leptin correlated strongly with HOMA-IR, BMI Z-score, LH, and bone age advancement, with the strongest associations seen across the overall cohort (Table 4).

Table 5 illustrates the multivariate logistic regression identified leptin (OR=1.28 per ng/mL), HOMA-IR (OR=1.42 per unit), and advanced bone age (OR=2.94 per year) as significant independent predictors of precocious puberty in obese children. Conversely, BMI Z-score and IGF-1 were non-significant. The robust model demonstrated excellent predictive power (AUC=0.89, Nagelkerke $R^2 = 0.73$).

Table No. 1: Baseline demographics and anthropometric measurements

Variable	Group A (Controls) [n=30]	Group B (Obese, No Puberty) [n=30]	Group C (Obese + CPP) [n=30]
Age (years)	7.2±1.4(A)	7.5±1.3*	7.8±1.2 (A)
Sex (Female/Male)	16/14 (A)	17/13*	18/12 (A)
Height (cm)	122.4±8.9 (A)	125.8±9.2* (A)	128.6±8.7 (B)
Weight (kg)	23.1±4.2 (A)	38.7±7.8** (B)	41.2±8.9**
Body mass index (kg/m²)	15.3±1.8 (A)	24.2±3.1 (B)	24.8±3.4**

*=Non significant, **b=p<0.05 versus Group A, **=p<0.05 versus Group B

Table No. 2: Metabolic hormones and growth factors and leptin concentrations and insulin resistance parameters

Variable	Group A	Group B	Group C
Reproductive Hormones			
LH (IU/L)	0.18±0.12	0.21±0.15	2.34±1.87
FSH (IU/L)	1.42±0.89	1.58±0.94	4.67±2.13
LH/FSH Ratio	0.13±0.08	0.14±0.09	0.52±0.31
Estradiol (pg/mL) - Girls	8.2±3.1	9.4±3.8	28.7±12.4
Testosterone (ng/dL) - Boys	12.4±4.2	14.1±5.3	89.3±34.7
Metabolic hormones and growth factors			
IGF-1 (ng/mL)	142.3±28.7	168.9 ± 34.2	198.4 ± 41.6
IGFBP-3 (μg/mL)	2.8±0.6	3.2 ± 0.7	3.9 ± 0.9
IGF-1/IGFBP-3 Ratio	51.2±8.9	53.1 ± 9.4	51.8 ± 8.7
TSH (mIU/L)	2.1±0.8	2.3 ± 0.9	2.2 ± 0.7
Free T4 (ng/dL)	1.2±0.2	1.1 ± 0.2	1.2 ± 0.2
Leptin concentrations and insulin resistance parameters			
Serum Leptin (ng/mL)	3.2±1.8	12.4±4.7[a]	18.9±6.3[a,b]
Fasting Glucose (mg/dL)	89.4±6.7	94.2±8.9[a]	97.8±9.4[a]
Fasting Insulin (μU/mL)	6.8±2.9	18.7±7.2[a]	24.3±8.9[a,b]
HOMA-IR	1.5±0.7	4.4±1.8[a]	5.9±2.3[a,b]

Table No. 3: Insulin resistance prevalence by group

HOMA-IR Cutoff	Group A (%)	Group B (%)	Group C (%)	χ^2 p-value	OR (95% CI) B vs A	OR (95% CI) C vs A
≥2.5	4 (13.3%)	24 (80.0%)	28 (93.3%)	<0.001	25.0 (6.8-92.1)	72.0 (8.7-595.2)
≥3.0	2 (6.7%)	19 (63.3%)	26 (86.7%)	<0.001	22.8 (4.8-108.3)	72.0 (13.8-375.8)
≥4.0	1 (3.3%)	12 (40.0%)	21 (70.0%)	<0.001	19.0 (2.3-157.4)	70.0 (8.4-583.2)

Table No. 4: Correlations between leptin, insulin resistance and pubertal parameters

Variable Pair	Overall (n=90)	Group A (n=30)	Group B (n=30)	Group C (n=30)
Leptin vs HOMA-IR	r = 0.78***	r = 0.42*	r = 0.61***	r = 0.54**
Leptin vs BMI Z-score	r = 0.82***	r = 0.38*	r = 0.59***	r = 0.47**
Leptin vs LH	r = 0.69***	r = 0.21	r = 0.34	r = 0.58***
Leptin vs Bone Age Advancement	r = 0.64***	r = 0.18	r = 0.41*	r = 0.52**
HOMA-IR vs LH	r = 0.58***	r = 0.19	r = 0.28	r = 0.49**
HOMA-IR vs Bone age advancement	r = 0.51***	r = 0.15	r = 0.33	r = 0.44*
BMI Z-score vs LH	r = 0.71***	r = 0.23	r = 0.31	r = 0.41*

*p<0.05, **p<0.01, ***p<0.001

Table No. 5: Logistic regression - predictors of precocious puberty in obese children (Groups B+C)

Variable	Univariate OR (95% CI)	Multivariate OR (95% CI)
Leptin (per ng/mL)	1.34 (1.18-1.52)	1.28 (1.09-1.51)
HOMA-IR (per unit)	1.67 (1.28-2.18)	1.42 (1.05-1.92)
BMI Z-score (per unit)	2.14 (0.89-5.15)	1.78 (0.68-4.67)
Bone Age Advancement (per year)	3.89 (2.12-7.14)	2.94 (1.45-5.96)
IGF-1 (per 10 ng/mL)	1.23 (1.08-1.40)	1.15 (0.98-1.35)
Sex (Female vs Male)	1.85 (0.67-5.12)	-

Area Under ROC Curve: 0.89 (95% CI: 0.81-0.97); Hosmer-Lemeshow χ^2: 6.34, p = 0.609 (good fit); Nagelkerke R^2: 0.73.

Table No. 6: ROC Analysis for predicting precocious puberty

Predictor	AUC (95% CI)	Optimal Cutoff	Sensitivity (%)	Specificity (%)
Leptin (ng/mL)	0.94 (0.88-1.00)	14.2	90.0	86.7
HOMA-IR	0.78 (0.66-0.90)	4.8	73.3	76.7
Combined Model	0.96 (0.92-1.00)	-	93.3	90.0

Table NO> 7: Primary parameter clinical diagnostic levels

Parameter	Clinical Cutoff	Sensitivity (%)	Specificity (%)	Clinical Utility
Leptin	14.2 ng/mL	90	87	Excellent screening tool
HOMA-IR	4.8	73	77	Good metabolic marker
LH	1.2 IU/L	87	93	Excellent pubertal marker
Combined Model	Probability > 0.7	93	90	Superior diagnostic accuracy

Serum leptin (AUC=0.94) and HOMA-IR (AUC=0.78) diagnose precocious puberty in obese children. Combining them improves accuracy (AUC=0.96, 93.3% sensitivity, 90.0% specificity), enhancing clinical potential (Table 6).

Leptin, HOMA-IR, and LH each showed clinical usefulness for diagnosing precocious puberty in obese children, and their combined model optimized accuracy (93% sensitivity, 90% specificity) for early clinical application (Table 7).

DISCUSSION

The current research showed that the values of leptin and HOMA-IR showed progressive rises in the obese children with and without central precocious puberty (CPP). These results are aligned with the already existing evidence indicating that leptin is a metabolic signal and permissive factor of pubertal activation. In agreement with our Group C findings, Badr et al[14] have noted the paradoxical effect of the resistance of leptin on obesity that increased leptin does not regulate appetite but promotes the activation of the hypothalamic–pituitary-gonadal (HPG) axis. On the same note, the reference level of leptin in healthy children as reported by Gajewska et al[15] were very similar to our control group, which confirms our methodology.

We also found that there were significant correlations between leptin and BMI Z-score, which is in accord with previous findings that leptin is a robust surrogate of adiposity.[16] The sex-related differences, where the girls had higher leptin concentrations, are in agreement with the literature in endocrinology studies in paediatrics.[17] Notably, our insulin resistance prevalence (93.3% among obese children with CPP) is in keeping with the international prevalence of 70-90% in obese pediatric cohorts.[18] Therefore, our results validate as well as expand the international literature by presenting data on an Iraqi pediatric sample.

Both leptin and insulin resistance and pubertal markers were strongly linked to the hypothesis that these factors are not mere side effects of obesity, but may also mediate early HPG axis activation. The positive correlations between leptin and LH and bone age advancement reinforce its dual role in metabolic and reproductive pathways. The pathophysiology of leptin and HOMA-IR is intertwined as their relationship was synergistic and represents a vicious cycle between adiposity, hormonal regulation, and maturation.[19]

Diagnostic analysis showed that leptin =14.2 ng/mL displayed a high discriminatory validity to CPP (AUC = 0.94), and a leptin model combined with HOMA-IR showed even better validity (AUC = 0.96). These findings indicate that leptin and insulin resistance can be considered practical biomarkers in early screening particularly in groups where clinical evaluation becomes difficult due to excess adiposity. Also, the CPP child metabolic syndrome clustering (60 percent) underscores that early puberty obesity is not a solitary endocrine occurrence, but a component of a more generalized metabolic risk factor.[20]

The present was conducted on leptin, insulin resistance and precocious puberty in obese children. This minimized bias because of the use of a clearly defined control group and inclusion/exclusion criteria. The standardized Tanner staging, bone age assessment, and radiological verification were used to clinical assessment which guaranteed diagnostic accuracy. Validated assays have been used to conduct laboratory analyses and more sophisticated statistical methods like multivariate logistic regression and ROC curve analysis have been used to enhance the strength of our findings. The use of both sexes permitted the investigation of gender peculiarities of correlations, which enriched the result set.[21]

CONCLUSION

Leptin and insulin resistance are confirmed as the independent predictors of central precocious puberty among the obese children. A leptin cut-off threshold at least 14.2 ng/mL and HOMA-IR of at least 4.8 were highly diagnostic, and both together provided better predictive validity. These results indicate that metabolic dysfunction is critically important in the premature pubertal activation and justify the incorporation of leptin and insulin resistance index into routine pediatric endocrine evaluation.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ghada Abd Arhman Taqa, Abeer Mansour Abdel Rasool
Drafting or Revising Critically:	Ghada Abd Arhman Taqa, Zeyad Khalaf Maded
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

The Effect of Thymus Vulgaris Extract on Pharmacological Activity of Acarbose in Diabetic Animal Model

Asma A. Alkafaji and Majid Kadhim Abbas

ABSTRACT

Objective: To investigate the therapeutic effects of acarbose alone and in combination with thymol on oxidative stress, β-cell function, and glycemic control in streptozotocin (STZ)-induced diabetic rats.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the College of Science at Babylon University, Iraq from 15th July 2025 to 14th December 2025.

Methods: Forty male albino rats (180–320 g) were maintained under controlled conditions (22–25°C, 50% humidity, free access to water). After 2 weeks, rats were randomly divided into four groups: negative (A), positive (B), acarbose-treated (C), and acarbose plus thymol-treated (D). After treatment, rats were sacrificed and blood samples were collected for biochemical and inflammatory analyses.

Results: Group B showed decreased superoxide dismutase (0.337±0.091) and C-peptide (863.17±250.14), with increased advanced oxidation protein products (430.37±77.85) and glucose (148±29.71) vs A ($p<0.05$). Group C increased superoxide dismutase (0.554±0.155) and C-peptide (1248.78±303.68) and reduced advanced oxidation protein products (271.42±120.38) and glucose (102.9±12.74) ($p<0.05$). Group D further increased superoxide dismutase (0.615±0.202) and C-peptide (1368.33±277.35) and decreased advanced oxidation protein products (269.74±59.11) and glucose (97±7.46) ($p<0.05$).

Conclusion: Acarbose improves oxidative stress and glycemic status, while thymol provides additional improvement.

Key Words: Effect, Thymus vulgaris extract, Pharmacological activity, Acarbose, Diabetic animal

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INTRODUCTION

Diabetes mellitus is a long standing metabolic condition characterized by sustained elevation in blood glucose levels arising from insufficient insulin secretion, insulin resistance, or both.[1] Persistent hyperglycemia contributes to enhance generation of reactive oxygen species (ROS), which promotes oxidative stress and plays a major role in the development of long- term diabetes complications, including retinopathy, nephropathy, and neuropathy.[2] Oxidative damage influences cellular lipids, proteins, and DNA, and accelerates β-cell dysfunction, and insulin resistance, making the reduction of oxidative stress a key therapeutic aim.[3]

Traditional treatments for DM, such as oral medications and insulin injections, may have certain side effects such as hypoglycemia (especially insulin) and the symptoms of hypoglycemia may include sweating, confusion, anxiety and dizziness. Phytochemicals found in medicinal plants have been found to have hypoglycemic properties, suggesting they may be useful in reducing the risk of hyperglycemia or may be useful in glycemic control.

Alpha-glucosidase inhibitor (acarbose) is group of medication use to control blood glucose level by inhibited a glucosides enzyme located in intestinal brush border, a glucosidase enzyme breaking down carbohydrate in to glucose that can be absorbed and elevated glucose level.[4]

Vegetables, in particular, are a cornerstone of nutritional strategies for both prevention and management of diabetes mellitus because of their unique composition of dietary fiber, antioxidants, vitamins, minerals, and phytochemicals.[5]

Thymus vulgaris (common thyme) is a small aromatic herb from the flowering plant Lamiaceae, which contains volatile oils rich in thymol and carvacol.[6] In addition to various phenolic components (phenolic acid).[7]

[1.] M.Sc. Scholar, Department of Pharmacology & Toxicology, Babylon University, Hillah, Iraq.
[2.] Professor, Department of Pharmacology, Babylon University, Hillah, Iraq.

Correspondence: Asma A. Alkafaji, M.Sc. Scholar, Department of Pharmacology & Toxicology, Babylon University, Hillah, Iraq.
Contact No: 07715067698
Email: asmaaabdalkareem1@gmail.com

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Medicinally, these constituents exhibit antimicrobial, antioxidant, anti-inflammatory capabilities, which have supported the traditional use of thyme in several health conditions, including metabolic diseases.[8]

Thymol, the principal phenolic monoterpene in thyme, demonstrates strong free radical scavenging activity, and has been reported to enhance endogenous antioxidant enzymes such as superoxide dismutase (SOD), and providing significant protection against oxidative and inflammatory injury, Studies have shown that thymol improves lipid profiles, reduces oxidative markers such as advanced oxidation protein products (AOPP), and preserves pancreatic β-cell integrity. Owing to its natural origin, high safety profile, and strong antioxidant capacity, thymol may be used as a complement to conventional antidiabetic drugs.[9]

Combining acarbose with a natural antioxidant, such as thymol, may offer synergistic therapeutic benefits by improving glycemic control while simultaneously enhancing antioxidant defenses. This combination has the potential to reduce oxidative damage, preserve β-cell function (as reflected by c-peptide levels), and mitigate diabetes-related biochemical alterations more effectively than acarbose alone.

METHODS

This experiment was conducted at College of Science at Babylon University, Iraq from 15th July 2025 to 14th December 2025 vide letter No. 4329 dated 6th July 2025 on 40 adults males, which were obtained from the animal facility of the. All rats were maintained under controlled laboratory conditions for two weeks prior to start of the study. The animals were kept in plastic cages (five rats per cage) and maintained under controlled environment, including a temperature (22–25°C), relative humidity of approximately adequate ventilation, and a 12 house light/dark cycle. Following acclimatization, the animals were randomly and manually allocated into four groups, (n=10 per group) (based on prior studies with similar effect sizes, n=10/group was deemed sufficient) using a simple randomization method to ensure unbiased group allocation.

Chemical and reagent: STZ from Heowns (China), acarbose from exit costar (Iran),and thymus vulgaris from natural (India).

Induction of T2DM: To establish the type 2 diabetes mellitus (T2DM) model characterized by increased adiposity, a modified protocol based on was employed, except for the control group, all rats were maintained on a high-fat diet (HFD) supplemented with butter and cholesterol powder for a 14-day pre-induction period to promote fat accumulation. Following an 8–10 hour fast, T2DM was induced via a single intraperitoneal (IP) injection of streptozotocin (STZ) at a dosage of 35 mg/kg prepared by dissolving 100 mg of STZ in 10 ml of normal saline. To ensure stability, the STZ solution was kept refrigerated and shielded from light until the moment of administration. Animals exhibiting fasting blood glucose levels 125 mg/dl were confirmed as diabetic and included in the experimental cohorts.[10]

Experimented design: Fourth adult male rats were randomly divided into 4 groups with ten animals in each group and the experimental animal groups received.

Group A was the negative control group (normal group), which is received distilled water and pellet feed for 12 days and injected with normal saline.

Group B was the positive group that received a high fat diet (cholesterol powder and butter) for 12 days and was then injected with streptozotocin (STZ) at 35mg/kg.[11]

Group C was the diabetic treated group that was injected with STZ (35 mg/kg) and then treated with 24mg/kg of acarbose for 14 days.[12]

Group D was the diabetic treated combination group that was injected with STZ (35 mg/kg) and then treated with acarbose (24 mg/kg) and Thymus vulgaris extract (0.4 ml/kg).[13]

After 14 days of this trial, the rats were euthanized with xylocaine (10 mg/kg) and ketamine (80 mg/kg).[14] Blood samples were collected from all rats that underwent laparotomy, and serum was prepared for chemical analysis. The kit was superoxide dismutase kit (SOD), C-peptide kit, advanced oxidation protein product kit (AOPP), and glucometer was used to measure blood glucose levels were used.

All analyses were performed using SPSS-26. Statistical evaluation was conducted using one-way analysis of variance (ANOVA) followed by Student's t-test where appropriate. A probability value of less than 0.05 was regarded as statistically significant.

RESULTS

The biochemical variables in groups are in Table 1. A statistically significant reduction in plasma SOD concentration was observed in the diabetic control group (Group B) when compared with both the negative control (Group A) and the combined treatment group (Group D). In contrast, the acarbose-treated group (Group C) did not demonstrate a statistically meaningful difference relative to Group B in Figure 1.

Table 2 shown the SOD levels in Group A were markedly higher than in Groups C and D ($p=0.001$). Additionally, Group D exhibited a significant improvement in SOD concentration compared with Group B ($p=0.035$). However, the difference between Groups C and B did not reach statistical significance ($p=0.098$). Statistical significance was defined at $p<0.05$

Plasma content of C-peptide: C-peptide levels were expressed as mean ± SD. The diabetic control group (Group B) showed a marked reduction compared with the negative control (Group A). Both treatment groups, acarbose (Group C) and acarbose plus thymol (Group D) in Figure 2, demonstrated significantly higher C-

peptide levels than Group B (p=0.002 and p=0.001, respectively). No significant differences were observed between Groups C or D and the negative control (p>0.05) [Table 3].

Table No. 1: Biochemical variables in the studied groups

Groups	SOD (pg/ml)	C- peptide (pg/ml)	AOPP (pg/ml)	Glucose (mg/dl)
A	1.093±0.64	1292.82±208.58	281.61±49.68	95.9 ± 18.69
B	0.337±0.091*	863.17±250.14*	430.37±77.85*	148±29.71*
C	0.554±0.155	1248.78±303.68*	271.42±120.38*	102.9±12.74*
D	0.615 ±0.202*	1368.33±277.35*	269.74±59.11*	97±7.46*

*p<0.05 (Significant)

Table No. 2: Comparison between the effects of treatment on superoxide dismutase level in studied groups

Variable	Group	Study groups	Mean±SD	P- value
Superoxide dismutase (pg/ml)	Group C (0.554±0.155)	Group A	1.093±0.64	0.001
		Group B	0.337±0.091	0.098
	Group D (0.615±0.202)	Group A	1.093±0.64	0.001
		Group B	0.337±0.091	0.035

P<0.05 (Significant)

Table No. 3: Comparison between the effect of treatment on C-peptide level in studied groups

Variable	Group	Study groups	Mean±SD	P- value
C-peptide (pg/ml)	Group C (1248.78±303.68)	Group A	1292.82±208.58	0.716
		Group B	863.17±250.14	0.002
	Group D (1368.33±277.35)	Group A	1292.82±208.58	0.534
		Group B	863.17±250.14	0.001

P<0.05 (Significant)

Table No. 4: Comparison between the effects of treatment on AOPP level in studied groups

Variable	Group	Study groups	Mean±SD	P- value
AOPP (pg/ml)	Group C (271.42±120.38)	Group A	281.61±49.68	0.763
		Group B	430.37±77.85	0.001
	Group D (269.74±59.11)	Group A	281.61±49.68	0.726
		Group B	430.37±77.85	0.001

P<0.05 (Significant)

Table No. 5: Comparison between the effects of treatment on Blood glucose level in studied groups

Variable	Group	Study groups	Mean±SD	P- value
Blood glucose (mg/dl)	Group C (102.9±12.74)	Group A	95.9±18.69	0.348
		Group B	148±29.71	0.001
	Group D (97±7.46)	Group A	95.9±18.69	0.887
		Group B	148±29.71	0.001

P<0.05 (Significant)

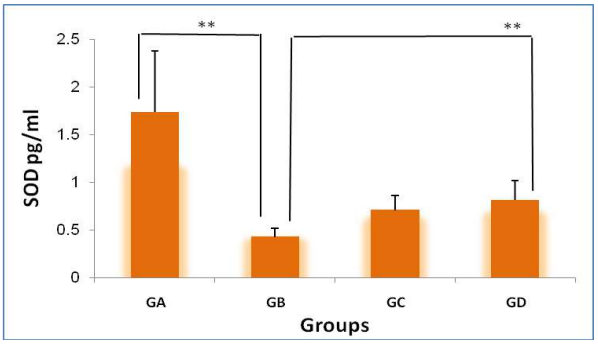

Figure No. 1: GA is controlled negative; GB is control positive (DM); GC is the treated group (Acarbose); GD is the treated group (Acarbose+Thymol), (p<0.05 compared to positive control)**

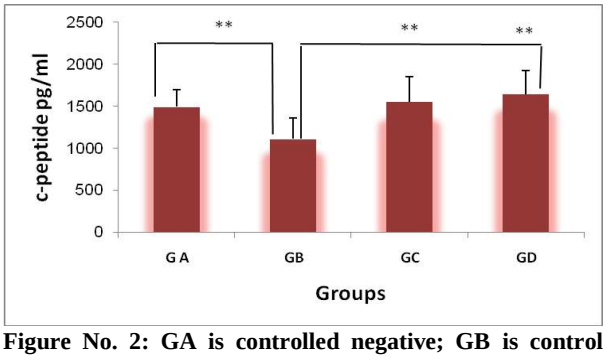

Figure No. 2: GA is controlled negative; GB is control positive (DM); GC is the treated group (Acarbose); GD is the treated group (Acarbose+Thymol), (p<0.05 compared to positive control)**

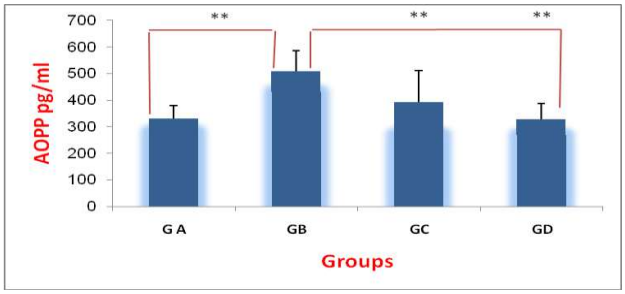

Figure No. 3: GA is controlled negative;GB is control positive (DM); GC is the treated group (Acarbose); GD is the treated group (Acarbose+Thymol), (p<0.05 compared to positive control)**

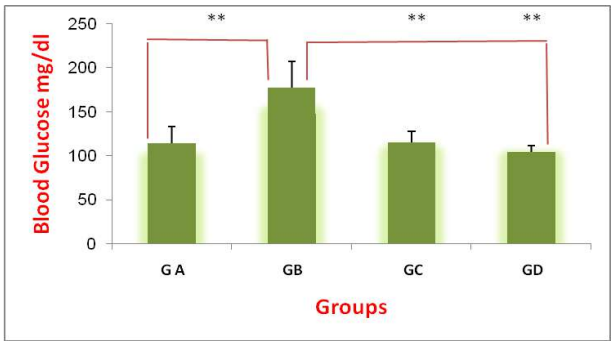

Figure No. 4: GA is controlled negative;GB is control positive (DM); GC is the treated group (Acarbose); GD is the treated group (Acarbose+Thymol), (p<0.05 compared to positive control)**

Plasma AOPP concentrations were markedly elevated in the diabetic control group (Group B) compared with the negative control (Group A). Both treatment groups, acarbose (Group C) and acarbose combined with thymol (Group D), showed a significant reduction in AOPP levels relative to Group B ($p=0.001$ for both comparisons). No statistically significant differences were detected between Groups C or D and the negative control ($p>0.05$). Statistical significance was defined at $p<0.05$ (Fig. 3, Table 4).

Blood glucose levels were significantly higher in the diabetic control group (Group B) than in the negative control group (Group A). Treatment with acarbose alone (Group C) or in combination with thymol (Group D) resulted in a significant decrease in glucose levels compared with Group B ($p = 0.001$). Neither treatment group differed significantly from the negative control ($p>0.05$). Statistical significance was set at $p<0.05$ (Fig. 4, Table 5).

DISCUSSION

The present study demonstrated that induction of diabetes by STZ produced clear metabolic and oxidative disturbances in rats, as evidenced by the significant elevation in blood glucose[15] and AOPP levels[16], and marked reduction in SOD[17] and C-peptide concentrations. These alterations reflected the well-established action of STZ, which are selective target pancreatic β-cell through DNA alkylation and over production of reactive oxygen species (ROS), leading to impaired insulin secretion and severe oxidative imbalance.[18]

Acarbose treatment (Group C) produced significant improvements in all measured biomarkers compared with the diabetic control (Group B). Blood glucose levels were significantly reduced, therefore, acarbose modulated oxidative stress[19] and enhance antioxidant stats.[20] These results are consistent with the known pharmacological actions of acarbose, which include the reduction of glucose absorption. The elevation of c-peptide suggests preserve β cell function.

Thymus vulgaris combined with acarbose (Group D), produced additional improvements in oxidative and glycemic parameters, consistently showing superior trends in increasing SOD activity, reducing AOPP levels, and improving glucose control. These enhancements likely reflect the potent antioxidant properties of thymol. As a phenolic monoterpene, thymol effectively neutralizes free radicals, stabilizes cellular membranes, prevents lipid and protein oxidation, and upregulates endogenous antioxidant enzymes.[21] Furthermore, thymol has been reported to improve glucose metabolism, reduce insulin resistance, and preserve pancreatic tissue integrity, which supports the improved C-peptide trends observed in this study.

The significant reduction in AOPP levels in the treatment groups highlights the efficacy of both acarbose and thymol in minimizing oxidative protein damage. AOPP is a well-established biomarker of oxidative stress and protein oxidation, and its levels are commonly elevated in diabetes due to persistent hyperglycemia and chronic inflammation.[22] The ability of the acarbose-thymol combination to reduce AOPP more effectively suggests enhanced antioxidant synergy, whereas thymol directly scavenges free radicals.

Similarly, the increase in SOD levels in combination therapy emphasizes the potential of thymol to stimulate endogenous antioxidant defenses. SOD is a critical enzyme that converts superoxide radicals into less harmful molecules. Its depletion in diabetic rats reflects increased oxidative burden, whereas its elevation following therapy indicates restoration of redox balance. The higher SOD activity in the acarbose + thymol group suggests a greater capacity to counteract oxidative injury than acarbose alone, although, the difference did not reach statistical significance.

Regarding glycemic control, all treatment groups showed substantial reductions in blood glucose levels compared to diabetic controls. However, the combination therapy group (D) demonstrated the most pronounced reduction. This improvement may be attributed not only to the pharmacological effects of acarbose on carbohydrate metabolism but also to

thymol's ability to modulate glucose uptake and enhance insulin receptor signaling.

The accompanying improvement in C-peptide levels further supports better β-cell performance or preservation under combination therapy.

CONCLUSION

The notable pancreatic protection effect in diabetes animal model, indicating the beneficial role of acarbose and thymol in reducing oxidative stress, restoring β-cell function, increasing antioxidant state, and improving glucose levels. Thymol enhances the antioxidant properties of acarbose. Therefore, Acarbose is effective in glycemic control and reducing oxidative stress. Combination with thymol shows additional therapeutic benefits.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Asma A. Alkafaji, Majid Kadhim Abbas
Drafting or Revising Critically:	Asma A. Alkafaji, Majid Kadhim Abbas
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.

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Original Article

Impact of Flaxseed Supplementation on Thyroid Function, Reproductive Hormones and Kidney Health in Women with Hypothyroidism

Amel S. Abdulredha[1], Riyadh K. Abdulah[2], Amal H. Anatheil[3] and Wafa S. Abdulredha[2]

ABSTRACT

Objective: To determine the extent of thyroid, reproductive, and renal functions in hypothyroid women after consuming supplements of flaxseeds.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Endocrinology and Oncology Center in Thi-qar from 1st April 2024 to 31st January 2025.

Methods: 41 women aged 30-55 were enrolled. They were divided in 4 groups: healthy women without supplements group, hypothyroid women without supplements group, hypothyroid women where flaxseed was added to medication group and healthy woman who consumed only flaxseed group. The duration was 5 weeks, 1 week acclimatization period to the new diet and 4 weeks of daily flaxseed consumption, with a stepwise increase of the flaxseed dosage to a fixed endpoint.

Results: The hypothyroid patients showed a prevalence of thyroid stimulating hormone, blood urea nitrogen, creatinine, and dysfunction of some reproductive hormones. Some renal parameters were evidenced to have decreased to normal values, follicle-stimulating horome, luteinizing hormone, estrogen, and testosterone were all restored, TSH was normal and flaxseed caused an elevation of T3. The healthy women's parameters were all normal. It is plausible that a positive effect was brought about by the omega-3 fatty acid which assists the conversion of T4 to T3, the antioxidant boosting effect of the lignans and the support of the gut microbiome.

Conclusion: Flaxseed could help reclaim these issues and restore hormones to a more healthy balance. Nevertheless, additional participants, larger samples, and extended studies are necessary to determine the efficacy of the treatment and assess the optimum dosage that can be administered alongside conventional thyroid medication.

Key Words: Creatinine, Estradiol, Flaxseed, Hypothyroidism, Levothyroxine

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INTRODUCTION

When the thyroid is underactive (produces low amount of hormones), it disrupts the normal metabolic processes that happen throughout the body. This condition is called hypothyroidism, and it happens to be a fairly common condition in the endocrine system. It happens to about 1 in 300 Americans[1] and affects more women and older people than men. Still the best way to restore hormonal balance and keep your metabolism steady is Levothyroxine, a synthetic version of T4.[2] More women than men are diagnosed with hypothyroidism in Iraq. In Basra, about 83% of the diagnosed hypothyroidism cases were women. In the Thi-qar Governorate, the results indicate that a little more than 80% of the diagnosed hypothyroidism cases were women.[3,4] The increased likelihood of developing hypothyroidism is due to the rapid hormonal changes that occur during and after pregnancy, as well as after menopause.[5] It is essential that we implement better early screening procedures in women to allow quick and efficient diagnosis to avoid negative consequences. More and more people are turning away from traditional treatments and instead using functional foods and medicinal plants to improve their hormonal and metabolic health.[6-8]

1. Lecturer, Department of Biology, College of Education for Pure Science, University of Basrah, Basrah 64001, Iraq.
2. Lecturer, Department of Pharmacognosy & Medicinal Plants, College of Pharmacy, University of Thi-qar, Thi-qar 64001, Iraq
3. Lecturer, Department of Pharmaceutical Chemistry, University of Thi-qar, Thi-qar 64001, Iraq.

Correspondence: Wafa S. Abdulredha, Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Thi-qar, Thi-qar 64001, Iraq.
Contact No: +964 780 345 3859
Email: wafaabdulredha81@utq.edu.iq

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Flaxseed (Linum usitatissimum L.) has many good things in it, including omega-3 fatty acid alpha-linolenic acid (ALA) and SDG lignans. The first two are turned into antioxidants in the stomach, but they don't do anything to estrogen. But these last two do help lower inflammation.[9-11] The components, which include phenolic acids, tocopherols, and important minerals, as well as the high fiber content (28-40%)[12-14], help with digestion, blood sugar control, lipid metabolism, lowering inflammation, and safely improving lipid profiles. Prior research has demonstrated that the phytoestrogenic characteristics of flaxseed may enhance cardiovascular health, lower triglyceride and cholesterol concentrations, and alter reproductive hormones.[15,16] These advantages indicate that flaxseed may be beneficial for women with hypothyroidism. More research is needed, though, to find out how they affect kidney function and hormones that control reproduction.

The purpose is to examine the impact of 30 days of flaxseed consumption on thyroid function, kidney markers, and reproductive hormones in hypothyroid women, compared with hypothyroid women not receiving the supplement and a group of healthy women.

METHODS

This experimental study was conducted at Endocrinology and Oncology Center in Thi-qar from 1st April 2024 to 31st January 2025 vide letter No. 21772/Approval/SKDFF dated 11th March 2024. Forty-one adult women aged 30-55 were enrolled. Based on their health and the dietary intervention, the participants were arranged into four groups: Control group consisted of twelve healthy women without any medical issues. Hypothyroid group: consisted of eleven women diagnosed with hypothyroidism.

Hypothyroid-flaxseed group: Ten women with hypothyroidism who took flaxseed powder in larger and larger amounts, up to 7 grams per day.

Healthy-flaxseed group: Eight women who did not have thyroid problems made up and took flaxseed at the same times as the hypothyroid-flaxseed group.

To safely and effectively evaluate the effects of flaxseed on thyroid function, it was administered gradually to mitigate the risk of gastrointestinal adverse effects, including bloating and diarrhea, and to observe for possible interactions with levothyroxine.[17,18] The hypothyroid and hypothyroid-flaxseed groups commenced the trial on an identical stable dosage of levothyroxine, which was maintained consistently throughout the study. Participants were excluded if they had chronic endocrine, renal, or cardiac disease; were pregnant or lactating; were taking medications that could affect thyroid function or lipid metabolism (excluding levothyroxine); or were unable to adhere to the prescribed flaxseed regimen.

Dietary supplementation and intervention protocol: The strategy for flaxseed supplementation were to take it for 30 days, then slowly stop taking it over the course of a week. Drinking milk or juice with freshly ground flaxseed at least 1.5 hours after taking levothyroxine helped keep the body from absorbing it. Starting with a dose of 2.6 grams, it increased to 7 grams by the 7th day, remaining at that level for all three doses. This process will be designed to minimize negative effects from the seeds while retaining positive effects such as lignans and omega-3 fatty acids, due to the slow introduction to the digestive system. The dose of flaxseed treatment is shown in Table 1.

Biochemical Assessments: Everyone's blood was drawn and analyzed for biochemical markers per the protocol after the 30 day intervention. Blood was drawn following an overnight fast. Thyroid function tests were done and blood levels of TSH, T3, and T4 were analyzed. For the evaluation of renal function blood urea and creatinine levels were assessed. In addition, the reproductive hormones were analyzed, including estrogen, testosterone, FSH, and LH. The assessments were made at the Biochemistry and Physiology Labs of the College of Pharmacy, University of Thi-qar. The results were kept reliable done by the use of internal quality control measures and commercial testing kits.

The data was analyzed through SPSS-25. The t-test and analysis of variance (ANOVA) were applied. $P<0.05$ is considered significant.

RESULTS

The hypothyroid group has higher TSH and significantly lower T3 and T4 compared to control ($P<0.05$). In hypothyroid women, T3 levels increased and TSH levels decreased in flaxseed consuming group less than those observed. With healthy women, Flaxseed consumed did not disturb the thyroid hormones balance, indicating a modulatory effect and not an adverse effect (Table 2).

Table No. 1: A titrated flaxseed dosage protocol for complementary therapeutic use

Time (day)	Total daily intake (g)	Average amount per Meal (3 meals/day)
Initial	2.6 g	0.87 g
2	3.6 g	1.2 g
3	4.6 g	1.53 g
4	5.1 g	1.7 g
5	6.1 g	2.03 g
6	6.6 g	2.2 g
7+	7.0 g (stable intake dose)	2.33 g

Hypothyroid women showed considerable alterations in levels of reproductive hormones after taking flaxseed supplements. In comparison to the healthy control group, the hypothyroid group had higher levels of FSH

and LH, lower levels of estradiol and higher levels of testosterone (P<0.05). In contrast, hypothyroid women consuming flaxseed had levels of FSH and LH which were lower, levels of estradiol which were higher, and levels of testosterone which were lower, to levels seen in normal healthy individuals (P<0.05). In healthy women, flaxseed had no significant influence on the levels of gonadotropins, but it did maintain estradiol levels in the normal range, indicating a potential stabilizing effect on this particular hormone. Flaxseed helped to resolve some reproductive hormone imbalances caused by hypothyroidism (Table 3)

There were significant differences in the renal function indices between study groups. Hypothyroidism involves renal function impairment which is shown by the increased BUN and creatinine levels in hypothyroid patients compared to the controls (P<0.05). In women with hypothyroidism, BUN and creatinine levels were considerably lower due to flaxseed supplementation compared to levels seen in healthy individuals (P<0.05). Flaxseed seems to be protective on the renal markers of healthy women by the absence of any negative effects and the maintenance of all values within the normal physiological ranges after the consumption of flaxseed (Table 4).

Table No. 2: Thyroid hormone modulation for study groups

Study groups	TSH (mU/L)	T3 (nmol/L)	T4 (nmol/L)
Control group (n=12)	1.62±0.34^{a}	2.01±0.26^{b}	109.42±10.36^{c}
Healthy-flaxseed group (n=11)	2.93±0.51^{c}	1.73±0.27^{b}	95.53±15.11^{b}
Hypothyroid group (n=8)	3.11±0.57^{c}	0.96±0.15^{a}	79.29±12.97^{a}
Hypothyroid-flaxseed group (n=10)	2.52±0.48^{b}	2.42±0.37^{c}	97.98±15.10^{b}

Statistically significant variations (p<0.05) found when distinct superscript letters (a, b, c) are present within a row

Table No. 3: Reproductive hormone level across study groups

Study Groups	FSH (ng/mL)	LH (ng/mL)	Estradiol (pg/mL)	Testosterone (ng/mL)
Control group (n=12)	9.6±1.8^{a}	4.9±1.01^{a}	72.4±8.2^{c}	22.4±2.8^{a}
Healthy-flaxseed group (n=11)	9.2±2.01^{a}	4.7±1.1^{a}	74.1±7.6^{c}	25.9±3.1^{b}
Hypothyroid group (n=8)	14.8±2.1^{c}	7.2±1.4^{c}	46.3±6.1^{a}	36.8±3.4^{c}
Hypothyroid-flaxseed group (n=10)	11.9±2.4^{b}	5.8±1.2^{b}	63.5±7.3^{b}	27.4±3.5^{b}

Statistically significant variations (p<0.05) exist when distinct superscript letters (a, b, c) are present within a row

Table No. 4: Renal function indicators across study groups

Parameter	Control group (n=12)	Healthy-flaxseed group (n=11)	Hypothyroid group (n=8)	Hypothyroid-flaxseed group (n=10)
BUN (mg/dL)	16.8±1.1^{a}	18.9±1.3^{a}	27.4±1.2^{c}	21.6±1.4^{b}
Creatinine (mg/dL)	0.75±0.08^{a}	0.71±0.06^{a}	0.92±0.10^{b}	0.68±0.07^{a}

Statistically significant variations (p<0.05) found when distinct superscript letters (a, b, c) are existing within a row

DISCUSSION

This study investigated the effects of hypothyroidism and flaxseed supplementation on thyroid and reproductive hormones, as well as kidney function in women. Hypothyroidism in females was associated with disturbances in thyroid, reproductive, and renal functions. Signs of impaired endocrine and ovarian function included low T3 and T4 levels, elevated testosterone, reduced estradiol and increased FSH and LH, along with other reproductive hormone irregularities.[19,20]

A lack of thyroid hormone is linked to less blood flow to the kidneys and less glomerular filtration. This is backed up by the higher kidney markers.[21] Adding flaxseed to the diet helped fix these problems: T3 levels went up, TSH levels got better, and reproductive hormones got back to normal levels. This improvement is probably because omega-3 helps the body turn T4 into T3, lignan helps receptors become more sensitive, antioxidants work, and the gut bacteria helps hormones break down.[22,23] The lowered BUN and creatinine levels show the renal protection attributed to the microcirculation and glomerular function enhancing effects of ALA and the antioxidant activity of SDG.[24] The results also show that flaxseed kept all the hormone and kidney markers in healthy women within the normal range, indicating that it kept the regulatory system intact and it did no harm to the women. These results support the idea that flaxseed could be a useful addition to a diet for improving the balance of hormones and kidneys, especially in women with hypothyroidism.

CONCLUSION

Flaxseed supplements assisted women with hypothyroidism improve their thyroid, reproductive, and kidney functioning by bringing hormone and renal indicators back to normal levels. It was safe and helped

healthy women stay stable because it kept all of their levels in the usual range. These results indicate that flaxseed may serve as an effective adjunctive technique for aiding hypothyroid women in sustaining healthy endocrine and renal systems.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Amel S. Abdulredha, Riyadh K. Abdulah
Drafting or Revising Critically:	Amal H. Anatheil, Wafa S. Abdulredha
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Hematological Abnormalities in Neonates Diagnosed with Neonatal Sepsis

Ammara Murtaza[1], Maheen Matloob[1], Nimra Gull[1] and Muhammad Yasin[2]

ABSTRACT

Objective: To determine the mean value of hematological parameters including red blood cell distribution width, platelet distribution width, mean platelet volume, white blood cell count, red blood cell count and platelet count in neonates diagnosed with neonatal sepsis.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Pediatric Medicine, Sahiwal Teaching Hospital, Sahiwal from 1st February 2025 to 31st July 2025.

Methods: In this study 70 neonates were enrolled. Blood samples were taken in a 3cc disposable syringe and sent to the laboratory for assessment of hematological parameters

Results: The mean age was 11.6±7.4 days, and 39 (55.7%) neonates were males. Early-onset sepsis was present in 42 (60%) cases. Raised red cell distribution width was observed in 46 (65.7%) neonates, abnormal white blood cell count in 38 (54.3%), raised platelet distribution width in 39 (55.7%), raised mean platelet volume in 34 (48.6%), and thrombocytopenia in 29 (41.4%). Late-onset sepsis was associated with significantly higher red cell distribution width, platelet distribution width, and mean platelet volume and a significantly lower platelet count.

Conclusion: Hematological abnormalities were frequent among neonates with sepsis. Red cell and platelet indices may provide useful supportive information, particularly in neonates with late-onset sepsis and low birth weight.

Key Words: Neonatal sepsis, Hematological abnormalities, Red cell distribution width, Mean platelet volume, Platelet distribution width, Thrombocytopenia

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INTRODUCTION

Sepsis is one of the most common infectious diseases of newborns, which mostly occurs in premature and low birth weight babies. Sepsis is the third most common cause of neonatal death with a high morbidity and mortality rate particularly in the developing countries, causing over a quarter of neonatal deaths (25%).[1,2] It may be diagnosed by the presence of a positive blood culture and/or cerebrospinal fluid culture.[3] The hemogram or blood count includes the quantitative (counting) and qualitative (formula) examination of the counted elements of blood (erythrocytes, leukocytes and platelets). The first years of life have a dramatic effect on the blood count parameters, which vary according to the developmental stage.[4]

[1.] Postgraduate Residents / Assistant Professor & Head of Department[2], Department of Paediatric Medicine, Sahiwal Teaching Hospital Sahiwal.

Correspondence: Ammara Murtaza, Postgradute Resident, Department of Paediatric Medicine, Sahiwal Teaching Hospital Sahiwal.
Contact No: 03356915999
Email: ammaramurtaza18@gmail.com

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Most common haematological abnormalities in neonatal sepsis are anaemia, leukocytosis, thrombocytopenia and shortened coagulation time. Mean platelet volume (MPV) and red blood cell distribution width (RDW), which are components of the complete blood count, are, however, routinely measured, inexpensive, and are readily available but there is little information on the haematological abnormalities in neonatal sepsis.[3] The correlation of MPV and RDW with inflammatory diseases. The mean platelet volume is a direct measure of platelet activation due to inflammation.[5] Red blood cell distribution width is one of the red blood cell indices that are rapidly and automatically calculated by all modern hematological analysers.[6] Ahmed et al[7], conducted a study in Sudan and found that among neonates with neonatal sepsis, the mean RDW was 18.366±2.1%, which was significantly higher than neonates without neonatal sepsis (17.464±18%). Similarly, mean MPV was 10.604±0.95 vs. 8.894±0.85 and platelet count was 60.289±52.0 vs 212.030±162.6.

Neonatal sepsis, a serious systemic infection during the first 28 days of life, is a major cause of the morbidity and mortality of the newborns worldwide.[8] The very immature immune responses and limited physiological reserves, as well as constant exposure to maternal, environmental and health care-associated pathogens

make newborns especially vulnerable. Clinical signs are often subtle and vague such as poor feeding, temperature instability, lethargy, respiratory symptoms, apnea, altered perfusion, and glucose disturbances.[9] This often results in progressive deterioration, multi-organ failure, disseminated intravascular coagulation and death within 24 hours of onset.[10] Currently, more than half a million neonates per year die from neonatal infections, such as sepsis, pneumonia and meningitis, with the highest rates in low- and middle-income countries (LMICs).[11]

METHODS

This was an observational cross-sectional study carried out in Department of Pediatric Medicine, Sahiwal Teaching Hospital, Sahiwal from 1st February 2025 to 31st July 2025. The study was approved by CPSP/REU/PED-2022-143-7117, dated 17 January 2025. The sample size was determined using a sample-size calculator from the World Health Organization. The minimum sample size of 70 neonates was determined by selecting a 95% confidence level with absolute precision 0.05, based on the mean red cell distribution width of 18.366±2.1 in neonates with sepsis reported earlier.[7] All neonates, irrespective of gender, diagnosed to have neonatal sepsis and meeting the eligibility criteria, during the study period, were included. Neonates (1-28 days) of either sex were included if they were clinically and laboratory based diagnosed as neonatal sepsis within the defined criteria. Neonates born before 37 weeks gestation were excluded. Induced abortions and neonates with congenital anomalies, inborn errors of metabolism, severe neonatal jaundice, respiratory distress syndrome, surfactant deficiency or extremely low birth weight (<1 kg) were also excluded. Mothers with pregnancy induced hypertension (BP > or = 140/90 mmHg and dipstick proteinuria > +1) did not have their babies included. Neonates who had birth asphyxia (Apgar score less than 7 at 5 minutes) were also excluded. Demographic and clinical data were recorded. Data provided included neonatal age, sex, birth weight, current weight, mode of delivery, gestational age at birth, and site of birth, as well as the Apgar score at 5 minutes and whether the baby had been diagnosed with early- or late-onset neonatal sepsis. Venous blood samples were drawn from each neonate after performing aseptic measures and placed into an appropriate ethylenediaminetetraacetic acid tube. The sample was sent straight to the hospital laboratory for full blood count and haematological examination. The parameters that were measured were red cell distribution width, platelet distribution width, mean platelet volume, white blood cell count, red blood cell count, and platelet count. Any laboratory data obtained was documented. The main outcome was the nature of hematological abnormalities in neonates who were diagnosed with neonatal sepsis. The hematological parameters assessed were red cell distribution width, platelet distribution width, mean platelet volume, white blood cell count, red blood cell count and platelet count. These parameters were also compared among appropriate demographic and clinical subgroups.

The data was analyzed through SPSS-25. Normality of quantitative variables was determined by Shapiro-Wilk test. Neonatal age, birth weight, current weight, gestational age, Apgar score, RDW, PDW, MPV, WBC, RBC and platelet count, all quantitative variables, were presented as mean ± SD (normally distributed). Data that did not fit the normal distribution were presented as median (interquartile range). Qualitative data such as gender, mode of delivery, place of birth and type of neonatal sepsis were represented as frequencies and percentages. Data were stratified by the following variables: place of birth, birth weight, current weight, neonatal sepsis (early- and late-onset), gender, mode of delivery, gestational age at birth and Apgar score. Mean hematological parameters were compared between two groups after stratification using independent-samples t-test. One-way analysis of variance was used to make comparisons between more than two groups. When the data were not parametric the Mann-Whitney U test or the Kruskal-Wallis test were used. A p-value of <0.05 was deemed as statistically significant.

RESULTS

Their mean age was 11.6±7.4 days. Males were slightly more common, accounting for 39 (55.7%) neonates, while 31 (44.3%) were females. The mean birth weight was 2.78±0.46 kg, whereas the mean current weight was 2.69±0.45 kg. The mean gestational age at birth was 38.6±1.1 weeks, and the mean Apgar score at 5 minutes was 8.1±0.8. Vaginal delivery was reported in 41 (58.6%) cases and caesarean section in 29 (41.4%). Most neonates were hospital-born, 44 (62.9%), while 26 (37.1%) were home-born (Tables 1-2).

Table No. 1: Baseline demographic and clinical characteristics of neonates with sepsis (n=70)

Variable	No.	%
Gender		
Male	39	55.7
Female	31	44.3
Clinical characteristics		
Vaginal delivery	41	58.6
Caesarean section	29	41.4
Hospital-born	44	62.9
Home-born	26	37.1
Early-onset sepsis	42	60.0
Late-onset sepsis	28	40.0

The mean red cell distribution width was 18.42±2.16%, with values ranging from 14.2% to 23.8%. The mean platelet distribution width was 16.91±2.37 fL, ranging

from 12.1 to 22.4 fL, while the mean platelet volume was 10.84±1.29 fL, with a range of 8.1-13.9 fL. The mean white blood cell count was 17.62±6.84 $\times 10^9$/L and ranged from 4.2 to 34.8 $\times 10^9$/L. The mean red blood cell count was 4.36±0.71 $\times 10^{12}$/L, with values between 2.8 and 5.9 $\times 10^{12}$/L (Table 3).

Table No. 2: Descriptive statistics of neonates with sepsis (n=70)

Variable	Mean±SD
Age (days)	11.6±7.4
Birth weight (kg)	2.78±0.46
Current weight (kg)	2.69±0.45
Gestational age (weeks)	38.6±1.1
Apgar score at 5 minutes	8.1±0.8

Raised platelet distribution width was present in 39 (55.7%), while raised mean platelet volume was identified in 34 (48.6%) cases. An abnormal total white blood cell count was found in 38 (54.3%) neonates, including leukocytosis in 27 (38.6%) and leukopenia in 11 (15.7%). A low red blood cell count was recorded in 22 (31.4%) neonates. Thrombocytopenia was present in 29 (41.4%) cases, including severe thrombocytopenia in 8 (11.4%) [Table 4].

The mean red cell distribution width was significantly higher in late-onset than early-onset sepsis, at 19.18±2.27% versus 17.91±1.94% (p=0.015). Similarly, platelet distribution width was higher in late-onset sepsis, 17.86±2.45 fL compared with 16.28±2.11 fL (p=0.006), while mean platelet volume was 11.47±1.22 fL versus 10.42±1.17 fL (p=0.001). The mean platelet count was significantly lower in late-onset sepsis than in early-onset sepsis, 148.9±76.2 $\times 10^9$L versus 194.8±75.4 $\times 10^9$/L (p=0.015) [Table 5].

The mean red cell distribution width was significantly higher than that of neonates weighing 2.5 kg or more, 19.41±2.28% versus 18.08±2.02% (p=0.021). The mean red blood cell count was significantly lower in the low-birth-weight group, 4.05±0.73 $\times 10^{12}$/L compared with 4.47±0.67 $\times 10^{12}$/L (p=0.028). Similarly, the mean platelet count was lower in neonates weighing below 2.5 kg, 139.3±70.1 $\times 10^9$/L versus 189.2±77.6 $\times 10^9$/L (p=0.018) [Table 6].

Table No. 3: Hematological parameters among neonates with sepsis (n=70)

Hematological parameter	Mean±SD	Minimum–Maximum
Red cell distribution width (%)	18.42±2.16	14.2-23.8
Platelet distribution width (fL)	16.91±2.37	12.1-22.4
Mean platelet volume (fL)	10.84±1.29	8.1-13.9
White blood cell count ($\times 10^9$/L)	17.62±6.84	4.2-34.8
Red blood cell count ($\times 10^{12}$/L)	4.36±0.71	2.8-5.9
Platelet count ($\times 10^9$/L)	176.4±78.6	38-356

Table No. 4: Frequency of hematological abnormalities among neonates with sepsis (n=70)

Hematological abnormality	No.	%
Raised red cell distribution width	46	65.7
Raised platelet distribution width	39	55.7
Raised mean platelet volume	34	48.6
Leukocytosis	27	38.6
Leukopenia	11	15.7
Abnormal total white blood cell count	38	54.3
Low red blood cell count	22	31.4
Thrombocytopenia	29	41.4
Severe thrombocytopenia	8	11.4

Table No. 5: Comparison of hematological parameters according to type of neonatal sepsis

Parameter	Early-onset sepsis (n=42)	Late-onset sepsis (n=28)	p-value
Red cell distribution width (%)	17.91±1.94	19.18±2.27	0.015
Platelet distribution width (fL)	16.28±2.11	17.86±2.45	0.006
Mean platelet volume (fL)	10.42±1.17	11.47±1.22	0.001
White blood cell count ($\times 10^9$/L)	18.54±6.41	16.24±7.35	0.171
Red blood cell count ($\times 10^{12}$/L)	4.48±0.68	4.18±0.72	0.083
Platelet count ($\times 10^9$/L)	194.8±75.4	148.9±76.2	0.015

Table No. 6: Comparison of hematological parameters according to birth weight

Parameter	Birth weight <2.5 kg (n=18)	Birth weight ≥2.5 kg (n=52)	p-value
Red cell distribution width (%)	19.41±2.28	18.08±2.02	0.021
Platelet distribution width (fL)	17.78±2.51	16.61±2.26	0.069
Mean platelet volume (fL)	11.34±1.31	10.67±1.25	0.054
White blood cell count ($\times 10^9$/L)	16.13±6.94	18.13±6.79	0.283
Red blood cell count ($\times 10^{12}$/L)	4.05±0.73	4.47±0.67	0.028

Platelet count ($\times 10^9$/L)	139.3±70.1	189.2±77.6	0.018

DISCUSSION

In the present study, the authors showed that neonates with neonatal sepsis had hematological abnormalities. The most common abnormalities were raised red cell distribution width (65.7%), abnormal total white blood cell count (54.3%), raised platelet distribution width (55.7%), raised mean platelet volume (48.6%) and thrombocytopenia (41.4%). The observations suggest the widespread impact of neonatal sepsis on red cells, white cells and platelets and reinforce the use of commonly available hematological parameters in the evaluation of affected neonates. The mean age of the study population was 11.6±7.4 days and 60.0% of cases were early onset sepsis. Such an excess could be due to maternal and perinatal transmission of infection in early neonatal period. Neonatal sepsis can cause a rapid onset of respiratory and systemic signs and symptoms because of underdeveloped immune system and reduced physiological reserves. Increased early onset sepsis is also highlighting the need for maternal risk assessment, as well as aseptic delivery and early postnatal surveillance.[12] Almost 2/3 of the neonates had elevated RDW levels, and the total mean RDW was 18.42±2.16%. RDW measures the variation in size of RBC and may rise in response to alterations in red cell survival, hemolysis, oxidative stress, impaired erythropoiesis or inflammation. Systemic infection may interfere with iron metabolism and may impair function of the bone marrow, leading to a release of cells of varying sizes into the blood. The present study showed elevated levels of RDW occurred frequently; this indicates that RDW might be a valuable supportive marker of haematological stress in neonatal sepsis. The mean RDW was also significantly higher in late onset compared to early onset sepsis (19.18±2.27% vs. 17.91±1.94%). This may be due to a longer period of inflammatory exposure, extended hospitalisation, repeated blood sampling, nutritional disturbances or the severity of illness in neonates with late onset infection. Late-onset sepsis is also linked to the use of healthcare-related organisms and is more likely to be associated with invasive procedures that could result in a greater systemic inflammatory response. Platelet indices also were significantly modified.[13] The mean platelet distribution width was 16.91±2.37 fL, while the mean platelet volume was 10.84±1.29 fL. 55.7% neonates had raised PDW and 48.6% had raised MPV. These indices measure the size of platelets and the release of newer and larger platelets from the bone marrow. Sepsis can trigger platelet activation, consumption, endothelial damage, and inflammatory cytokines, which can lead to the production of larger platelets. Thus, elevated MPV and PDW can be a sign of the elevated platelet turnover and inflammation.[14] Both PDW and MPV were significantly higher among neonates with late-onset sepsis. Mean PDW increased from 16.28±2.11 fL in early-onset sepsis to 17.86±2.45 fL in late-onset sepsis, while MPV increased from 10.42±1.17 fL to 11.47±1.22 fL. These results indicate that platelet activation and consumption may be increased in the late onset disease. Additionally, the more significant shifts in platelet indices could be due to a longer duration of infection, invasive procedures, and increased severity of illness.[15]

The rate of neonates with thrombocytopenia was 41.4% and severe thrombocytopenia was 11.4%. Sepsis can decrease platelets in a variety of ways: peripheral destruction, platelet consumption, endothelial activation, disseminated intravascular coagulation, immune-mediated destruction, and decreased bone-marrow production. The clinical significance of thrombocytopenia is that it may be associated with greater bleeding tendency and more severe infections. The mean platelet count was significantly lower in late-onset and early-onset sepsis groups and was 148.9±76.2 $\times 10^9$/L and 194.8±75.4 $\times 10^9$/L respectively. This is again evidence of more platelet consumption in the late onset disease. Neonatal anemia (RBC <4.5 x 106/ul) was found in 31.4% of the neonates.[16] The anemia or decrease in RBC in neonatal sepsis may result from depressed erythropoiesis, hemolytic process, shortened red cell survival, multiple phlebotomy, bleeding or nutritional deficits. The mean RBC count was also lower in late onset sepsis, but this did not achieve statistical significance. The lack of significance could be due to the relatively small number of infants studied, disease duration, or because very premature and extremely low birth neonates were excluded, which are more susceptible to anemia.[17]

The results of the present study have clinical relevance, as these are the parameters that are currently available in the routine laboratory examination of automated analysis of complete blood count These parameters are cost-effective and can be measured quickly, which is useful in situations where use of advanced biomarkers or rapid microbiological tests is restricted. None of these indices, however, are stand-alone and should not be used alone.[18] The most important use of these is to complement clinical evaluation, to facilitate the identification of high-risk neonates, and to monitor for changes occurring while in treatment. Late onset sepsis was associated with increased RDW, PDW, and MPV and decreased platelet count, thus indicating that platelet and red cell indices might be indicators of the severity or duration of systemic inflammation. Likewise, the low-birth-weight neonates had poorer hematological data, which further underscores the need to closely monitor this vulnerable population. A single value may not be as informative as serial measurements, which can show a trend of progression, treatment response or complication.[19,20]

CONCLUSION

Hematological abnormalities were common among neonates diagnosed with neonatal sepsis. Raised red cell distribution width was the most frequent finding, while altered platelet indices, abnormal white blood cell count, reduced red blood cell count, and thrombocytopenia were also frequently observed. Neonates with late-onset sepsis had significantly higher red cell distribution width, platelet distribution width, and mean platelet volume, together with a lower platelet count, indicating more pronounced hematological disturbance. Low-birth-weight neonates also showed higher red cell distribution width and lower red blood cell and platelet counts.Routine complete blood count parameters may therefore provide useful and readily available supportive information in the assessment of neonatal sepsis, particularly where advanced diagnostic facilities are limited.

Limitations: This was undertaken in one centre, and the relatively small number of children may restrict the findings to a relatively small population. The cross-sectional design did not permit the evaluation of time course changes in hematological parameters nor the relationship of the parameters to treatment response. The study did not include any septic neonates in a non-septic control group and there was no assessment of microbiological organisms, inflammatory biomarkers, disease severity, transfusion history or mortality outcome. There were also some hematological abnormalities that could have been affected by nutritional status, maternal factors or undiagnosed clinical conditions.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ammara Murtaza, Maheen Matloob
Drafting or Revising Critically:	Nimra Gull, Muhammad Yasin
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Narrative Review

Treatment of Delirium in Septic Shock Patients: A Focused Evidence Review

Delirium in Septic Shock Patients

Muhammad Asim Rana[1], Moayad Nasser Alkhlewi[1], Kaouthar Sifaoui[1], Meshal Alharbi[2], Hector Emanuel Arias[1] and Andres Saravia-Moya[1]

ABSTRACT

Objective: To review pharmacological and non-pharmacological strategies for established delirium in adults with septic shock.

Place and Duration of Study: This review was conducted Intensive Care Medicine, Department of Adult Intensive Care Medicine, King Abdullah Specialized Hospital, Ministry of National Guards Health Affairs, Qassim, KSA.from January to March 2026.

Methods: PubMed/MEDLINE was searched for studies published between 2021 and 2026 on septic shock, delirium, sepsis-associated encephalopathy, dexmedetomidine, antipsychotics, the ABCDEF bundle, early mobilization, and melatonin.

Results: Dexmedetomidine may improve some organ-related outcomes but increases hypotension and has no consistent mortality or delirium benefit. Quetiapine and haloperidol show comparable efficacy for hyperactive delirium. ABCDEF bundle implementation improves long-term functional outcomes. Early mobilization appears feasible in hemodynamically stable patients, while melatonin improves sleep but has not consistently reduced delirium.

Conclusion: No pharmacological therapy has demonstrated a clear mortality or delirium-resolution benefit in septic shock.

Key Words: Septic shock; Delirium; Sepsis-associated encephalopathy

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INTRODUCTION

The reason is biological, not just statistical. Sepsis-associated encephalopathy (SAE) is increasingly recognized as a distinct clinical entity rather than a generic label for "ICU confusion."[1,2] Systemic cytokine release, blood-brain barrier disruption, microglial activation, and impaired neurovascular coupling combine to injure the frontal cortex, hippocampus, and brainstem in ways that differ mechanistically from postoperative or trauma-related delirium.[3–5]

This matters clinically because guidelines built around pooled ICU populations, most visibly the Society of Critical Care Medicine's Pain, Agitation, Delirium, Immobility, and Sleep (PADIS) recommendations delivered through the ABCDEF bundle, offer blanket advice that was never designed to answer a sepsis-specific question: does treating delirium in a hemodynamically unstable, cytokine-driven illness require a different approach than treating it after elective surgery?[3,5]

This review asks that question directly. We focus on adult patients with septic shock, examine both pharmacological agents (dexmedetomidine, antipsychotics, and corticosteroids as an unavoidable confounder) and non-pharmacological strategies (the ABCDEF bundle, early mobilization under vasopressor support, and sleep optimization), and close by identifying where the evidence base still falls short.

METHODS

We searched PubMed/MEDLINE for studies published between 2021 and 2026, combining the terms septic shock, sepsis, delirium, sepsis-associated encephalopathy, dexmedetomidine, antipsychotic,

[1.] Consultant Physician, Intensive Care Medicine, Department of Adult Intensive Care Medicine, King Abdullah Specialized Hospital, Ministry of National Guards Health Affairs, Qassim, KSA.

[2.] Clinical Pharmacist, Department of Pharmacy, King Abdullah Specialized Hospital, Ministry of National Guards Health Affairs, Qassim, KSA

Correspondence: Muhammad Asim Rana, Consultant Physician, Intensive Care Medicine, Department of Adult Intensive Care Medicine, King Abdullah Specialized Hospital, Ministry of National Guards Health Affairs, Qassim, KSA.
Contact No: +92 343 5807006
Email: ranamu@mngha.med.sa

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ABCDEF bundle, ICU Liberation, early mobilization, and melatonin from January 2026 to March 2026. Because several of the systematic reviews and meta-analyses retrieved through this strategy had themselves searched Embase, Cochrane CENTRAL, Scopus, Web of Science, and CINAHL, evidence from those databases is represented indirectly within this synthesis even though each platform was not independently queried. Randomized controlled trials, meta-analyses, and large cohort or registry studies enrolling adult patients with sepsis or septic shock were prioritized.

PATHOPHYSIOLOGY

Why Septic Shock Delirium Is Not Generic ICU Delirium

Sepsis-associated encephalopathy begins with a systemic inflammatory cascade rather than a focal insult. Circulating cytokines including IL-1β, IL-6, and TNF-α activate cerebral endothelium and disrupt tight-junction proteins such as claudin-5, allowing peripheral immune signaling to cross into the CNS.(3,4,6)microglial activation follows, and recent work using pharmacological CDK9 inhibition in a caecal ligation and puncture model showed that blocking this inflammatory kinase reduced both microglial activation and blood-brain barrier breakdown, evidence that the pathway is druggable in principle even though no such agent is yet in clinical use.[6]

Beyond inflammation, cerebral perfusion itself is deranged. Systemic inflammation impairs the brain's glymphatic clearance system, and in an experimental model, restoring cerebral blood flow with levosimendan normalized glymphatic flux and reduced neuroinflammation, pointing to hypoperfusion, not inflammation alone, as an independent contributor to SAE.[7] Consistent with this, ascorbate depletion, common in sepsis and disproportionately affecting the metabolically demanding frontal cortex, has been proposed as a modifiable contributor to microvascular dysfunction and encephalopathy, an area currently more mechanistic than clinical.[8] Endothelial phenotyping adds a further layer: a specific circulating endothelial subset (CD32b-positive cells) independently predicted 28-day delirium in a prospective cohort of postoperative septic and non-septic ICU patients, with an adjusted area under the curve of 0.89, suggesting endothelial injury tracks with brain dysfunction risk beyond what organ-failure scores alone capture.[9] Recent reviews now describe SAE not as a single syndrome but as a family of mechanism-based subphenotypes, ischemic-hypoxic, inflammatory-dominant, and metabolic-degenerative, each with a distinct clinical trajectory.[10] Blood-based biomarkers including neurofilament light chain, glial fibrillary acidic protein (GFAP), UCH-L1, and Tau correlate with both delirium and structural brain injury on imaging, and GFAP in particular improved prediction of unfavorable neurological outcome and 90-day mortality beyond age, SOFA, and APACHE II alone in a recent exploratory analysis.[11]

PHARMACOLOGICAL INTERVENTIONS

Sedation Strategy and Dexmedetomidine

Sedation is unavoidable in ventilated septic shock, and current sedation reviews recommend an analgesia-first approach, adding sedatives only as needed, with propofol and dexmedetomidine preferred over benzodiazepines.[12] The trial-level picture is more mixed than the mechanistic rationale suggests. A 2026 meta-analysis of eight RCTs (702 patients) found that dexmedetomidine significantly reduced day-2 SOFA score and acute kidney injury incidence, but it also significantly increased hospital length of stay and the incidence of hypotension, with no significant difference in 28-day mortality, mechanical ventilation duration, ICU length of stay, or delirium incidence.[13] A separate meta-analysis of a similar trial set (eight RCTs, 662 patients) reached the same conclusion on mortality, no significant difference across in-hospital, ICU, or long-term endpoints, and again found dexmedetomidine significantly increased hospital length of stay, with the authors grading certainty of evidence as low throughout.[14] The model, incorporating age, BMI, sepsis status, SOFA score, and cumulative opioid, propofol, and benzodiazepine dosing, achieved an area under the receiver-operating curve of 0.83 on derivation and 0.70 on external validation, reasonable discrimination for a bedside tool once prospectively tested.[15] A separate retrospective cohort reinforces the same point from the opposite direction: a system-wide shift from benzodiazepines toward dexmedetomidine did not change annual delirium incidence, while septic shock, vasopressor use, corticosteroid use, and antipsychotic use all remained independent correlates of delirium on multivariate analysis.[16]

ANTIPSYCHOTICS

Neither of the two agents in routine use, haloperidol and quetiapine, has demonstrated superiority for treating established delirium, and the newest evidence continues that pattern. A 2026 meta-analysis with trial sequential analysis pooling four RCTs (292 hospitalized adults) found no significant difference in delirium severity reduction (DRS-R-98), ICU or hospital length of stay, or mortality between quetiapine and haloperidol; quetiapine showed a non-significant trend toward fewer extrapyramidal symptoms and more sleep, but GRADE certainty was very low across all outcomes and the trial sequential analysis confirmed the evidence base remains underpowered.[17] A dedicated RCT in 100 patients with hyperactive delirium found an essentially identical response rate between the two drugs (92% overall, no significant between-group difference), though the quetiapine group had a modestly

shorter ICU stay (10.1 versus 11.7 days) and more sleep per night.[18]

CORTICOSTEROIDS

An Unavoidable Confounder

Low-dose corticosteroids are frequently used in refractory septic shock for hemodynamic support, and this creates a direct tension with delirium management that is easy to overlook. A comprehensive narrative review of corticosteroid use across critical illness, including septic shock, documents agitation and delirium among the recognized adverse effects, alongside genuine benefits in shock reversal and reduced mechanical ventilation duration.[19] Yet the relationship is not uniformly harmful. In a retrospective cohort of 583 patients undergoing emergency surgery for gastrointestinal obstruction or perforation, in which septic shock was among the strongest independent risk factors for postoperative delirium (adjusted odds ratio 8.57), low-dose dexamethasone was independently protective (adjusted odds ratio 0.41).[20]

Table No. 1: Key trials and meta-analyses of dexmedetomidine in septic shock

Study	Design / N	Key findings
Wolfkind et al. (2026)(13)	Meta-analysis, 8 RCTs, n=702	↓ Day-2 SOFA, ↓ AKI; ↑ hospital LOS, ↑ hypotension; no significant difference in mortality, delirium, or MV duration
Alsagban et al. (2025)(14)	Meta-analysis, 8 RCTs, n=662	No difference in-hospital, ICU, or long-term mortality; ↑ hospital LOS; low certainty of evidence throughout
Prendergast et al. (2025)(15)	Derivation n=836; external validation n=340	Prediction model for sedative-associated delirium (age, BMI, sepsis, SOFA, drug doses); AUROC 0.83 derivation, 0.70 external validation

Table No. 2: Antipsychotic trials for ICU and hospital delirium

Study	Design / N / Comparator	Key findings
de Oliveira et al. (2026)(17)	Meta-analysis with TSA, 4 RCTs, n=292; quetiapine vs haloperidol	No significant difference in DRS-R-98 reduction, LOS, or mortality; GRADE very low certainty; TSA confirms evidence is inconclusive
Zakhary et al. (2024)(18)	RCT, n=100, hyperactive delirium; quetiapine vs haloperidol	Equal response rate (92%); quetiapine associated with shorter ICU stay and more sleep; no mortality difference

THE ABCDEF / ICU LIBERATION BUNDLE

The ABCDEF bundle, translating the PADIS guidelines into a daily checklist covering pain assessment, spontaneous awakening and breathing trials, choice of sedation, delirium assessment, early mobility, and family engagement, remains the backbone of non-pharmacological delirium management.[21] Its long-term value was tested in a secondary analysis of the MIND-USA trial cohort, in which median proportional bundle compliance was high (97%), and greater compliance was associated with better twelve-month functional independence and health-related quality of life, though not with better long-term cognition, mental health, or survival.[22] That distinction matters: the bundle appears to protect physical and functional recovery more reliably than it protects the brain itself, at least on the outcomes measured so far.

In clinical practice, adherence to the individual components of the bundle remains inconsistent. A retrospective cohort study from Chile reported that early mobilization, represented by element E, had the highest compliance rate at 67%, whereas spontaneous awakening trials (B1) and family engagement (F) were rarely implemented.[23] The same concerns were found in a study conducted by the Society of Critical Care Medicine in 2026 that questioned 456 clinicians and healthcare leaders. The survey revealed an even greater drop in compliance with the bundles after the onset of COVID-19. The chief problems were lack of support from leadership, lack of communication within healthcare teams, and lack of staffing. Standardized order sets, checklists and incorporation into EHRs were perceived as helpful tools to facilitate adherence, in contrast[24] Although efforts are being made to standardize the documentation of bundle components and link them with common data models for more consistent auditing, these approaches have not yet become part of routine clinical practice.[25]

EARLY MOBILIZATION IN VASOPRESSOR-DEPENDENT PATIENTS

Recent evidence does not support the traditional fear that mobilizing patients on vasopressors is unsafe. In the 64% of patients that were mobilized on low-dose vasoactive medications, and in the 30% of patients that were mobilized on moderate-dose vasoactive drugs, and even 7% of patients that were mobilized on high-dose vasoactive drugs, the overall rate of adverse events was 2%.[26] A large retrospective cohort of over 12,000 ICU patients receiving more than 59,000 mobilization sessions identified specific, actionable norepinephrine dose thresholds: safe mobilization out of bed up to approximately 0.20 mcg/kg/min, and in-bed mobilization up to 0.33 mcg/kg/min, without a significant increase in adverse events within these limits.[27] Nurse-led quality improvement work in a cardiovascular ICU replicated this safety signal for

post-cardiac surgery patients on vasoactive medication, with mobility scores improving significantly after a structured educational intervention.[28] A prospective multicenter protocol currently underway in Colombia should further clarify adverse event rates and dose-response relationships.[29]

Whether this translates into less delirium specifically, rather than just less ICU-acquired weakness, is now better supported than it once was. A large 2025 meta-analysis of 59 RCTs (8,462 patients) found that enhanced mobilization activities reduced ICU-acquired weakness (moderate certainty), reduced delirium duration by just over a day on average (low certainty), and shortened mechanical ventilation duration, with little to no increase in adverse events.[30]

Table No. 3: Priority research gaps in delirium management for septic shock

Gap	Description
Subgroup reporting gap	Septic shock is rarely a prespecified primary population in delirium treatment trials
Phenotyping deficit	Hyperactive/hypoactive and SAE mechanistic subphenotypes are not used to stratify treatment trials
Biomarker-treatment integration	NfL, GFAP, endothelial CD32b$^+$ subsets, and mNUTRIC associate with delirium but remain untested as treatment-response markers
Corticosteroid paradox	Protective against delirium in one septic-surgical cohort, listed as a risk factor in general critical illness

CONCLUSION

No pharmacological agent reviewed here, dexmedetomidine, quetiapine, haloperidol, or melatonin, has shown a clear delirium-resolution or mortality advantage over its comparator, and most of that evidence still comes from mixed ICU cohorts rather than septic shock specifically. Non-pharmacological bundle care, paired with hemodynamically informed early mobilization and basic sleep hygiene, currently carries the more consistent signal, primarily for functional and quality-of-life recovery rather than a proven cognitive or mortality effect.

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

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Narrative Review

Childhood Obesity, Epidemiology, Metabolic Consequences and Evidence-Based Preventive Strategies

Childhood Obesity, Epidemiology, Metabolic Consequences

Sahreen Gul Qureshi

ABSTRACT

Objective: To review the epidemiology, metabolic consequences, preventive measures, and evidence-based management strategies for childhood obesity.

Study Design: Structured narrative review

Place and Duration of Study: This study was conducted through searches of PubMed/MEDLINE, Scopus, Web of Science, Cochrane Library, and Google Scholar for relevant publications from January 2016 to June 2026.

Methods: Guidelines and reports from recognized organizations, including the World Health Organization and American Academy of Pediatrics, were also reviewed.

Results: The reviewed evidence demonstrated a substantial global rise in childhood obesity, particularly in low- and middle-income countries experiencing urbanization, dietary transition, reduced physical activity, and increased sedentary behavior. Childhood obesity was consistently associated with insulin resistance, dyslipidemia, hypertension, type 2 diabetes mellitus, metabolic dysfunction-associated steatotic liver disease, obstructive sleep apnea, and adverse psychosocial outcomes.

Conclusion: Childhood obesity requires a comprehensive, non-stigmatizing, and stepped-care approach. Early prevention, structured lifestyle treatment, timely pharmacotherapy, and metabolic/bariatric surgery for eligible adolescents can reduce obesity-related complications and improve long-term health outcomes.

Key Words: Childhood obesity; epidemiology; metabolic consequences; insulin resistance; dyslipidemia; physical activity; preventive strategies; pediatric obesity.

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INTRODUCTION

Childhood obesity has emerged as one of the most serious public health challenges of the twenty-first century, affecting millions of children and adolescents worldwide[1]. Defined as excessive accumulation of body fat that adversely affects health, childhood obesity has increased dramatically over the past few decades in both developed and developing countries[3]. The rapid rise in prevalence has transformed childhood obesity from an individual health concern into a global epidemic with significant medical, social, and economic consequences[2]. The condition is now recognized as a major contributor to the growing burden of non-communicable diseases across all age groups[4].

Consultant, Department of pediatrics, King Khalid Hospital, Majmaah, Saudi Arabia.

Correspondence: Dr. Sahreen Gul Qureshi, Consultant, Department of pediatrics, King Khalid Hospital, Majmaah, Saudi Arabia.
Contact No: +966503185630
Email: sahreenkashif@gmail.com

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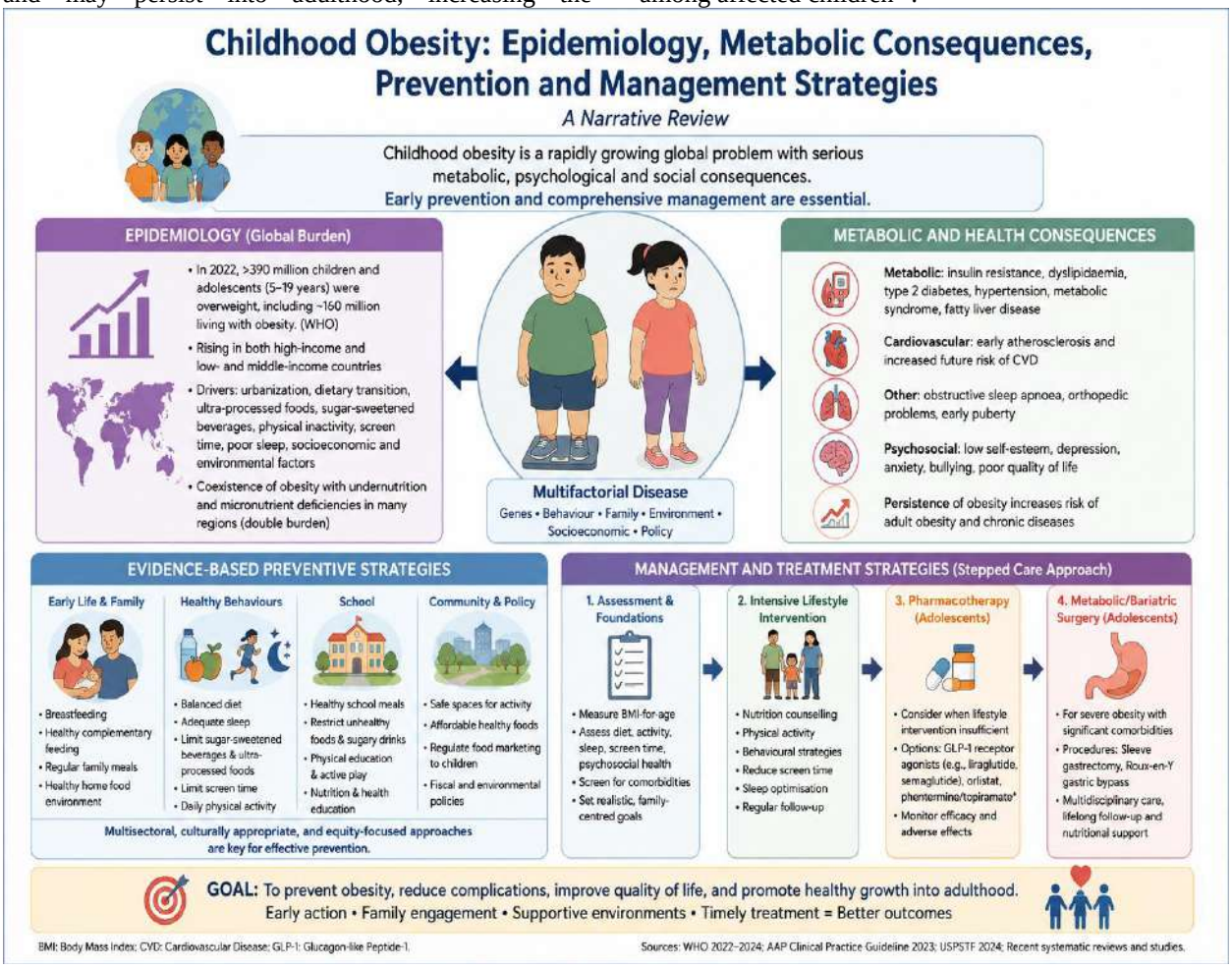
The epidemiology of childhood obesity has undergone substantial changes due to urbanization, technological advancement, dietary transitions, and increasingly sedentary lifestyles[5]. According to international estimates, the prevalence of overweight and obesity among children has risen steadily, particularly in urban populations where access to energy-dense foods and reduced physical activity are common[6]. Developing countries are experiencing a particularly rapid increase in childhood obesity as nutritional patterns shift from traditional diets toward highly processed foods rich in sugars, fats, and calories[7-9].

Childhood obesity results from a complex interaction of genetic, environmental, behavioral, and socioeconomic factors[10]. Excessive caloric intake, inadequate physical activity, prolonged screen time, poor sleep habits, parental obesity, and socioeconomic influences all contribute to the development of obesity during childhood[11]. In addition, modern lifestyles characterized by reduced outdoor activity and increased consumption of processed foods have further accelerated the problem[12]. Early-life factors, including maternal obesity, gestational diabetes, infant feeding practices, and rapid weight gain during infancy, have also been associated with increased obesity risk later in life[13]. The metabolic consequences of childhood obesity are extensive and often begin long before adulthood[14-16]. Obese children are at increased risk of

developing insulin resistance, type 2 diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, and non-alcoholic fatty liver disease[17]. These metabolic abnormalities contribute to early cardiovascular risk and may persist into adulthood, increasing the likelihood of chronic disease development[18]. Furthermore, obesity has been associated with hormonal disturbances, sleep disorders, orthopedic complications, and impaired physical functioning among affected children[19].



METHODS

This article was conducted as a structured narrative review to synthesize current evidence on the epidemiology of childhood obesity, its metabolic consequences, and evidence-based preventive strategies.

Literature Search Strategy

A comprehensive literature search was conducted in PubMed/MEDLINE, Scopus, Web of Science, Cochrane Library, and Google Scholar. Relevant reports, recommendations, and policy documents from the World Health Organization, United Nations Children's Fund, American Academy of Pediatrics, Centers for Disease Control and Prevention, and other recognized public-health organizations were also reviewed. The search included articles published from January 2014 to June 2026; however, selected older landmark studies were included where they provided important background evidence on the epidemiology, pathophysiology, or long-term consequences of childhood obesity. The search terms included "childhood obesity," "pediatric obesity," "adolescent obesity," "overweight in children," "epidemiology," "prevalence," "risk factors," "metabolic consequences," "insulin resistance," "type 2 diabetes mellitus," "dyslipidemia," "hypertension," "metabolic syndrome," "fatty liver disease," "sleep apnea," "cardiovascular risk," "prevention," "physical activity," "dietary intervention," "screen time," "family-based intervention," "school-based intervention," "community intervention," and "public health policy." Boolean operators "AND" and "OR" were used to combine search terms.

Eligibility Criteria

Eligible publications included original research studies, systematic reviews, meta-analyses, clinical practice guidelines, policy documents, and high-quality narrative reviews addressing childhood and adolescent obesity among individuals aged 0–19 years. Articles

reporting the prevalence, determinants, metabolic or psychosocial consequences, prevention, or management of childhood obesity were included. Only English-language publications with accessible full text were considered. Studies published between January 2016 and June 2026 were prioritized, while selected older landmark publications were included where relevant to the review objectives. Articles were excluded when they focused exclusively on adult obesity without separate pediatric findings, were case reports, small case series, conference abstracts, editorials, letters, or opinion articles without adequate supporting evidence. Studies focusing solely on bariatric surgery, pharmacotherapy, or rare genetic obesity syndromes without relevance to population-level prevention were also excluded. All retrieved records were initially screened based on title and abstract. Potentially relevant studies were subsequently assessed through full-text review, and duplicate records were removed before final inclusion. Articles were selected according to their relevance to the review objectives, methodological quality, recency, sample size, population representativeness, and applicability to children and adolescents.

Data Extraction and Evidence Appraisal

Data from eligible publications were extracted using a structured format. The extracted information included the author's name, year of publication, country, study design, population characteristics, sample size, obesity definition, prevalence estimates, associated risk factors, metabolic outcomes, preventive intervention type, key findings, and study limitations. Greater emphasis was placed on systematic reviews, meta-analyses, multicenter studies, nationally representative surveys, and evidence-based guidelines. The quality and applicability of the evidence were assessed critically, with attention to study design, sampling methods, diagnostic criteria for overweight and obesity, outcome definitions, representativeness of the study population, and potential risk of bias.

Data Synthesis

The findings were synthesized narratively under three principal domains: the epidemiology and global burden of childhood obesity; its metabolic and psychosocial consequences; and evidence-based preventive strategies at the individual, family, school, community, healthcare-system, and policy levels. Evidence was compared across geographical regions and socioeconomic settings, with particular consideration of low- and middle-income countries where childhood obesity may coexist with undernutrition and micronutrient deficiencies.

Epidemiology and Burden of Childhood Obesity

The evidence showed a marked rise in childhood and adolescent overweight and obesity across both high-income and low- and middle-income countries. Global estimates indicated that more than 390 million children and adolescents aged 5–19 years were living with overweight in 2022, including approximately 160 million with obesity[20]. The prevalence of overweight, including obesity, in this age group increased from approximately 8% in 1990 to 20% in 2022. The rise was observed in both boys and girls and was particularly concerning in countries experiencing rapid urbanization, dietary transition, and reduced opportunities for physical activity.

The reviewed studies highlighted the double burden of malnutrition in many low- and middle-income countries, where childhood obesity coexists with undernutrition, micronutrient deficiencies, and food insecurity. Increased consumption of energy-dense, nutrient-poor, and ultra-processed foods; frequent intake of sugar-sweetened beverages; reduced outdoor activity; prolonged screen exposure; inadequate sleep; and limited access to safe recreational spaces were repeatedly identified as important contributors to excess weight gain. Parental obesity, lower socioeconomic status in some settings, and unhealthy food marketing directed at children were also associated with a higher risk of childhood obesity[21].

Metabolic and Health Consequences

The reviewed literature demonstrated that childhood obesity is strongly associated with early cardiometabolic abnormalities. Excess adiposity contributes to chronic low-grade inflammation, insulin resistance, altered lipid metabolism, and endothelial dysfunction. Consequently, children and adolescents with obesity have an increased risk of impaired glucose tolerance, type 2 diabetes mellitus, dyslipidemia, hypertension, metabolic syndrome, and metabolic dysfunction-associated steatotic liver disease[22]. Persistent obesity from childhood to adulthood was consistently associated with a substantially greater risk of adult obesity, cardiovascular disease, type 2 diabetes mellitus, and premature morbidity. These findings reinforce the importance of recognizing childhood obesity early and assessing affected children for associated medical and psychosocial complications[23].

Evidence-Based Preventive Strategies

The evidence supported prevention strategies beginning early in life and continuing throughout childhood and adolescence. At the individual and family level, effective approaches included promoting breastfeeding, ensuring appropriate complementary feeding, encouraging regular family meals, reducing consumption of sugar-sweetened beverages and ultra-processed foods, increasing fruit and vegetable intake, promoting adequate sleep, limiting recreational screen time, and supporting regular age-appropriate physical activity. Family-based interventions were more effective when they focused on achievable behavioral changes, involved caregivers actively, and avoided blame or stigmatizing language[24]. School-based interventions were repeatedly identified as important

because schools influence dietary intake, physical activity, and health literacy. The reviewed evidence supported provision of healthier school meals, restrictions on the sale of sugar-sweetened beverages and energy-dense snacks, access to safe drinking water, nutrition education, regular physical education, and opportunities for active play. Multicomponent school programs that involved teachers, parents, students, and local health services appeared more sustainable than isolated educational interventions[25]. At the population level, the evidence supported policies that improve access to affordable healthy foods, regulate child-directed marketing of unhealthy foods and beverages, strengthen nutrition standards in schools, promote active transport, and create safe spaces for physical activity. Healthcare providers also have a central role in routine growth monitoring, early identification of excess weight, assessment for obesity-related comorbidities, and delivery of family-centered behavioral counselling. Overall, the literature indicated that isolated interventions have limited impact, whereas comprehensive, multisectoral, and life-course strategies are more likely to prevent childhood obesity and reduce its metabolic consequences[26].

Management and Treatment Strategies for Childhood Obesity

The reviewed literature emphasized that childhood obesity requires early, longitudinal, family-centered, and non-stigmatizing management rather than delayed or isolated advice. Initial clinical assessment should include body mass index-for-age classification, dietary and physical-activity patterns, sleep duration, screen exposure, psychosocial well-being, family history, and assessment for obesity-related complications. Blood pressure measurement and targeted screening for dyslipidemia, abnormal glucose metabolism, liver disease, obstructive sleep apnea, musculoskeletal problems, and mental-health concerns should be considered according to the child's age, obesity severity, and clinical presentation. Intensive health behavior and lifestyle treatment remains the foundation of management. Effective programs combine nutrition counselling, age-appropriate physical activity, behavioral modification, sleep optimization, and reduction of sedentary screen-based behavior. Family involvement is essential because parents and caregivers influence food availability, meal patterns, physical activity, and treatment adherence[27]. For adolescents with obesity who do not achieve adequate response to intensive lifestyle intervention alone, anti-obesity pharmacotherapy may be considered as an adjunct to behavioral treatment under specialist supervision. Available options may include glucagon-like peptide-1 receptor agonists, such as liraglutide and semaglutide, phentermine/topiramate extended-release, and orlistat, depending on age, clinical indication, local availability, contraindications, and regulatory approval[28]. Medication use requires regular monitoring of treatment response, adverse effects, adherence, nutritional status, mental well-being, and obesity-related comorbidities. Pharmacotherapy should not replace lifestyle treatment; instead, it should be integrated into a comprehensive management plan. Metabolic and bariatric surgery may be considered for adolescents with severe obesity, particularly those with major obesity-related complications such as type 2 diabetes mellitus, severe obstructive sleep apnoea, hypertension, or significant fatty liver disease[29].

DISCUSSION

Childhood obesity is now recognised as a chronic, complex, and multifactorial disease with important immediate and lifelong health implications. The reviewed evidence demonstrates that its increasing prevalence is driven by the interaction of dietary transition, reduced physical activity, sedentary behaviour, inadequate sleep, family influences, socioeconomic inequalities, and exposure to unhealthy food environments. The burden is particularly concerning in low- and middle-income countries, where childhood obesity increasingly coexists with undernutrition and micronutrient deficiencies. This double burden requires preventive and therapeutic strategies that are sensitive to local dietary practices, food affordability, cultural norms, and access to healthcare services. The metabolic effects of childhood obesity are substantial and may begin early in life. Excess adiposity promotes insulin resistance, chronic low-grade inflammation, dyslipidaemia, endothelial dysfunction, and abnormal glucose metabolism. These mechanisms contribute to the early development of hypertension, impaired glucose tolerance, type 2 diabetes mellitus, metabolic dysfunction-associated steatotic liver disease, and other cardiovascular risk factors. The likelihood of complications increases with the severity and persistence of obesity, particularly when obesity continues from childhood into adolescence and adulthood. Therefore, childhood obesity should not be viewed solely as an aesthetic concern but as an important predictor of future cardiometabolic morbidity.

The reviewed literature also highlights that obesity affects psychological and social health. Children and adolescents with obesity may experience body-image dissatisfaction, low self-esteem, social isolation, bullying, anxiety, depressive symptoms, and weight-related stigma. These psychosocial consequences can negatively affect adherence to treatment and may further promote unhealthy eating patterns or physical inactivity[30]. For this reason, obesity management should use respectful, non-stigmatising, and family-centred communication. Healthcare professionals should avoid blame-based approaches and instead support realistic, achievable behavioural goals that

improve health, functioning, and quality of life. Lifestyle modification remains the cornerstone of childhood obesity management. The evidence supports structured interventions combining dietary counselling, regular physical activity, reduction in sedentary screen time, behavioural strategies, and sleep optimisation.

CONCLUSION

Childhood obesity was highly prevalent among the study population, affecting nearly one-third of participants and demonstrating strong associations with adverse metabolic consequences including elevated blood pressure, impaired glucose metabolism, dyslipidemia, and insulin resistance. Excessive screen time, low physical activity, unhealthy dietary behaviors, inadequate sleep, and family history of obesity were identified as significant predictors of obesity. These findings highlight the urgent need for comprehensive evidence-based prevention strategies focusing on healthy nutrition, promotion of physical activity, reduction of sedentary behaviors, adequate sleep, and family-centered interventions.

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

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Case Report

Umbilical Endometriosis

Umbilical Endometriosis: A Case Study

Husam Zain

ABSTRACT

Background: Umbilical endometriosis is a rare form of extrapelvic endometriosis characterized by the presence of functional endometrial tissue within the umbilical region.

Case Presentation: We report the case of a 29-year-old Sudanese nulliparous woman who presented with a two-year history of cyclical dull umbilical pain associated with deep red discharge, worsening during menstruation. She also reported dyspareunia and primary infertility. There was no history of abdominal surgery or trauma. Physical examination revealed a 2 cm bluish, firm, tender, and irreducible umbilical nodule. Ultrasonography demonstrated a superficial avascular hypoechoic mass suggestive of umbilical endometriosis. **Conclusion:** Umbilical endometriosis should be considered in women of reproductive age presenting with cyclical umbilical pain and bleeding, even in the absence of prior surgical history.

Key Words: Umbilical endometriosis, Villar's nodule, Extrapelvic endometriosis, GnRH therapy

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INTRODUCTION

Endometriosis is a long-term gynaecological disorder that is typified by the presence of functional endometrial glands and stroma located outside the uterine cavity. It usually involves the structures of the pelvis, including the ovaries, uterosacral ligaments and peritoneum, and is often referred to as being the cause of dysmenorrhea, chronic pelvic pain, and infertility[1,2]. Endometrial tissue will most commonly proliferate on or around the reproductive organs like the ovaries and fallopian tubes, on the external environment of the womb, or on the tissues surrounding the womb and the ovaries. It may also develop on other organs within the pelvic area such as the bowels, stomach, bladder or cervix. It is also a frequent occurrence in other parts of the body, rarely[3]. It is DM's complication called Diabetic Nephropathy that all extra genital endometriosis cases are associated with, and is extraordinarily uncommon[4]. 6-10% of women of reproductive age is the approximate number of the global incidence of endometriosis, and most likely underdiagnosed[5]. Endometrial cells may proliferate via lymphatic or venous pathways and implant in other locations, such as the umbilicus, lungs, and pleura[6].

Department of Obstetrics and Gynecology, College of Medicine, Majmaah University, Al-Majmaah, Saudi Arabia.

Correspondence: Husam Zain, Department of Obstetrics and Gynecology, College of Medicine, Majmaah University, Al-Majmaah 11952, Saudi Arabia.
Contact No: +966 53 481 7294
Email: h.mahmoud@mu.edu.sa

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CASE REPORT

A 29-year-old Sudanese woman, married for four years, presented with a two-year history of cyclical, dull aching pain localized to the umbilical region, associated with deep red discharge from the umbilicus. The pain characteristically intensified during menstruation and was partially relieved with combined hormonal contraceptives and analgesics. She was nulliparous and had been attempting to conceive throughout the duration of her marriage. There was no history of previous abdominal surgery, trauma, or laparoscopic procedures. She also reported dyspareunia. On physical examination, her vital signs were stable, with normal pulse rate, blood pressure, and temperature. Abdominal and pelvic examinations were unremarkable except for a 2 cm bluish nodular lesion at the umbilicus. The lesion was firm, irreducible, and tender on palpation. The cyclical nature of pain and bleeding, along with the history of infertility and dyspareunia, raised a strong clinical suspicion of endometriosis. Ultrasonography of the umbilical region demonstrated a superficial, avascular, irregular hypoechoic mass with posterior acoustic shadowing, findings consistent with umbilical endometriosis. However, a definitive diagnosis could not be established based on imaging alone. Magnetic resonance imaging (MRI) was not available at the time of evaluation. In view of the high clinical suspicion, the patient was started on a two-month trial of gonadotropin-releasing hormone (GnRH) analogues, considering their established efficacy in the management of pelvic endometriosis. The patient showed an excellent clinical response, with marked reduction in pelvic pain and noticeable decrease in the size of the umbilical mass. Following the favorable response to medical therapy, surgical excision was

planned. The patient underwent surgery under general anesthesia. A supra-umbilical incision was made, and careful dissection was carried out. A 2.5 cm mass was excised along with a margin of normal surrounding umbilical tissue to ensure complete removal. The specimen was sent for histopathological examination. Histopathology revealed fibro-adipose and connective tissue containing endometrial glands and stroma, thereby confirming the diagnosis of primary umbilical endometriosis.

DISCUSSION

Umbilical endometriosis is a rare type of extrapelvic endometriosis, which has a small percentage of the cases of abdominal wall endometriosis. It can be either primary (spontaneous) disease, which has not been preceded by any previous surgery, or secondary endometriosis, which appears after abdominal or pelvic operations. Here, there is no history of surgery or trauma, which helps in identifying the diagnosis of primary umbilical endometriosis, also known as Villar nodule[7]. The aetiology of primary lesions is not fully understood, and it has been postulated that the disease is either lymphatic or hematogenous, or may involve metaplastic differentiation of embryonic elements in the umbilical area. Skin manifestation in endometriosis is very rare with only up to 0.5-1 being reported in various studies. It affects 15% of women in their active sexual life, i.e. between the ages of 20 and 40 years of age, whereas 5% of cases have been found in women who are post menopausal. Endometriosis is typically present in the ovary, uterus, as well as the peritoneum and vagina[8]. Extragenital endometriosis has numerous theories to explain its occurrence. The lesions can be single or multiple findings and can be located across a broad anatomical distribution. It is common to face diagnostic challenges, as patients often display uncharacteristic or unanticipated symptoms[9].

CONCLUSION

Umbilical endometriosis is a rare but important differential diagnosis in women of reproductive age presenting with cyclical umbilical pain and bleeding. The absence of prior abdominal surgery does not exclude the diagnosis, as primary umbilical endometriosis can occur spontaneously. Clinical suspicion based on cyclical symptoms is crucial, while histopathological examination remains the gold standard for confirmation.

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