

Vol. 37, No. 9 September, 2026

ISSN 1029 - 385 X (Print)

ISSN 2519 - 7134 (Online)



MEDICAL FORUM MONTHLY

RECOGNISED BY
PMDC & HEC

APNS
Member

CPNE
Member

ABC
Certified

On OJS

Scopus, Open Access,
Online, Peer Reviewed Journal

Journal of all Specialities

“Medical Forum” Monthly Recognised, Indexed and Abstracted by

- ☞ PMDC with Index Pakistan No.48 since 1998
- ☞ HEC since 2009
- ☞ Pakmedinet Since 2011
- ☞ Medlip (CPSP) Since 2000
- ☞ PASTIC & PSA Since 2000
- ☞ NLP Since 2000
- ☞ WHO, Index Medicus (IMEMR) Since 1997
- ☞ EXCERPTA MEDICA, Netherlands Since 2000
- ☞ EMBASE SCOPUS Database Since 2000
- ☞ Registered with International Standard Serial Number of France bearing ISSN 1029-385X (Print), ISSN 2519-7134 (Online) Since 1992
- ☞ Registered with Press Registrar Govt. of Pak bearing No.1221-B Copr. Since 2009
- ☞ ABC Certification Since 1992
- ☞ On Central Media List Since 1995
- ☞ Medical Forum Affiliated with Medical Academic Foundation (MAF)
- ☞ On OJS, SCOPUS, Open Access, Online, Peer Reviewed Journal
- ☞ EScience Press (CrossRef DOI)
- ☞ Email: med_forum@hotmail.com, medicalforum@gmail.com
- ☞ website: www.medicalforummonthly.com, www.medforum.pk

MEDICAL FORUM MONTHLY	ISSN 1029 - 385 X (Print)	ISSN 2519 - 7134 (Online)	
	APNS Member	CPNE Member	ABC Certified
	Online Journal	Published Since 1989	
	«Online» www.medicalforummonthly.com		

on OJS	Scopus	Open Access	Peer Reviewed	Affiliation With: Medial Academic Foundation (MAF) (Regd.)
------------------	---------------	------------------------	--------------------------	---

Recognized, Indexed & Abstracted by	PMDC-IP-0048 (1998), HEC-Y-Category (2009), Excerpta Medica Netherlands (2000), EMBASE SCOPUS Database (2000), Index Medicus (IMEMR) WHO (1997), Cross Ref (DOI), SJR, HJRS, SCI Journal, Research Gate, Resurchify, Editage, Enago, Research Bib, Research Bite, Pastic and PSA, NLP, Pakmedinet & CPSP	doi Ease of Access in Article through doi in One Click doi:10.60110/medforum

Editorial Executives

Patron-in-Chief Prof. Mahmood Ali Malik Medicine	Editor-in-Chief Prof. Azhar Masud Bhatti Public Health Specialist & Nutritionist	Managing Editor Prof. Nasreen Azhar Consultant Gynaecologist
Co-Editors Tahir Masud Jan (Canada) Dr. Meshaal Azhar (Pak) Dr. Faryal Azhar (Pak)	Editor Dr. Mohsin Masud Jan	Associate Editors Prof. Syed Mudassar Hussain (Pak) Prof. M. Mohsin Khan (Pak) Dr. Iftikhar A. Zahid (Pak)

National Editorial Advisory Board

Prof. Abdul Hamid	Forensic Medicine	Sialkot	03239824782	drabdulhamid12345@hotmail.com
Prof. Abdul Khaliq Naveed	Biochemistry	Rawalpindi	03215051950	khalignaveed2001@yahoo.com
Prof. AftabMohsin	Medicine	Lahore	03314101516	aftabmohsin@yahoo.com
Prof. Anjum Habib Vohra	Neurosurgery	Lahore	03008443218	omer@brain.net.pk
Prof. Asad Aslam Khan	Ophthalmology	Lahore	03008456377	prof.asad.a.khan@gmail.com
Prof. Haroon Khurshid Pasha	Paed. Surgery	Multan	03008633433	haroonkpasha@hotmail.com
Prof. Haroon Nabi	Dermatology	Lahore	03004000216	haroonnabi@hotmail.com
Prof. Javed Akram	Medicine	Lahore	03008450505	jakramsmc@gmail.com
Prof. Israr Ahmad Akhund	Physiology	Jamshoro	0333-3691556 0333-3909264	israrakhund65@gmail.com
Prof. Kh. M. Azeem	Surgery	Lahore	03334242122	khawaja.azeem@sihs.org.pk
Prof. Khalid Masood Gondal	Surgery	Lahore	03328483823	khalidmasoodgondal@yahoo.com
Prof. M. Amjad	ENT	Lahore	03334254695	professoramjad@yahoo.com
Prof. M. Amjad Amin	Surgery	Multan	03336103262	dramjadamin@gmail.com
Prof. M. Sabir	Anatomy	Sialkot	03005183021	raosabirdr62@gmail.com
Prof. Mahmood Nasir Malik	Medicine	Lahore	03009487434	nasirphysician@yahoo.com
Prof. Majeed Ahmad Ch.	Surgery	Lahore	03008440415	prof_abdulmajeed@hotmail.com
Prof. Mian Rasheed	Forensic Medicine	Rawalpindi	03025033559	drmian1000@hotmail.com

Prof. Naseer Khan Bangulzai	Pharmacology	Khuzdar	0336-2149615 0311-6131609	naseerbaloch19@gmail.com
Prof. Pervez Akhtar Rana	Forensic Medicine	Lahore	03009422511	pzrana@gmail.com
Prof. Rukhsana Majeed	Community Medicine	Quetta	03337808138	majidrukhsana@hotmail.com
Prof. Rashid Mahmood	Physiology	Peshawar	0321-9037286	drrashid62@gmail.com
Prof. Safdar Ali Shah	Urology	Lahore	03334391474	drsafdar-ali@hotmail.com
Prof. SardarFakhar Imam	Medicine	Lahore	03008451843	drfakhar@lhr.paknet.com.pk
Prof. Shahid Mehmood	Surgery	Rawalpindi	03215001120	shahid63@gmail.com
Prof. Syed M. Awais	Orthopaedics	Lahore	03334348716	awais@kemu.edu.pk
Prof. Syed Nazim Hussain Bukhari	Medicine & Chest	Lahore	03009460515	nhbokhari@yahoo.com

International Editorial Advisory Board

Dr. Tahir Abbas	Oncology	Canada	+13067178592	drtgabbas@hotmail.com
Dr. Amjad Shad	Neurosurgery	UK	447963442419	amjad.shad@uhcw.nhs.uk
Dr. Ghazanfar Ali	Gastroenterology	UK	447800760008	ghazanfarali@hotmail.com
Dr. Haider Abbas	Urology	UK	447816149374	haidersyed@hotmail.com
Dr. Khalid Rashid	Cardiology	UK	447740477756	khalid.rashid@cht.nhs.uk
Dr. M. Shoaib Khan	Medicine	UAE	00971503111420	mkskd2000@yahoo.com
Dr. Basil Nouman Hashmi	Surgery	UK	00447806611517	basilhashmi@doctor.net.uk
Dr. Sohail Saied	Surgery	UK	00441923285114	sohailsaied@gmail.com
Dr. Safdar Ali	Cardiology	USA	0016307816668	safdarali@sbcglobal.net
Dr. Parashu Ram Mishra	Surgery & Gastroenterology	Nepal	+9779841233450	drparashuram.mishra@gmail.com
Dr. Mansoor M. Mian	Psychiatry	USA	+1 (972)375 7821	mmian2000@yahoo.com
Dr. Sohail Qureshi	Orthopaedic	UK	00447734329666	quraishisohail@yahoo.com
Dr. Mushtaq Ahmad Mughal	Orthopaedics	UK	00447971886006	mahmed01@blueyonder.co.uk
Dr. Mansoor Tahir	Radiology	UK	00447921838093	drmansoortahir@yahoo.com

Business Manager: Nayyar Zia Ch.

Legal Advisors: Kh. EjazFeroz (Barrister),
Kh. Mazhar Hassan &Firdos Ayub Ch. (Advocates)

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

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

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

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

Rate per Copy: Rs.3000.00

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

CONTENTS

Editorial

Alzheimer's Disease – Research Progress and Achievements _____	1-5
Prof. Dr. Azhar Masud Bhatti	

Original Articles

1. Repurposing Metformin to Combat Multidrug-Resistant Proteus Mirabilis in Diabetic Foot Ulcers: Insights into Antibiotic Resistance and Some ABC Efflux Pump Genes _____	6-10
1. Nahida Fitnan Ghadban 2. Hawraa Wahab Al-Kaim	
2. Prevalence and Associated Risk Factors of Tech Neck Syndrome Among Medical Students Using Digital Devices: A Cross-Sectional Study _____	11-15
1. Maryam Shan 2. Maria Iram 3. Aqsa Zahid 4. Alizeh Ejaz Pasha 5. Iqra Awan 6. Zarmeen Nadeem	
3. In-Vitro Evaluation of effect of Pre-Heating on Degree of Conversion and Surface Micro-Hardness of Bulk-Fill Composite at Different Depths _____	16-20
Shahzad Ali Shah	
4. Enhancing Nurses' Knowledge through Meleis' Transition Theory-Based Educational Program for First-Time Mothers _____	21-25
1. Eman Tammuz Mahmood 2. Salwa Hazim Al Mukhtar	
5. The Effect of Melatonin on Oxidative Biomarkers (MDA,SOD,GSH) in Patients with Stable Angina _____	26-30
1. Zahraa R. Hadi 2. Layth Jabbar	
6. The Effect of Empagliflozin on the Brain Toll Like Receptor-4 Gene Expression in the Experimental Models of Parkinson's Disease _____	31-35
1. Riham Fadhil Jawad Kadhim 2. Selman Mohammed Selman	
7. Effectiveness of Diaphragmatic Breathing Technique on Stress Reduction among Patients with Acute Heart Failure: A Quasi-Experimental Study _____	36-41
1. Amer J. Kadush 2. Diaa F. Jrood 3. Malak Hassan Sallal 4. Maath Ahmed Khalaf 5. Maher Abdulameer Atiyah	
8. Advancing Respectful Maternity Care: Evaluating Nursing Perspectives and Clinical Practices in Safeguarding Birthing Rights during Complex Labors _____	42-46
1. Doaa Sami Rashash 2. Layla Oun AlWassiti 3. Fatima Jaber Shinjar	
9. Prevalence, Biofilm-Forming Capacity and Expression of Matrix-Associated Genes in Clinical Pseudomonas Aeruginosa Isolates from Babylon, Iraq _____	47-51
1. Manar Fadhil Obaid 2. Ali Hmood Al-Saadi	
10. Role of Anisotropic Analytical Algorithm and Acura's XB Algorithm in the Treatment of Breast Cancer using Volumetric Modulated Arc Therapy Technique _____	52-56
1. Reem Aref Abd Allateef 2. Numan Salman Dawood	
11. Salivary Cortisol and the Reproductive Endocrine Profile in Infertile Iraqi Men, with Preserved Sperm Chromatin Maturity: A Cross-Sectional Study _____	57-61
1. Layla Kadhum Radeef 2. Manal Nasih Ahmed Hamdan 3. Thulfiqar Ibrahim Raheem 4. Amina Abbas Alwan 5. Ali Ibrahim Al-Dulaimi	
12. The Role of TP53 Genetic Mutation in Breast Cancer Pathogenesis _____	62-66
1. Soura Alaa Hussein 2. Ahmed Mudher Al- Khaykanee 3. Tiba A.H. Al-Rubaye	
13. Prevalence of Entamoeba Gingivalis and Trichomonas Tenax among Nursing Staff and Students: Study their Relationship with Some Health Parameters _____	67-71
1. Wijdan Dhaidan Shnain Al-Abbas 2. Kawther Alqaseer 3. Ohood Aqeed Radhi 4. Nadia Habeeb Sarhan	
14. Effectiveness of an Educational Program in Improving Knowledge of Pregnancy Danger Signs among Primigravida Women _____	72-76
1. Zuhoor Jasim Ahmed 2. Suhaila Fadhil Al-Shaikh	
15. Enhancing Empathy and Caring Behavior among Nursing Interns: An Interventional Study with Gender-Based Analysis _____	77-82
1. Ali Hamza 2. Shima Sadat Aghahosseini 3. Sarfraz Masih	

16. Effect of an Instructional Antenatal Care Program on Knowledge Enhancement among Primigravida Women _____	83-88
1. Sumaya Abdulhakeem Hasan 2. Bushra Jaber Omran AL-Rubayae	
17. The Role of Soluble Tumor Necrosis Factor Receptor II as a Prognostic Marker in Severe COVID-19 _____	89-93
1. Muayad AL-Dulaimi 2. Saleh A.H 3. Mohammad Mutlag Salih	
18. Assessment of TCF7L2 Gene rs7903146 Polymorphism as Type 2 Diabetes Genetic Risk Factor within Southern Iraqi Population _____	94-98
1. Manar Qasim Mohammed 2. Afrodet A. Saleh	
19. Study of Communication Skills among Psychiatric Nurses in Psychiatric Care Units _____	99-104
1. Hawraa Haider Al-Zghaibi 2. Muna Abdulwahab Khaleel	
20. Effectiveness of Educational Program regarding Children Mental and Personal Developmental Stages of Kindergarten Teachers Knowledge _____	105-110
1. Mohammed Hassan Aloda 2. Sahar Adham Ali	
21. Evaluation of Nurses' Knowledge regarding Correct Electrocardiographic Procedures in Medical Wards of Thi-Qar Governorate Hospitals _____	111-115
1. Hiba Jasim Hammood 2. Harith Fathi Khudhayer Al-Aubaidy 3. Nasir Muwfaq Younis	
22. Comparison of Extension of Analgesic Effect of Bupivacaine Alone and Bupivacaine with Dexmedetomidine in Caudal Anesthesia amongst Children _____	116-120
1. Ayesha Mirza 2. Amjad Nadeem	
23. Nutritional Profile and Quality of Sleep among Adult Patients on Maintenance Hemodialysis _____	121-125
1. Hina Rahim 2. Zahid Nabi 3. Humayun Rashid 4. Hifsa Akram	
24. Serum Ferritin an Indicator of Iron Deficiency Anemia in Children with Normal Hemoglobin _____	126-131
1. Hafsa Hashmi 2. Sughra Wahid 3. Maliha Akhtar Qureshi	
25. Diagnostic Accuracy of Upper Lip Bite Test Versus Modified Mallampati for Predicting Difficult Airway in Patients coming for Surgery in a Tertiary Care Hospital _____	132-137
1. Asma Zareen 2. Muhammad Zameer Rajput 3. Muhammad Ashraf	
26. Diagnostic Accuracy of Computed Tomography and Magnetic Resonance Urography in Detection of Bladder Tumors taking Histopathology as Gold Standard in Patients presenting with Hematuria at Tertiary Care Hospital Karachi _____	138-143
1. Nida Asad 2. Sana Shaikh 3. Muhammad Rehan Alamgir 4. Kashaf Anwar Arain 5. Pooja Devi 6. Munazzah Ibrar	
27. Effects of Objective Structured Clinical Examination on Clinical Competency and Self-Efficacy among Nursing Students _____	144-148
1. Muhammad Jawad Khan 2. Azeem Kaleem, Muhammad Saifullah	

Narrative Review

28. Non-Pharmacological and Non-Surgical Options to Combat Obesity _____	149-153
Abdul Qadeer Memon	

Editorial

Alzheimer's Disease – Research Progress and Achievements

Prof. Dr. Azhar Masud Bhatti

Editor-in-Chief

Introduction

Worldwide 57 million people living with dementia with nearly 10 million new cases diagnosed every year in which 60-70% are thought to have Alzheimer's disease. The most common form of dementia, Alzheimer is a brain disorder characterized by the slow erosion of a person's memory and thinking skills.

Alzheimer disease (AD) is the most prevalent type of dementia, accounting for at least two-thirds of cases in individuals aged 65 and older. AD is a neurodegenerative condition with insidious onset and progressive impairment of behavioral and cognitive functions. These functions include memory, comprehension, language, attention, reasoning, and judgment.

Scientists discover a major brain shift between ages 50 and 75, when many of the brain's original immune cells declined and were replaced by cells with more inflammatory characteristics. Researchers also found weakening of cells that help maintain the blood-brain barrier, along with widespread deterioration in the genome's three-dimensional organization.

According to CDC data, AD is ranked as the seventh leading cause of death in the United States in 2022, while COVID-19 ranked fourth. Before the COVID-19 pandemic, AD was the sixth leading cause of death following stroke¹.

The significant progress has been made in developing biomarkers for specific and early diagnosis of AD over the past decade. These biomarkers include neuroimaging markers obtained through amyloid and tau PET scans, cerebrospinal fluid (CSF), and plasma markers, such as amyloid, tau, and phospho-tau levels.² In recent years, there have been significant advancements in the development of medications that aim to moderate the progression of the disease, particularly with the discovery of new disease biomarkers.²

The symptoms of AD can vary depending on the stage of the disease. AD is classified into different stages based on the level of cognitive impairment and disability experienced by individuals. These stages include the preclinical or presymptomatic stage, mild cognitive impairment, and dementia stage. The dementia stage is further divided into mild, moderate, and severe stages. AD Stages from Preclinical to Severe Disease).³

The short-term memory loss is the initial and most common presenting symptom of typical AD. Individuals may have difficulty retaining new information while still recalling long-term memories.

Individuals may experience problem-solving, judgment, executive functioning, and organizational skills impairments following short-term memory loss.

They may struggle with tasks that require multitasking and abstract thinking. In the early stages of the disease, executive functioning impairments can range from subtle to significant. Instrumental activities of daily living such as driving, financial management, cooking, and detailed activity planning are affected relatively early in their dementia.

These early signs of cognitive decline are followed by language disorder and impaired visuospatial skills. Neuropsychiatric symptoms like apathy, social withdrawal, disinhibition, agitation, psychosis, and wandering are also common in the moderate to late stages. Difficulty performing learned motor tasks (dyspraxia), olfactory dysfunction, sleep disturbances, and extrapyramidal motor signs like dystonia, akathisia, and Parkinsonian symptoms occur late in the disease. Primitive reflexes, incontinence, and total dependence on caregivers follow this.⁴

Etiology

Alzheimer disease is characterized by gradual and progressive neurodegeneration caused by neuronal cell death. The neurodegenerative process typically begins in the entorhinal cortex within the hippocampus. Genetic factors have been identified to contribute to both early and late-onset AD.

AD is a multifactorial condition associated with many known risk factors. The most significant factor is age, with advancing age being the primary contributor. The prevalence of AD approximately doubles with every 5 years increase in age starting from age 65.

Cardiovascular diseases (CVD) are recognized as significant risk factors for AD. CVD is increasingly recognized as a modifiable risk factor for AD.⁵

Obesity and diabetes are also important modifiable risk factors for AD.⁶

Traumatic head injury, depression, smoking, family history of dementia is also included in the risk factors of AD.⁷

Epidemiology

The global prevalence of dementia was reported to be 20.3 million in 1990, and it significantly increased to 43.8 million in 2016, representing a remarkable rise of 116%. From 1990 to 2019, the incidence and prevalence of Alzheimer disease and other dementias rose by 147.95% and 160.84%, respectively. Projections indicate that the number of people affected

by dementia is expected to reach 150 million by 2050, representing a 4-fold increase.⁸

The incidence of Alzheimer's disease doubles every 5 years after age 65. Age-specific incidence rates significantly increase from less than 1% yearly before 65 to 6% per year after 85. Prevalence rates increase from 10% after the age of 65 to 40% after 85. Incidence rates of Alzheimer's disease are slightly higher for women, especially after 85.⁹

Pathophysiology

Alzheimer disease is characterized pathologically by an accumulation of abnormal neuritic plaques and neurofibrillary tangles in the brain. These pathological changes are accompanied by a loss of neurons, particularly cholinergic neurons in the basal forebrain and the neocortex.

Two prominent pathophysiological hypotheses have been proposed based on these pathological findings:¹⁰

1. **The Cholinergic Hypothesis** proposes that the reduced levels of acetylcholine (ACh) in the brain, resulting from neuronal loss in the Nucleus Basalis of Meynert, play a significant role in AD development.¹¹
2. **The Amyloid Hypothesis** is currently the most widely accepted pathophysiological mechanism for AD, especially in cases of inherited AD. The amyloid hypothesis suggests that amyloid beta (A β) peptide is derived from amyloid precursor protein (APP) through the actions of β - and γ -secretase enzymes.¹²

Brain Immune Cells Undergo a Major Midlife Shift. Between approximately ages 50 and 75, the researchers found a sharp decline in microglia that originate during embryonic development.

That result challenges a long-standing assumption that microglia established early in development remain in the brain for a person's entire life. The replacement microglia-like cells also showed stronger inflammatory signatures, raising the possibility that they could contribute to chronic inflammation in the aging brain.

"Microglia are critical for maintaining brain homeostasis,"

Alzheimer's damage may begin outside the brain. Immune cells in lymph nodes appear to kick-start inflammation. Destructive immune cells tied to Alzheimer's disease may get their marching orders from cells outside the brain rather than within it.

For years, researchers have gone all in on efforts to remove toxic tangles of proteins like amyloid and tau from the brain as a way of halting Alzheimer's, with limited success. But the immune system also plays an important role in neurodegeneration. What exactly that role is — protector or antagonist — has been unclear.

In 2023, neurologist David Holtzman and colleagues showed that immune system T cells accumulate around tau tangles — implying that they might cause damage. Halting these cells' entry into the brain reduced

inflammation, and also reduced neuronal damage, says Holtzman, of Washington University School of Medicine in St. Louis.

New study shows Alzheimer's disease can be reversed to achieve full neurological recovery—not just prevented or slowed—in animal models

For more than a century, people have considered Alzheimer's disease (AD) an irreversible illness.

In this study, the researchers used two of these mouse models: One carried multiple human mutations in amyloid processing; the other carried a human mutation in the tau protein.

Amyloid and tau pathology are two of the major early events in AD.

After finding that NAD⁺ levels in the brain declined precipitously in both human and mouse AD, the research team tested whether preventing loss of brain NAD⁺ balance before disease onset or restoring brain NAD⁺ balance after significant disease progression could prevent or reverse AD, respectively.

Histopathology

The typical histopathology of AD is characterized by the presence of 3 main elements:¹³

Neuritic Plaques: Neuritic plaques are spherical microscopic lesions featuring a core of extracellular amyloid beta-peptide (A β) surrounded by enlarged axonal endings.

Neurofibrillary Tangles: Neurofibrillary tangles are fibrillary intracytoplasmic structures that form inside neurons and are composed of a protein called tau.

Cortical Neuronal Degeneration: Among these degenerative changes, granulovacuolar degeneration of hippocampal pyramidal neurons is commonly observed.

Evaluation of Alzheimer disease

From a primary care provider standpoint, when evaluating a patient suspected to be suffering from AD, the tasks are essential like review and confirm the medical and family history, review the patient's medication list, perform a bedside cognitive assessment test and blood tests to rule out any reversible causes of dementia.

Routine laboratory tests do not reveal any specific abnormalities in AD. Complete blood count (CBC), complete metabolic panel (CMP), thyroid-stimulating hormone (TSH), and vitamin B12 levels are commonly conducted to exclude other potential causes of cognitive impairment.¹⁴

A brain computed tomography (CT) may reveal findings of cerebral atrophy and a widened third ventricle in individuals with AD.

More recently, volumetric MRI has emerged as a valuable tool for precisely measuring changes in brain volume. In AD, volumetric MRI can reveal shrinkage in the medial temporal lobe, particularly the hippocampus. This hippocampal atrophy is considered a characteristic feature of AD and is associated with memory decline.

Functional brain imaging techniques such as Positron Emission Tomography (PET), functional MRI (fMRI),

and Single-Photon Emission Computed Tomography (SPECT) are becoming increasingly valuable in mapping patterns of dysfunction in smaller brain areas of the medial temporal and parietal lobes.

Neuropsychological testing is the most reliable method for detecting mild cognitive impairment (MCI) in its early stages clinically.

Cerebrospinal fluid (CSF) analysis is a helpful diagnostic tool in the preclinical stage of AD. It serves as a biomarker for AD pathology and can detect changes well before the onset of overt clinical symptoms. However, it does not help determine the severity of AD.

Biomarkers for Alzheimer's disease

In the past year, blood tests for Alzheimer's have improved significantly, notably due to the incorporation of measurements of a specific phosphorylated form of the tau protein known as p-tau217.

The blood test results outperformed the accuracy of brain imaging and were comparable to results using the current standard test — spinal fluid biomarkers. Notably, the p-tau217 assay was highly accurate in identifying tau pathology in individuals with brain amyloid plaques. This suggests that this assay may help screen participants for anti-amyloid immunotherapies.

The ADHD link

What they found was that the ADHD adults had more iron in certain regions of their brain, along with higher levels of neurofilaments in their blood, both of which are markers for dementia and Alzheimer's. While it was a small case study, it nevertheless paves the way for further research to determine if a reduction of iron levels in the brain of a person with ADHD could reduce the likelihood of developing dementias like Alzheimer's down the line.

Treatment / Management of Alzheimer disease

Symptomatic Therapies of AD

Traditionally, there has been no cure for AD, and symptomatic treatment remains the primary approach in everyday clinical practice.¹⁵

Two categories of drugs are approved for treating AD: cholinesterase inhibitors and partial N-methyl D-aspartate (NMDA) antagonists.

Cholinesterase Inhibitors

Cholinesterase inhibitors function by increasing the levels of acetylcholine, a neurotransmitter that plays a role in nerve cell communication and is involved in learning, memory, and cognitive functions. Within this category, 3 drugs have received FDA approval for treating AD: **Donepezil**, **Rivastigmine**, and **Galantamine**.¹⁶

Partial N-Methyl D-Aspartate (NMDA) Memantine

Partial N-Methyl D-aspartate (NMDA) antagonist memantine blocks NMDA receptors, slowing down intracellular calcium accumulation.

Disease Modifying Therapies of AD

In the past, the treatment of AD has primarily focused on managing the symptoms. Now a days, new and

specific disease-modifying therapies are being developed and FDA has recently approved some drugs for example:

- **Aducanemab** received accelerated FDA approval in June 2020. It was shown to reduce amyloid-beta plaque in the brain. However, it missed the primary phase III trial endpoint of clinical improvement.¹⁷
- **Lecanemab** received accelerated FDA approval in January 2023. It reduced the amyloid-beta burden in the brain. The phase III trial showed a 27% slowing of disease progression.¹⁸
- **Donanemab** is expected to receive FDA approval in 2023. It reduced the amyloid-beta burden in the brain and slowed cognitive decline by 35%.¹⁹ About the above-said drugs Alzheimer's Society found that the drugs could slow down disease progress by 60% if given at early stage.

Other Management Strategies in AD

In managing AD, addressing accompanying symptoms such as anxiety, depression, and psychosis is essential, especially in the mid to late stages of the disease.

It is advisable to avoid tricyclic antidepressants because of their anticholinergic activity, which can worsen cognitive impairment. Antipsychotic medications should be used cautiously for acute agitation when other interventions have been exhausted and the patient's or caregiver's safety is at risk. Other medications are generally tried before consideration of antipsychotics. These generally include SSRI antidepressants such as **Citalopram** and **Anticholinesterase** such as **Donepezil**.

The general principle in using antipsychotics is to use the lowest dose that may help the patient's agitation. Benzodiazepines should not be used because they may worsen delirium and agitation in these patients.

Preventing and treating Alzheimer's disease and related dementias

In recent years, there has been significant progress for the treatment of early Alzheimer's, marked by the U.S. Food and Drug Administration's (FDA) approval of the use of specific antibodies against beta-amyloid (termed "anti-A β " or "anti-amyloid immunotherapies") to slow progression of the disease. While NIH did not fund the clinical trials that led to these approvals. Although the FDA approvals of these therapies for the treatment of early Alzheimer's represent a significant scientific milestone, further research is needed to develop additional interventions that are effective for treatment and prevention of disease for all who are at risk.

NIH is investing in a range of potential therapeutics to treat and prevent dementia.

NIH investments to develop new treatments for dementia explore a wide range of therapeutic approaches and support multiple stages of drug development research, from basic science to clinical trials.

For example, NIH has funded nearly a decade of research leading to the development of a small

molecule drug, called **CT1812**, that shows promise for treating multiple types of dementia. NIH-funded basic and preclinical research has shown that **CT1812** may help prevent neurotoxicity associated with dementia by displacing toxic protein aggregates at synapses, the spaces between brain cells that facilitate communication.

NIH also invests the drive research progress in drug development.

- Development status of drug candidates in clinical trials:
 - Phase I trials: 18
 - Phase II trials: 6
 - Phase III trials: 1 (**buntanetap**)

Buntanetap development and testing: A journey of public and private sector collaborative discovery

- Buntanetap (also known as posiphen) is an orally administered drug candidate that inhibits the production of key proteins involved in the pathogenesis of Alzheimer's, Parkinson's disease, and LBD.

Gene therapies

As a potentially innovative way to prevent or treat dementia, NIH is investing in research on gene therapy, an emerging technology that treats or reverses conditions by correcting problems with DNA.

Anti-amyloid immunotherapies

In parallel to trials testing a range of potential therapeutic options, NIH also seeks to better understand how to effectively and safely use the current FDA-approved anti-amyloid immunotherapies, i.e., lecanemab and donanemab. Efforts include studying the effects of these drugs at different stages of disease, in different populations, and in combination with other potential treatments.

New Blood Thinner Benefits for AD

These blood thinners may have an unexpected benefit for Alzheimer's patients. Newer blood thinners may do more than prevent strokes in people with Alzheimer's disease and atrial fibrillation. Researchers found that patients taking NOACs experienced a slower decline in cognitive function than those using warfarin or no blood thinners. The effect was modest each year but could add up over time.

People who have both atrial fibrillation and Alzheimer's disease may experience slower cognitive decline when treated with newer NOAC blood thinners, according to research from Karolinska Institutet published in the European Heart Journal.

Atrial fibrillation is a common heart rhythm condition in older adults and also affects many people living with Alzheimer's disease.

Behavioral, lifestyle, and other interventions

Multiple lines of research find that several forms of non-pharmacological interventions may also offer promise in reducing dementia risk factors and improving cognition and memory. Results from

randomized control trials indicate that interventions with blood pressure control, hearing aids, multivitamins, and personalized health coaching can reduce dementia risk in specific populations, but more research is needed.

NIH researchers are also continuing to build on past scientific discoveries on the link between diet and dementia risk. As one example, NIH-funded investigators found that individuals who consumed a modified Mediterranean ketogenic diet, composed of 5–10% carbohydrate, 60–65% fat, and 30% protein, for six weeks had significant changes in both blood and spinal fluid biomarkers associated with Alzheimer's risk. Specifically, individuals following this diet exhibited improvements in several risk factors, including increases in beneficial (high density lipoprotein, or HDL) cholesterol and reductions in body mass index and systemic inflammation. This study is the first exploration of diet-related metabolic changes in spinal fluid and provides evidence of the effects of diet on modifiable risk factors for Alzheimer's.

REFERENCES

1. Ahmad FB, Cisewski JA, Xu J, Anderson RN. Provisional Mortality Data - United States, 2022. *MMWR Morb Mortal Wkly Rep* 2023; 72(18):488-492.
2. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatr* 2021; 26(1):296-308.
3. Porsteinsson AP, Isaacson RS, Knox S, Sabbagh MN, Rubino I. Diagnosis of Early Alzheimer's Disease: Clinical Practice in 2021. *J Prev Alzheimers Dis* 2021;8(3):371-386.
4. Tang Y, Lutz MW, Xing Y. A systems-based model of Alzheimer's disease. *Alzheimers Dement* 2019;15(1):168-171.
5. Santos CY, Snyder PJ, Wu WC, Zhang M, Echeverria A, Alber J. Pathophysiologic relationship between Alzheimer's disease, cerebrovascular disease, and cardiovascular risk: A review and synthesis. *Alzheimers Dement (Amst)* 2017;7:69-87.
6. Anjum I, Fayyaz M, Wajid A, Sohail W, Ali A. Does Obesity Increase the Risk of Dementia: A Literature Review. *Cureus* 2018; 10(5):e2660.
7. Nicolas G, Acuña-Hidalgo R, Keogh MJ, Quenez O, Stehouwer M, Lelieveld S, et al. Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer's disease. *Alzheimers Dement* 2018;14(12): 1632-1639.
8. Li X, Feng X, Sun X, Hou N, Han F, Liu Y. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2019. *Front Aging Neurosci* 2022; 14:937486.

9. Qiu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. *Dialogues Clin Neurosci* 2009;11(2):111-28.
10. Breijyeh Z, Karaman R. Comprehensive Review on Alzheimer's Disease: Causes and Treatment. *Molecules* 2020;25(24).
11. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* 2018; 141(7):1917-1933.
12. Paroni G, Bisceglia P, Seripa D. Understanding the Amyloid Hypothesis in Alzheimer's Disease. *J Alzheimers Dis* 2019;68(2):493-510.
13. Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological alterations in Alzheimer disease. *Cold Spring Harb Perspect Med* 2011;1(1):a006189.
14. Haapasalo A, Hiltunen M. A report from the 8th Kuopio Alzheimer Symposium. *Neurodegener Dis Manag* 2018;8(5):289-299.
15. Hussein W, Sağlık BN, Levent S, Korkut B, Ilgin S, Özkay Y, et al. Synthesis and Biological Evaluation of New Cholinesterase Inhibitors for Alzheimer's Disease. *Molecules* 2018;23(8).
16. Kandiah N, Pai MC, Senanarong V, Looi I, Ampil E, Park KW, et al. Rivastigmine: the advantages of dual inhibition of acetylcholinesterase and butyrylcholinesterase and its role in subcortical vascular dementia and Parkinson's disease dementia. *Clin Interv Aging* 2017;12:697-707.
17. Padda IS, Parmar M. StatPearls [Internet]. StatPearls Publishing; Treasure Island (FL): Aducanumab; Feb 26, 2024.
18. van Dyck CH, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in Early Alzheimer's Disease. *N Engl J Med* 2023;388(1): 9-21.
19. Rashad A, Rasool A, Shaheryar M, Sarfraz A, Sarfraz Z, Robles-Velasco K, et al. Donanemab for Alzheimer's Disease: A Systematic Review of Clinical Trials. *Healthcare (Basel)* 2022;11(1).

Repurposing Metformin to Combat Multidrug-Resistant *Proteus Mirabilis* in Diabetic Foot Ulcers: Insights into Antibiotic Resistance and Some ABC Efflux Pump Genes

Metformin
against *Proteus*
Mirabilis isolated
from Diabetic
Foot Ulcers

Nahida Fitnan Ghadban and Hawraa Wahab Al-Kaim

ABSTRACT

Objective: To evaluate the antibiotic resistance profile, distribution of ABC efflux pump genes, and the in vitro antibacterial activity of metformin against *Proteus mirabilis* isolated from diabetic foot ulcers.

Study Design: Cross-sectional laboratory-based study

Place and Duration of Study: This study was conducted at the Department of Biology, College of Science for Women, University of Babylon, in collaboration with Al-Marjan Teaching Hospital and Al-Kafeel Specialized Hospital, Iraq, from January 2026 to April 2026.

Methods: Clinical specimens from 188 patients with diabetic foot ulcers were cultured for bacterial isolation. *Proteus mirabilis* isolates were identified using standard microbiological methods. Antibiotic susceptibility testing was performed according to CLSI guidelines. The *macA*, *macB*, and *tolC* genes were detected by PCR, while the antibacterial activity and minimum inhibitory concentration (MIC) of metformin were determined using the broth microdilution method.

Results: Of 286 bacterial isolates recovered, 30 (10.49%) were identified as *P. mirabilis*. High multidrug resistance (MARI 0.38–0.84) was observed. The *macA*, *macB*, and *tolC* genes were detected in 90%, 86.7%, and 100% of isolates, respectively. Metformin exhibited dose-dependent antibacterial activity with an MIC of 100 mg/mL.

Conclusion: MacAB-TolC efflux pumps are strongly associated with antimicrobial resistance in *P. mirabilis* isolated from diabetic foot ulcers. Metformin demonstrated promising adjunctive antibacterial activity and warrants further investigation as a potential therapeutic strategy.

Key Words: *P. mirabilis*; Diabetic foot ulcer; *macA*; *macB*; *tolC*; Metformin

Citation of article: Ghadban NF, Al-Kaim HW. Repurposing Metformin to Combat Multidrug-Resistant *Proteus Mirabilis* in Diabetic Foot Ulcers: Insights into Antibiotic Resistance and Some ABC Efflux Pump Genes. Med Forum 2026;37(9):6-10. doi:10.60110/medforum.370901.

INTRODUCTION

DFUs are a serious complication of diabetes, affecting approximately 18.6 million people annually and associated with a five-year mortality rate exceeding 70%⁽¹⁾. These ulcers are often accompanied by multimicrobial infections due to factors such as weakened immunity and poor blood supply⁽²⁾.

P. mirabilis is commonly found in cultures of chronic DFUs⁽³⁾, and the therapeutic challenge is compounded by the increasing number of multidrug-resistant (MDR)

strains, contributing to a global antibiotic resistance crisis projected to cause up to 10 million deaths annually by 2050⁽⁴⁾. ATP efflux pumps play a crucial role in actively exporting antimicrobial agents and reducing intracellular drug concentrations. Overexpression of these pumps contributes to treatment failure and the emergence of MDR patterns⁽⁵⁾.

Drug repurposing can identify new antimicrobial applications for existing medications. Metformin, primarily used to treat type 2 diabetes, shows promise due to its anti-inflammatory and antibacterial effects. Research suggests it may enhance antibiotic efficacy, modulate bacterial metabolism, reduce biofilm, and boost the immune response, indicating its potential use as an adjunctive therapy against pathogens^(6,7).

Given the join between DFUs and *P. mirabilis* infection, metformin is a promising biological applicant as an adjuvant and warrants further evaluation. Particular the limited molecular data on ABC efflux pump in *P. mirabilis* from Iraqi DFU patients, the current study aimed to characterize the molecular distribution of *macA*, *macB* and *tolC* genes among these isolates, and evaluate antibacterial activity of metformin, in vitro.

Department of Biology, College of Science for Women, University of Babylon, Iraq.

Correspondence: Hawraa Wahab Al-Kaim, Department of Biology, College of Science for Women, University of Babylon, Iraq.

Contact No: +964 782 264 6627

Email: wsci.hawraa.w@uobabylon.edu.iq

ORCID: <https://orcid.org/0000-0001-6528-4911>

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

METHODS

Study design

This study included collecting 188 clinical samples from patients suffering from DFUs at Al-Marjan Teaching and Al-Kafeel Specialized Hospitals in Iraq, during the period between January 2026 to April 2026.

Bacterial identification

Wound specimens were collected from patients with DFUs. Each specimen was directly cultured onto a range of suitable media and incubated at 37°C for 18-24 h. Colonies were identified based on their morphology with initial identification using standard microbiological procedures. Final bacterial identification was confirmed using the VITEK® 2 Compact (bioMérieux, France) according to the manufacturer's instructions.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for *P. mirabilis* was achieved using the disk diffusion technique, according to CLSI guidelines. The method included using of 13 antibiotics (Information regarding antibiotics will be provided in detail in the results section). The Multiple-Antibiotic Resistance Index (MARI) is calculated using the formula A/B, where A represents the number of antibiotics the isolate is resistant to, and B is the total number of antibiotics studied. A MARI value of 0.2 or higher indicates exposure to high-risk antibiotic sources, while a value < 0.2 indicates infrequent or no antibiotic use⁽⁸⁾.

DNA Extraction

The DNA extraction kit from Mebep Bioscience (Mebep Bioscience/China) was used to separate genomic DNA from *P. mirabilis* isolates according to the manufacturer's protocol.

Polymerase Chain Reaction (PCR)

All *P. mirabilis* isolates underwent molecular characterization by detecting the *macA*, *macB*, and *tolC* genes using specific primer pairs (Table 1). DNA amplification was achieved in a 25 µL reaction mixture comprising forward/reverse primers (10 pmol/1 µL each), template DNA (50–500 ng/4 µL), nuclease-free water (6.5 µL), and Accustart™ Taq PCR SuperMix (12.5 µL). PCR products (5 µL) were observed using electrophoresis on agarose gel, stained with a safe red dye, exposed under UV-transilluminator and photographed, the sizes of the products were determined using a 100-base pair DNA ladder^(9,10).

Determination of the Minimum Inhibitory Concentration (MIC) for Metformin

This study evaluated the MIC and efficacy of metformin using a 96-well microplate method⁽¹¹⁾. Bacterial inoculum were added to each well, with negative controls (broth only) to ensure sterility and positive controls to confirm bacterial growth in the absence of metformin. Test rows included different metformin concentrations (12.5, 25, 50, 100, and 200 mg/mL) and were incubated at 37°C for 18 h, after which the optical density (OD) was measured to determine the MIC.

RESULTS

Distribution of Bacterial Isolates Recovered from DFUs samples

Through microbiological examination of the 188 DFU samples, 286 bacterial isolates were obtained, 10.49% were of *P. mirabilis*, and 89.51% were of other bacterial species (*S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *E. faecalis*), as shown in Figure (1).

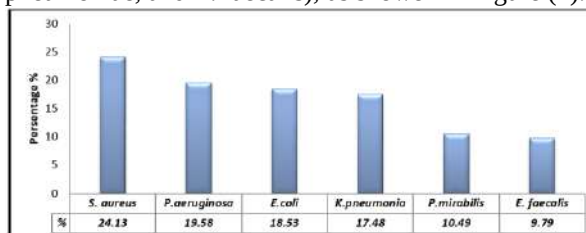


Figure No.1: Distribution of Bacterial Isolates Recovered from DFUs specimens

Resistance Profiles of *P. mirabilis*: Tests conducted on 13 antibiotics against *P. mirabilis* isolates showed that, not exhibit resistance to meropenem and levofloxacin, while resistance rates to amikacin, cefotaxime, chloramphenicol, tetracycline, doxycycline, and ceftazidime were significantly high. All strains were resistant to amoxicillin-clavulanate (Table 2).

ABC Efflux Pump Genes: The distribution of ABC efflux pump genes among the isolates showed that the *tolC* gene was present in 100% of the 30 isolates tested. Additionally, 90% of the samples were positive for the *macA* gene and 86.7% for the *macB* gene (Figure 2). Gel analysis confirms these findings; the *macA* gene was found at 255 bp, the *macB* gene at 364 bp, and the *tolC* gene amplifier at 596 bp, consistent with the lengths predicted from the primers used in the study (Figures 3).

Table No. 1: The PCR conditions and Primers sequences for genes

Primer	Sequence(5' - 3')	Product (bp)	Annealing (°C)	Ref.
MacA	F: GCACAACAAGCACCGAACAT R:CATATCCAGCCGCAGCAAAC	255	58.3°C	(12)
MacB	F: GCGTGAGCGGCGATTATTTT R: AAACGCGTGAGTTGCTGTTC	364	57.2°C	
TolC	F: CGATCGTGATGCTGCCTTTG R: GGTTCGTTTTTCGGCTTCT	596	58.3°C	

Table No. 2: Antibiotic resistance patterns of *P. mirabilis* (n=30)

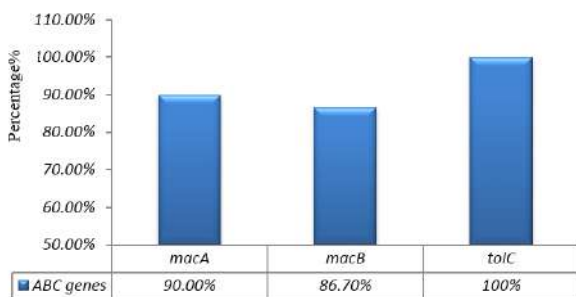
Antibiotics agents	Sensitive (n/%)		Resistance (n/%)		Total (n/%)
TE/Tetracycline 30 µg	1	3%	29	97%	30 (100%)
DXT/Doxycycline 30 µg	1	3%	29	97%	30 (100%)
CAZ/ ceftazidime 30 µg	1	3.3%	29	97%	30 (100%)
CTX/Cefotaxime 30 µg	2	7%	28	93%	30 (100%)
CN/gentamicin 10 µg	13	43%	17	57%	30 (100%)
AK/amikacine 30 µg	5	17%	25	83%	30 (100%)
LEV/levofloxacin 5 µg	27	90%	3	10%	30 (100%)
OFX/ ofloxacin 5 µg	26	87%	4	13%	30 (100%)
AMC/Amoxicillin-clavulanate 20/10 µg	0	0%	30	100%	30 (100%)
ATM/ Aztreoname 30 µg	24	80%	6	20%	30 (100%)
C/Chloramphenicol 30 µg	2	7%	28	93%	30 (100%)
IPM/ imipenem 10 µg	24	80%	6	20%	30 (100%)
MEM/meropenem 10 µg	29	97%	1	3%	30 (100%)

The MARI revealing resistance levels ranging from 0.38 to 0.84 (Table 3). All isolates (100%) had MARI values > 0.2.

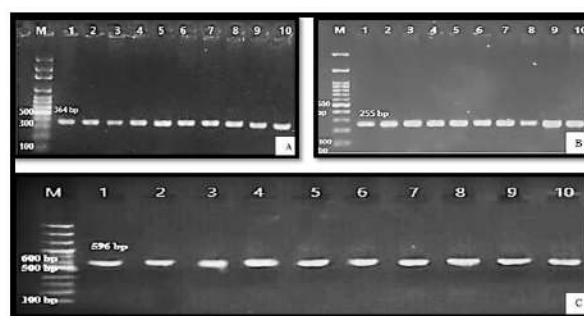
Table No.3: Multiple antibiotic resistance index values of *P. mirabilis* isolates (n=30).

Isolate code	MARI	n (%)
PM 3	0.38	1 (3.3)
PM 2, PM 6, PM 12, PM 28	0.46	4 (13.4)
PM1, PM4, PM5, PM9, PM17-23, PM27	0.53	12 (40)
PM7, PM10, PM13, PM15, PM16, PM24-26, PM 30	0.61	9 (30)
PM29	0.64	1 (3.3)
PM11, PM14	0.76	2 (6.7)
PM8	0.84	1 (3.3)

PM, *P. mirabilis*

**Figure No.2: Percentage of ABC efflux pump genes in *P. mirabilis*.**

The study demonstrated a strong association between antibiotic resistance in *P. mirabilis* and the *macA*, *macB*, and *tolC* genes, with detection rates ranging from 75% to 100%. The *tolC* gene was present in all resistance groups (100%). The significance of these results was high ($P < 0.0001$), indicating stable gene expression among isolates. However, no significant changes in gene distribution were observed among the different antibiotic resistance classes ($P = 0.818$ to 1.000).

**Figure No.3: Agarose gel electrophoresis of A; *macB* (364bp), B; *macA* (255bp), C; *tolC* (596bp) genes amplicon.**

Determination of Metformin Minimum Inhibitory Concentrations (MIC): This study investigated the effect of metformin on the growth of ten MDR *P. mirabilis* isolates at concentrations ranging from 12.5 to 200 mg/ml, using OD at 625 nm (Figure 4). The results indicate inhibition of bacterial growth in dose-dependent manner: at positive control: Maximum growth ($OD_{625} \approx 1.34-1.52$), at 12.5 mg/ml: Moderate inhibition ($\approx 0.82-0.97$). At 25 mg/ml: Further

reduction ($\approx 0.58-0.74$), at 50 mg/ml: Significant inhibition ($OD_{625} \approx 0.23-0.44$), at 100 mg/ml: Strong inhibition ($\approx 0.18-0.30$). At 200 mg/ml: no growth was recorded ($\approx 0.05-0.07$), equal to negative control. The MIC was found to be 100 mg/ml after sub- culturing.

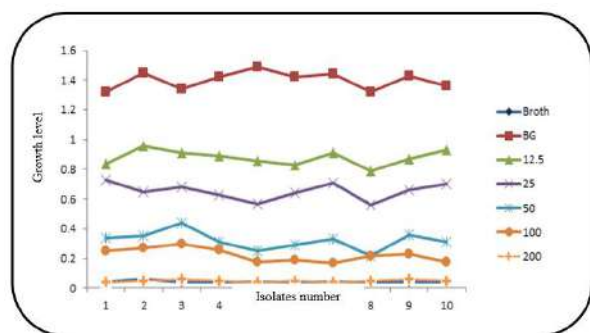


Figure No.4: Result of OD to bacterial growth with different concentration of metformin (12.5-200 mg/ml) at 625 nm. Control (BG): growth of *P.mirabilis* without drug. (n= 10)

DISCUSSION

The bacterial profile of current study is consistent with findings from studies conducted in Iraq, where *S. aureus* emerged as the most frequently isolated bacterial species, followed by *P. mirabilis* and then *E. coli*, indicating a similar distribution of pathogens in DFUs⁽¹²⁾. This aligns with the recommendations of the International DF Working Group/Infectious Diseases Society of America (IDWA), which classifies *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *P. mirabilis* as common pathogens in moderate to severe DFUs⁽¹³⁾. The high proportion of isolated Gram-negative bacteria may be attributed to chronic ulceration, repeated antibiotic therapy, prolonged hospital stays, and compromised patient immunity. It has been observed that *P. mirabilis* carries many virulence genes with resistance factors, and identified as one of the predominant Gram-negative pathogens associated with DFUs worldwide^(14, 15).

The current study indicate resistance rates ranging from 3 to 100%, characterizing these isolates with MDR phenotype, which is particularly concerning in DFU infections treated without prior culture⁽¹⁶⁾. Notably, complete resistance to amoxicillin-clavulanate and tetracyclines, along with 57% resistance to gentamicin, possibly due to specific aminoglycoside-modifying enzymes is consistent with previous findings in Iraqi clinical settings⁽¹⁷⁾. Carbapenemase-producing *P. mirabilis* have been reported regionally in cases of DFUs, with non-producing strains achieving reduced susceptibility through overexpression of the efflux pumps⁽¹⁸⁾.

The high values of MARI suggesting significant selective pressure resulting from inappropriate or prolonged antibiotic use confirming their MDR nature. This aligns with previous research on DFUs, which

highlighted elevated MARI and MDR resulting from intensive antibiotic exposure⁽¹⁹⁾. Current international guidelines recommend that antibiotic treatment for DFU infections be based on culture and susceptibility testing to enhance treatment efficacy and reduce the spread of antibiotic resistance⁽²⁰⁾. Antibiotic resistance is meaningfully associated with efflux pumps, which showed high prevalence rates in this study, consistent with local Iraqi evidence. At the international level, genomic characterization of the *P. mirabilis* SCDR1 strain revealed the co-occurrence of the MacAB-TolC pumps, which prone to overexpression under sustained antibiotic pressure. Our study demonstrated that ABC antibiotic ejection pump genes are broadly expressed across different classes of antibiotics; no specific antibiotic resistance pattern significantly affected their gene expression. The genes responsible for antibiotic resistance in *P. mirabilis* are essential for its survival under the stressful conditions of diabetic foot. The absence of the MacA, MacB, and tolC genes impairs the formation of a macrolide-specific export system, as supported by genomic data⁽²⁰⁾.

CONCLUSION

Current research underscores the importance of *P.mirabilis* in DFUs and calls for local antibiotic treatment guide in Iraq. It also highlights a research gap, noting that many studies focus solely on antibiotic susceptibility, neglecting other non-antibacterial agents such as metformin. The study suggests a reassessment of metformin as an adjunctive antimicrobial therapy and urges further research into its molecular interactions with antibiotics and their combined efficacy in patient care.

Acknowledgments: We express our gratitude to the College of Science for Women and the Department of Biology at the University of Babylon for their support, to the medical staff for allowing the collection of samples, and to the patients who participated in the research.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Nahida Fitnan Ghadban, Hawraa Wahab Al-Kaim
Drafting or Revising Critically:	Nahida Fitnan Ghadban, Hawraa Wahab Al-Kaim
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. BHD-2025- B251007(71)/QM/ Approval/JSDJNEHU Dated 05.01.2026

REFERENCES

1. Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic foot ulcers: A review. *JAMA* 2023;330(1): 62-75. doi: <https://doi.org/10.1001/jama.2023.10578>
2. Wang Q, Liu C, An J, Liu J, Wang Y, Cai Y. Mechanisms of microbial infection and wound healing in diabetic foot ulcer: pathogenicity in the inflammatory proliferative phase, chronicity, and treatment strategies. *Front Endocrinol* 2025;16:1657928. doi:10.3389/fendo.2025.1657928
3. Tirumala N, Rani K L. Bacteriological profile of diabetic foot ulcer with special reference to biofilm formation. *Cureus* 2025;17(3):e80974. doi: <https://doi.org/10.7759/cureus.80974>
4. Algammal AM, Hashem HR, Alfifi KJ, Hetta HF, Sheraba NS, Ramadan H, et al. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. *Scientific Reports* 2021;11:9476.
5. Huo T, Zheng W, Yu Z, Wu Y, Ren Q, Cao J, et al. In situ structure and assembly of the ABC-type tripartite pump MacAB-TolC. *Communications Biol* 2025;8:851. doi: <https://doi.org/10.1038/s42003-025-08236-z>
6. Pryor R, Cabreiro F. Repurposing metformin: An old drug with new tricks in its binding pockets. *Biochem J* 2015;471(3):307–322. doi: <https://doi.org/10.1042/BJ20150497>
7. Foretz M, Guigas B, Viollet B. Metformin: Update on mechanisms of action and repurposing potential. *Nature Reviews Endocrinol* 2023;19(8):460–476. doi: <https://doi.org/10.1038/s41574-023-00833-4>
8. Ayandele AA, Oladipo EK, Oyebisi O, Kaka MO. Prevalence of multi-antibiotic resistant *Escherichia coli* and *Klebsiella* species obtained from a tertiary medical institution in Oyo State, Nigeria. *Qatar Med J* 2020;9:1-6.
9. Sambrook J, Russell D. *Molecular Cloning: A laboratory manual.* (3-Volume Set). *Immunol* 2001;49:895-909.
10. Ali SA, Al-Dahmoshi HOM. Molecular study on Efflux pumps of *Klebsiella pneumoniae* Isolated from patients with Cystitis. *Research J Pharm Tech* 2022;15(10):4559-4564.
11. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens* 2021;10(2):165.
12. Mahmoud H, Abid Ali S, Jaddue D. Prevalence of bacterial types in Wagner grade three of diabetic foot ulcer. *Iraqi J Science* 2023;55(3B):1232–1235.
13. Senneville É, Albalawi Z, van Asten SA, Abbas ZG, Allison G, Aragón-Sánchez J, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF/IDSA 2023). *Diabetes/Metabolism Research and Reviews* 2024;40(3):e3687. doi: <https://doi.org/10.1002/dmrr.3687>
14. Hamid SF, Taha AB, Abdulwahid MJ. Distribution of blaTEM, blaSHV, blaCTX-M, blaOXA, and blaDHA genes in *Proteus mirabilis* isolated from diabetic foot infections in Erbil, Iraq. *Cellular and Molecular Biol* 2020;66(1):75–81.
15. Maity S, Leton AK, Nayak S, Jha AK, Anand A, Thompson B, et al. A systematic review of diabetic foot infections: Pathogenesis, diagnosis, and management strategies. *Frontiers Clinical Diabetes and Healthcare* 2024;5:1393309.
16. Uçkay I, Gariani K, Dubois-Ferrière V, Suvà D, Lipsky BA. Diabetic foot infections: recent literature and cornerstones of management. *Current Opinion in Infectious Diseases* 2016;29:145–152.
17. Jaber AH, Almiyah SAF. Antibiotic susceptibility of *Proteus mirabilis* isolates of diabetic foot ulcers in Al-Diwaniyah Hospital. *Al-Qadisiyah J Pure Sci* 2022;27(1):15-25. doi: <https://doi.org/10.29350/qjps.2022.27.1.1528>
18. Saleem M, Moursi SA, Altamimi TNA, Alharbi MS, Alaskar AM, Hammam SAH, et al. Prevalence and Molecular Characterization of Carbapenemase-Producing Multidrug-Resistant Bacteria in Diabetic Foot Ulcer Infections. *Diagnostics* 2025;15:141. doi: <https://doi.org/10.3390/diagnostics15020141>
19. Shoaib NNM, Chitra E, Ong J R, et al. Predominance of multidrug-resistant bacteria with high resistance to empiric antibiotics in diabetic foot ulcers: A cross-sectional study. *Scientific Reports* 2026;16:13043. doi: <https://doi.org/10.1038/s41598-026-42418-x>
20. Lipsky BA, Senneville É, Abbas ZG, Aragón-Sánchez J, Diggle M, Embil JM, et al. Guidelines on the diagnosis and treatment of diabetes-related foot infections. *Clin Infectious Dis* 2023 (IWGDF 2023 update)

Prevalence and Associated Risk Factors of Tech Neck Syndrome Among Medical Students Using Digital Devices: A Cross-Sectional Study

Prevalence Of Neck Pain And Evaluate Its Association With Digital-Device Use

Maryam Shan¹, Maria Iram², Aqsa Zahid², Alizeh Ejaz Pasha³, Iqra Awan³ and Zarmeen Nadeem³

ABSTRACT

Objective: To determine the prevalence of neck pain and evaluate its association with digital-device use, ergonomic practices, and Tech Neck Syndrome among undergraduate medical students.

Study Design: Descriptive cross-sectional study

Place and Duration of Study: This study was conducted at the Anatomy Department, Rawal Institute of Health Sciences, Islamabad from 1st March 2026 till 30th May 2026

Methods: A descriptive cross-sectional study was conducted among 100 undergraduate medical students using a structured self-administered questionnaire. Information regarding demographic characteristics, digital-device use, ergonomic practices, physical activity, sleep duration, and neck-related symptoms was collected. Data were analyzed using IBM SPSS Statistics version 26. Descriptive statistics were presented as frequencies and percentages. Associations between neck pain and categorical variables were assessed using Pearson's Chi-square test or Fisher's exact test where appropriate. A p-value <0.05 was considered statistically significant.

Results: Among 100 participants, 48% reported neck pain and 51% reported no neck pain. Smartphones were the predominant device (97%). Significant associations with neck pain were observed for screen position ($\chi^2=111.102$, $p<0.001$), taking regular breaks ($\chi^2=35.015$, $p<0.001$), frequency of neck pain episodes ($\chi^2=17.921$, $p=0.001$), and neck and shoulder stiffness ($\chi^2=11.726$, $p=0.003$). No significant associations were observed for device type, daily screen time, purpose of use, posture, device position, back support, physical activity, sleep duration, mobile-phone use before sleep, or previous history of neck pain.

Conclusion: Neck pain was common among the medical students studied. Specific ergonomic factors, particularly screen position and taking regular breaks, were significantly associated with neck pain. Ergonomic awareness and healthy device-use practices should be encouraged.

Key Words: Tech Neck Syndrome, Neck Pain, Digital Devices, Ergonomics, Medical Students, Smartphone

Citation of article: Shan M, Iram M, Zahid A, Pasha AE, Awan I, Nadeem Z. Prevalence and Associated Risk Factors of Tech Neck Syndrome Among Medical Students Using Digital Devices: A Cross-Sectional Study. Med Forum 2026;37(9):11-15. doi:10.60110/medforum.370902.

INTRODUCTION

The present study was conducted to determine the prevalence of neck pain and to assess its association with digital-device use, ergonomic practices and selected lifestyle factors among undergraduate medical students¹.

¹. Professor and HOD Anatomy, Rawal Institute of Health Sciences, Islamabad.

². Assistant Professor Anatomy / Demonstrator Anatomy³, Rawal Institute of Health Sciences, Islamabad.

Correspondence: Prof. Dr. Maryam Shan, Professor and HOD Anatomy, Rawal Institute of Health Sciences, Islamabad.
Contact No: 03215185830
Email: drmaryamshan@gmail.com

Received: June, 2026
Reviewed: July, 2026
Accepted: August, 2026

Neck pain is a common musculoskeletal complaint that may affect individuals at different stages of life. Although it can begin during childhood or adolescence, its occurrence often follows a recurrent or episodic pattern throughout adulthood, similar to the clinical course observed with low back pain². Previous research among university and medical students has demonstrated a considerable burden of neck and musculoskeletal symptoms associated with digital-device use.³ Gao et al. identified multiple risk factors for neck pain among college students, highlighting the multifactorial nature of this condition in young adults.⁴ The epidemiology of neck pain is also well established in the general population, with multiple demographic, physical and psychosocial factors contributing to its occurrence.⁵ However, local evidence concerning digital-device-related neck symptoms among Pakistani medical students remains limited⁶. Ergonomic practices, including appropriate screen positioning and interruption of prolonged static activity, are potentially

modifiable factors that may be addressed through health education. Nevertheless, the relationship between individual device-use practices and neck pain may vary according to population characteristics, exposure patterns and study methodology².

Tech Neck Syndrome is commonly used to describe neck pain and musculoskeletal discomfort associated with prolonged forward-head posture during electronic-device use. Previous research has reported that Tech Neck Syndrome affects more than one-quarter of medical students, with most affected students experiencing mild degrees of neck-related disability. The same study identified significant associations between Tech Neck Syndrome and the use of multiple electronic devices, inadequate physical activity, and prolonged sedentary behavior⁷. Sustained cervical flexion may increase mechanical loading of the cervical region and place additional demands on the cervical and shoulder musculature. The frequent academic and recreational use of smartphones and other digital devices may therefore place medical students at particular risk of neck-related symptoms⁸.

The widespread use of smartphones, tablets, laptops and other digital devices has become an integral part of contemporary education, communication and entertainment. Medical students, in particular, rely extensively on digital devices for academic activities, online learning, information retrieval and social interaction⁹. Prolonged use of these devices, especially when accompanied by sustained cervical flexion and suboptimal posture, may contribute to neck pain and other musculoskeletal symptoms.

METHODS

A descriptive cross-sectional study was conducted among undergraduate medical students at the Rawal Institute of Health Sciences (RIHS), Islamabad, from March 2026 to May 2026. Participants were recruited using a convenience sampling technique. The study was conducted following institutional ethical approval.

Ethical approval was obtained from the Institutional Review Board (IRB) of the Rawal Institute of Health Sciences, Islamabad (Ref. No. RIHS/IRB/20/2026; dated 01 March 2026). All participants were informed about the purpose and procedures of the study, and written informed consent was obtained prior to participation. Participation was voluntary, and confidentiality of the collected information was maintained throughout the study.

Undergraduate medical students who regularly used digital devices for academic or recreational purposes and provided informed consent were eligible for inclusion. Students with a history of cervical spine trauma, congenital spinal deformities, inflammatory musculoskeletal disorders, or previous cervical spine surgery were excluded from the study.

Data were collected using a structured, self-administered questionnaire developed following a review of relevant literature, including the standardized Nordic Musculoskeletal Questionnaire framework. The questionnaire comprised sections addressing demographic characteristics, commonly used digital devices, average daily screen time, purpose of device use, sitting and working posture, screen position, ergonomic practices, level of physical activity, sleep duration, mobile phone use before sleep, previous history of neck pain, neck and shoulder stiffness, and other factors potentially associated with Tech Neck.

The primary outcome of the study was the presence of neck pain. Information regarding neck pain and other relevant musculoskeletal symptoms was obtained through participant self-report using the structured questionnaire.

The collected data were coded, entered, and analyzed using IBM SPSS Statistics, version 26. Descriptive statistics were used to summarize the study variables and were presented as frequencies and percentages. Associations between the presence of neck pain and categorical variables were assessed using Pearson's chi-square test. Fisher's exact test was applied where the assumptions for the chi-square test were not met. A two-sided p -value of <0.05 was considered statistically significant.

RESULTS

A total of 100 undergraduate medical students participated. Forty-eight percent reported neck pain and 51% reported no neck pain. Smartphones were the most commonly used devices (97%) shown in table 1. The most common daily screen-time category was 2–4 hours (40%), while social media (40%) and academic activities (38%) were the main purposes of use. Most participants reported using screens below eye level (71%), using devices while lying on the bed (43%), and using mobile phones before sleep (83%) figure 2. Neck and shoulder stiffness was reported by collecting different data 43% figure 1.

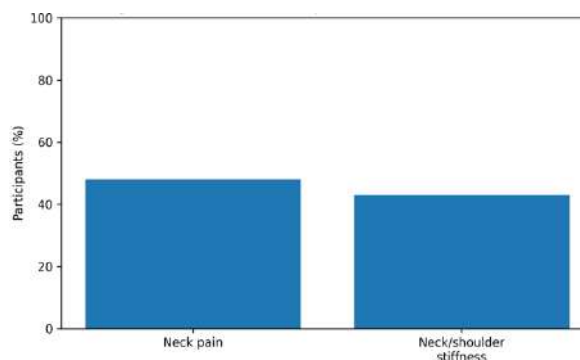
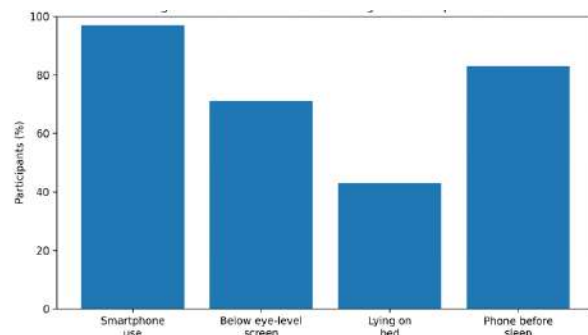


Figure No.1: Prevalence of neck pain and neck/shoulder stiffness.

Table No.I: Selected digital-device, ergonomic and lifestyle characteristics (n=100)

Characteristic	Category	n (%)
Frequently used device	Smartphone	97 (97. ⁰)
	Tablet	2 (2. ⁰)
	Laptop	1 (1. ⁰)
Daily screen time	<2 h	18 (18. ⁰)
	2–4 h	40 (40. ⁰)
	4–6 h	28 (28. ⁰)
	>6 h	14 (14. ⁰)
Purpose	Academic study	38 (38. ⁰)
	Social media	40 (40. ⁰)
	Watching videos	16 (16. ⁰)
	Gaming	6 (6. ⁰)
Posture	Sitting upright	28 (28. ⁰)
	Slouched sitting	26 (26. ⁰)
	Lying on bed	43 (43. ⁰)
	Standing	3 (3. ⁰)
Screen position	At eye level	21 (21. ⁰)
	Below eye level	71 (71. ⁰)
Physical activity	None	15 (15. ⁰)
	Occasional	70 (70. ⁰)
	Regular	15 (15. ⁰)
Sleep duration	<5 h	18 (18. ⁰)
	5–7 h	64 (64. ⁰)
	>7 h	18 (18. ⁰)
Mobile phone before sleep	Yes	83 (83. ⁰)
	No	17 (17. ⁰)
Neck/shoulder stiffness	Yes	43 (43. ⁰)
	No	57 (57. ⁰)

**Figure No.2: Prevalence of selected digital device practice**

Chi-square analysis showed significant associations between neck pain and screen position ($\chi^2=111.102$, $p<0.001$), taking regular breaks during device use ($\chi^2=35.015$, $p<0.001$), frequency of neck pain episodes ($\chi^2=17.921$, $p=0.001$), and neck and shoulder stiffness ($\chi^2=11.726$, $p=0.003$). No significant associations were observed for frequently used device, average daily screen time, purpose of use, posture, neck flexion during phone use, device position, back support,

ergonomic workstation setup, pillow use, physical activity, sleep duration, mobile-phone use before sleep, or previous history of neck pain (all $p>0.05$).

DISCUSSION

Despite these limitations, the study provides local information regarding neck pain and digital-device-related practices among medical students. The significant associations observed with screen position and regular breaks identify potentially modifiable behaviors that may be addressed through ergonomic education. Larger studies using objective posture assessment, standardized neck-disability measures and prospective designs are recommended.

The study has several limitations. The sample consisted of 100 undergraduate medical students recruited by convenience sampling, which limits generalizability. Exposure and symptom information was self-reported and may therefore be subject to recall or reporting bias. The cross-sectional design prevents assessment of temporal or causal relationships. In addition, several exposure categories were markedly unequal, particularly device type, which may have reduced statistical power. Other potentially relevant factors, including psychosocial stress and academic workload, were not comprehensively assessed.

Our findings are consistent with previous studies reporting musculoskeletal symptoms among university, medical and nursing students who use smartphones and other electronic devices. Differences in study populations, definitions of exposure, assessment methods and patterns of digital-device use may account for variations in reported prevalence and associations between studies.

Compared with other major contributors to pain and disability, the evidence base for the management of neck pain remains relatively limited, with comparatively few randomized controlled trials available to support treatment decisions. Consequently, clinical recommendations for neck pain are frequently informed by evidence derived from related musculoskeletal conditions⁵. Further well-designed clinical trials are required to evaluate the efficacy and comparative effectiveness of existing treatment approaches, with particular attention to interventions for neuropathic neck pain and surgical management of mechanical causes.

Neck pain represents a substantial global health burden and is among the important causes of disability worldwide. Although acute neck pain commonly improves spontaneously over several weeks to months, a considerable proportion of individuals may develop persistent or recurrent symptoms. Current evidence suggests that available treatments have limited influence on the natural progression of acute neck pain.⁵ A careful clinical history and physical examination remain important for identifying potential

underlying causes and guiding appropriate management.

Screen position demonstrated a statistically significant association with neck pain in the present study. Most participants reported using screens below eye level. A lower screen position may encourage sustained cervical flexion and forward-head posture, thereby increasing the mechanical demands placed on the cervical and shoulder musculature. This finding supports the inclusion of appropriate screen positioning in ergonomic education.¹⁰ However, because of the cross-sectional design, a causal relationship cannot be established.

Previous research has reported that tech neck syndrome is relatively common among medical students, with more than one-quarter of students meeting the criteria for the condition. Among those affected, mild neck disability was the most frequently observed level of functional impairment. The occurrence of tech neck syndrome was significantly associated with the use of multiple digital devices, lower levels of physical activity, and prolonged sitting, particularly for more than three hours per day. These findings indicate that frequent digital device use combined with sedentary behavior and insufficient physical activity may be important factors associated with neck-related dysfunction among medical students.⁷

An important distinction should be made between the prevalence of potentially unfavorable behaviors and their statistical association with neck pain. A behavior may be common in a population without demonstrating a significant association with the outcome in a particular sample.¹¹

Ergonomic measures show prevention of work-related neck and upper-limb musculoskeletal disorders.¹² Nevertheless, ergonomic education emphasizing neutral cervical posture, appropriate screen positioning and regular movement breaks remains a reasonable preventive approach.¹³

In contrast, no statistically significant associations were observed between neck pain and frequently used device, average daily screen time, purpose of device use, posture, neck flexion during phone use, device position, back support, ergonomic workstation setup, pillow use, physical activity, sleep duration, mobile-phone use before sleep or previous history of neck pain. These negative findings should be interpreted cautiously. In particular, the very high proportion of smartphone users in the sample resulted in very small comparison groups for tablet and laptop users, limiting meaningful comparisons. Similarly, the absence of a significant association with screen time may reflect the sample size and distribution of exposure categories rather than the absence of a relationship.

The frequency of neck pain episodes and the presence of neck and shoulder stiffness were also significantly associated with neck pain.¹⁴ These findings suggest that

recurrent neck symptoms and adjacent shoulder discomfort commonly coexist in this population.¹⁵ They should be interpreted as associated clinical characteristics rather than as independent causal factors. Taking regular breaks during device use was also significantly associated with neck pain. Interrupting prolonged static activity may permit changes in posture.¹⁶ Regular break for the continuous activity on digital device may reduce sustained cervical loading.¹⁷ Evidence-based recommendations for the assessment and management of neck pain by doing proper exercise helps to decrease the effect.¹⁸ Research established the reliability and validity of the Neck Disability Index for assessing functional disability associated with neck pain.

The present study found that 48% of the participating medical students reported neck pain, while 43% reported neck and shoulder stiffness. Smartphones were the predominant digital device used by participants (97%). Potentially unfavorable ergonomic practices, including below-eye-level screen positioning, use of devices while lying on the bed and mobile-phone use before sleep, were also common. These findings demonstrate that neck-related symptoms and potentially modifiable device-use behaviors are prevalent among medical students.

CONCLUSION

Neck pain was reported by 48% of the medical students studied. Significant associations were observed with screen position, taking regular breaks, frequency of neck pain episodes, and neck and shoulder stiffness. Other assessed digital-device, ergonomic and lifestyle variables were not statistically significantly associated with neck pain. These findings support the need for ergonomic awareness among medical students and further prospective research into modifiable factors related to Tech Neck Syndrome.

Study Limitations: The study was limited by its small convenience sample, self-reported exposure and symptom measures, cross-sectional design and unequal distribution across some exposure categories. The findings therefore should not be generalized beyond comparable student populations or interpreted causally.

Ethical Approval: Ethical approval was obtained from the Institution Review Board (IRB), Rawal Institute of Health Sciences Islamabad, under Ref. No. RIHS/IRB/20/2026, dated 01-03-2026. The approval letter authorizes conduct and publication of the research "Prevalence and Risk Factors of Tech Neck Syndrome among Students Using Digital Devices" and is valid for one year.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Maryam Shan, Maria Iram, Aqsa Zahid
--	-------------------------------------

Drafting or Revising Critically:	Alizeh Ejaz Pasha, Iqra Awan, Zarmeen Nadeem
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. RIHS/IRB/20/2026

Dated 01.03.2026

REFERENCES

1. Elvan A, Cevik S, Vatansever K, Erak I. The association between mobile phone usage duration, neck muscle endurance, and neck pain among university students. *Scientific Reports* 2024;14(1):20116.
2. Hoy D, Protani M, De R, Buchbinder R. The epidemiology of neck pain. *Best Pract Res Clin Rheumatol* 2010;24(6):783-92.
3. Phuyal N, Pandey N, Pandeya A, Mishra AK. Smartphone overuse and its impact on musculoskeletal pain in the neck and wrists/hands in medical and nursing undergraduate students: a cross-sectional study. *Scientific Reports* 2025;15(1):44945.
4. Gao Y, Chen Z, Chen S, Wang S, Lin J. Risk factors for neck pain in college students: a systematic review and meta-analysis. *BMC Public Health* 2023;23(1):1502.
5. Cohen SP. Epidemiology, diagnosis, and treatment of neck pain. *Mayo Clin Proc* 2015;90(2):284-99. doi: 10.1016/j.mayocp.2014.09.008.
6. Briggs AM, Woolf AD, Dreinhöfer K, Homb N, Hoy DG, Kopansky-Giles D, et al. Reducing the global burden of musculoskeletal conditions. *Bulletin World Health Organization* 2018;96(5):366.
7. Salameh MA, Boyajian SD, Amaireh EA, Jamal B, Alrfooh H, AbuKhalaf K, et al. Prevalence of text neck syndrome, its impact on neck dysfunction, and its associated factors among medical students: A cross-sectional study. *Work* 2024;79(3):1111-9.
8. Ariëns GA, van Mechelen W, Bongers PM, Bouter LM, van der Wal G. Psychosocial risk factors for neck pain: a systematic review. *Am J Industrial Med* 2001;39(2):180-93.
9. Organization WH. WHO guidelines on physical activity and sedentary behaviour: World Health Organization; 2020. <https://www.who.int/publications/i/item/9789240015128>
10. Riebe D, Ehrman JK, Liguori G, Magal M. ACSM's guidelines for exercise testing and prescription: American College of Sports Medicine; 2018. https://library-search.imperial.ac.uk/discovery/fulldisplay/alma9956102497801591/44IMP_INST:ICL_VU1
11. Hogg-Johnson S, van der Velde G, Carroll LJ, Holm LW, Cassidy JD, Guzman J, et al. The burden and determinants of neck pain in the general population. *J Manipulative Physiological Therapeutics* 2009;32(2S):S46-S60.
12. Buckle P, Devereux J. European Agency for Safety and Health at Work. Work-related neck and upper limb musculoskeletal disorders Luxemburgo [Consultado el 10 de abril del 2013].
13. Organization IL. Safety and Health at the Heart of the Future of Work: Building on 100 Years of Experience. International Labour Organization Geneva, Switzerland; 2019. https://www.ilo.org/sites/default/files/wcmsp5/groups/public/@dgreports/@dcomm/documents/publication/wcms_686645.pdf
14. Fejer R, Kyvik KO, Hartvigsen J. The prevalence of neck pain in the world population: a systematic critical review of the literature. *Eur Spine J* 2006;15(6):834-48.
15. Schenk R, Donaldson M, Parent-Nichols J, Wilhelm M, Wright A, Cleland JA. Effectiveness of cervicothoracic and thoracic manual physical therapy in managing upper quarter disorders—a systematic review. *J Manual Manipulative Therapy* 2022;30(1):46-55.
16. Organization WH. Rehabilitation 2030: meeting report, Geneva, Switzerland, 10-11 July 2023: World Health Organization; 2024. <https://www.who.int/publications/i/item/9789240087392>
17. Black NL, Neumann WP, Noy I. 21st Congress of the International Ergonomics Association (IEA 2021). DOI:10.1007/978-3-030-74614-8.
18. Côté P, Wong JJ, Sutton D, Shearer HM, Mior S, Randhawa K, et al. Management of neck pain and associated disorders: a clinical practice guideline from the Ontario Protocol for Traffic Injury Management (OPTIMA) Collaboration. *Eur Spine J* 2016;25(7):2000-22.

In-Vitro Evaluation of effect of Pre-Heating on Degree of Conversion and Surface Micro-Hardness of Bulk-Fill Composite at Different Depths

Shahzad Ali Shah

Pre-Heating on Degree of Conversion and Surface Micro-Hardness of Bulk-Fill Composite

ABSTRACT

Objective: This in-vitro study objective is to evaluate whether preheating improves degree of conversion and surface micro-hardness of the Filtek bulk-fill posterior composite.

Study Design: An in-vitro experimental laboratory study

Place and Duration of Study: This study was conducted at the Conservative Dental Sciences Department, College of Dentistry, Qassim University, KSA from 20 May 2026 to 30 June 2026.

Methods: This study was conducted to evaluate the effect of preheating temperature on the degree of conversion and Vickers microhardness of Filtek™ Bulk Fill Composite. Sixty specimens were prepared in 5mm diameter, 4mm depth molds, and assigned to 23°C, 37°C, or 54°C groups (n=20 each). Each group was divided for ATR-FTIR degree of conversion and Vicker's micro-hardness testing. Specimens were stored in distilled water at 37°C for 24 hours before analysis. The composite was cured for 30 seconds using LED light at 1200mW/cm². **Results:** Preheating significantly increased degree of conversion and micro-hardness with 54°C producing the highest values and 23°C the lowest. In all groups, the top surface showed higher values than the bottom surface, and the bottom to top hardness ratios remained clinically acceptable. Degree of conversion (DC) strongly and positively correlates with micro-hardness.

Conclusion: Preheating the tested bulk fill composite improved polymerization efficiency and surface hardness, particularly at 54°C, and may enhance performance in deeper restorations. The Data also showed a clear depth effect; top surface values exceeded bottom surface values across temperatures, suggesting that preheating improved but did not eliminate light attenuation through the 4mm increment. Because the correlation between conversion and hardness was strong, micro-hardness may serve as a practical indirect marker of polymerization in this material.

Clinical Relevance: Within the limits of this single material in-vitro study, preheating appears to be useful strategy for improving bulk fill composite polymerization especially when deeper restorations are planned with clinically relevant chairside implications for practice.

Key Words: Composite resins, Dental materials, Temperature, Micro-hardness, Spectroscopy, Fourier transform infrared

Citation of article: Shah SA. In-Vitro Evaluation of effect of Pre-Heating on Degree of Conversion and Surface Micro-Hardness of Bulk-Fill Composite at Different Depths. Med Forum 2026;37(9):16-20. doi:10.60110/medforum.370903.

INTRODUCTION

Resin-based composites (RBCs) have become the material of choice for direct posterior restorations due to their favorable esthetics and adhesive capabilities. ⁽¹⁾

Associate Professor, Conservative Dental Sciences Department. College of Dentistry, Qassim University, KSA.

Correspondence: Shahzad Ali Shah, Associate Professor, Conservative Dental Sciences Department. College of Dentistry, Qassim University, KSA.

Contact No: 00966-580548188/ 00923339115856

Email: s.mukhtiarshah@qu.edu.sa

Received: July, 2026

Reviewed: August, 2026

Accepted: August, 2026

However, conventional incremental placement is technique-sensitive, causes polymerization shrinkage, and prolongs clinical time. ⁽²⁾ Many of these issues have been addressed by bulk-fill composites, allowing up to 4 mm placement with adequate depth of cure and reduced shrinkage stress. These materials are specifically engineered with modified monomer systems, increased translucency, and advanced photo initiators to enhance light penetration and polymerization efficiency. ⁽³⁾

One important factor influencing resin composites' mechanical, biological, and physical performance is the degree of conversion (DC), which is the percentage of monomer converted to polymer. Increased clinical lifespan, decreased solubility, and greater mechanical strength are all strongly correlated with higher DC. ⁽⁴⁾ Crucially, DC and microhardness are depth-dependent characteristics that frequently decrease from top surface

to deeper layers as a result of curing light attenuation, particularly in bulk-fill restorations.⁽⁵⁾

Preheating composite resins has gained attention as an adjunctive technique to optimize polymerization, reducing viscosity of the composite, enhances flowability, and improves adaptation to cavity walls. More importantly, it increases molecular mobility, facilitating greater monomer conversion during polymerization. Several recent in-vitro studies have demonstrated that preheating can enhance DC and, in some cases, improve micro-hardness.⁽⁶⁾ However, the effect appears to be material-dependent, with some studies reporting no significant changes in microhardness despite improvements in other mechanical properties.⁽⁷⁾⁽⁸⁾⁽⁹⁾

Despite growing interest, the literature presents conflicting evidence regarding the influence of preheating on bulk-fill composites, particularly when evaluated at different depths.⁽¹⁰⁾ Studies report that preheating may enhance polymerization and deep-layer hardness, but effects vary with composite formulation and testing conditions. Furthermore, existing studies either focus on surface properties alone or do not comprehensively correlate DC with microhardness across multiple depths within the same experimental design.^(11, 12)

Given the growing use of bulk-fill composites, the effect of preheating on degree of conversion and surface micro-hardness at different depths remains unclear. This gap is clinically relevant because inadequate polymerization in deeper layers may compromise restoration durability, leading to secondary caries, marginal breakdown, and restoration failure.

Thus, the purpose of this in-vitro experimental laboratory study was to assess how preheating affects bulk-fill composite resins' degree of conversion and surface micro-hardness at various depths. By correlating these two fundamental properties under controlled conditions, the study aims to provide better insights into whether preheating can be recommended as a reliable clinical strategy to enhance the performance and longevity of bulk-fill composite restorations.

METHODS

This study was conducted at the Conservative Dental Sciences Department, College of Dentistry, Qassim University, over a period of approximately two months, from 20th May to 30th JUNE 2026, following ethical approval from the Institutional Review Board (IRB) with approval number #26-21-4. The specimens were prepared using cylindrical silicon molds having a diameter of 5 mm and depth of 4 mm respectively. The bulk-fill composite was placed in a single increment (4 mm thickness) and cured for 30 seconds using LED

blue phase N LED light. (Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm² light intensity). Three experimental groups (n=20) were assigned a total of 60 standardized specimens according to the preheating temperature. Group I was considered as control, with 23°C and no preheating. Group II was preheated to 37 °C (mild heating) and group III was preheated to 54°C (High preheating) using Calset Composite Warmer (Ad Dent Inc., Danbury, CT, USA). For Vicker micro-hardness testing and degree of conversion analysis, each group was further split into two subgroups (n=10). Before testing, each specimen was kept in distilled water at 37°C for the whole day.

An attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectrometer (IRPrestige-21, Shimadzu Corporation, Kyoto, Japan) was used to measure the degree of conversion. Comparing uncured and cured composite spectra. (Fig 1C) Absorbance peaks at 1638 cm⁻¹ (aliphatic C=C) and 1608 cm⁻¹ (aromatic C=C) were analyzed, and DC (%) was calculated using peak ratio changes before and after curing. Each specimen's top and bottom surfaces were measured.

Surface micro-hardness was assessed using Vickers microhardness tester (Shimadzu Corporation, Kyoto, Japan) under standardized loading conditions. Three indentations were made on both the top and bottom surfaces of each specimen, and the mean was calculated. The efficacy of polymerization at depth was then evaluated by calculating the bottom to top ratio.

Tuckey's post hoc test and one-way analysis of variance were used to analyze the data, with $p < 0.05$ was set as statistically significant.

RESULTS

All specimens' preparations and analysis using FTIR, followed the approval protocol, and no specimen was excluded from the analysis. (Fig 1A-C).

The mean degree of conversion values obtained from the FTIR analysis and Tukey's HSD Post-hoc Comparisons are presented in Table 1. Composite preheating significantly affected the degree of conversion ($p < 0.05$). The lowest degree of conversion was observed at 23°C, reflecting higher composite viscosity and reduced monomer mobility. For all groups, the top surface demonstrated significantly higher DC values than the corresponding bottom surface. Tukey's post hoc analysis revealed that specimens preheated to 54°C exhibited significantly high degree of conversion than those preheated 37°C and 23°C. Likewise, specimens at 37°C exhibit significantly higher degree of conversion than those at 23°C. All pair wise comparisons demonstrated statistically significant differences ($p < 0.001$).

Table No.1: Degree of Conversion (DC%) at the Top and Bottom Surfaces and Tukey's HSD Post-hoc Comparisons.

Temp (°C)	Top Surface *DC% (Mean ± ***SD)	Bottom Surface DC% (Mean ± SD)	Top–Bottom Diff	Pairwise Comparison	Top DC Mean Difference (95% ** CI)	Bottom DC Mean Difference (95% CI)	p-value
23°C	69.2 ± 2.5	60.6 ± 2.7	8.6	23°C vs 37°C	5.9 (3.6–8.2)	7.7 (5.3–10.1)	<0.001
37°C	75.1 ± 2.3	68.3 ± 2.4	6.8	23°C vs 54°C	12.1 (9.8–14.4)	16.7 (14.3–19.1)	<0.001
54°C	81.3 ± 2.1	77.3 ± 2.2	4.0	37°C vs 54°C	6.2 (3.9–8.5)	9.0 (6.6–11.4)	<0.001

*DC = Degree of Conversion, **CI = confidence Interval, ***SD = Standard deviation



Figure No. 1: Composite resin specimen preparation and degree of conversion (DC%) assessment. (1A) Customized silicone molds (5×4 mm); (1B) polymerized bulk-fill composite specimen removed from the mold; and (1C) FTIR-ATR analysis for DC% determination.

Table No.2: Vickers micro-hardness (VHN) and bottom to top hardness ratio.

Group	Top VHN	Bottom VHN	Bottom/Top VHN Ratio (%)
23 °C	58.4	46.8	80.1%
37 °C	63.7	54.2	85.1%
54 °C	69.9	63.8	91.3%

Table No.3: Correlation between degree of conversion and Vickers micro-hardness.

Variables	Correlation coefficient (r)	p-value
Top DC vs Top VHN	0.982	< 0.001
Bottom DC vs Bottom VHN	0.989	< 0.001

Surface micro-hardness results are summarized in Table 2. Preheating significantly influenced Vicker hardness values ($p < 0.05$). The group with the highest preheating temperature had the highest hardness ratings, whereas the room temperature group demonstrated the lowest values. Within each experimental group, the top surface exhibited significantly greater Vicker hardness than the bottom surface. The calculated bottom to top hardness ratio remained within the acceptable range for all groups.

The degree of conversion and Vickers micro-hardness showed a statistically significant positive link, according to Pearson's correlation analysis (Table 3).

Specimens with higher degrees of conversion generally exhibited greater surface micro-hardness, indicating a positive association between polymerization efficiency and mechanical performance.

DISCUSSION

Thermal energy reduces composite viscosity, increases segmental molecular mobility of the monomer network, and lowers the activation energy barrier for the free radical polymerization collectively enabling higher conversion rates of aliphatic C=C bonds during photocuring.

The observation that the 54°C group achieved significantly higher DC than both the 37°C and 23°C groups are consistent with several prior investigations. Keles and Tarim,⁽¹³⁾ studying Tetric Evo Ceram Bulk fill and Sonic fill2 preheated to 55°C reported enhanced DC and micro-hardness relative to room temperature controls, with the effects most pronounced at the bottom surface, a finding that parallels depth-dependent results. Similarly, Singha et al⁽¹⁴⁾ testing Filtek Bulk fill among other materials preheated to 68°C, documented a statistically significant increase in Vickers micro-hardness from 60 to 70HV, confirming the favorable correlation shown in this investigation between preheating temperature and mechanical characteristics observed in the present research. Prasanna et al,⁽¹⁵⁾ demonstrated using ATR-FTIR spectroscopy, that DC increased monotonically as preheating temperature was elevated, providing foundational thermodynamic

support for the temperature-response relationship, this study reported.

As photons scatter and are absorbed by the resin matrix and filler particles, irradiance diminishes with depth, reducing the number of photo-initiator radical events available to propagate the polymerization front. Preheating partially compensates for these phenomena, explaining why bottom surface DC improvement was proportionality greater in the preheated groups than at the top surface, a pattern also reported by Theobaldo et al, ⁽¹⁶⁾ for 4mm increment bulk fill specimens cured with both LED and QTH units.

The significant positive Pearson correlation between DC and Vickers micro-hardness cross experimental conditions supports the long-standing mechanistic proposition that surface hardness is a valid indirect indicator of polymerization efficiency in resin-based composites. As Prasanna et al ⁽¹⁵⁾ demonstrated across three nanohybrid composites, microhardness was systematically improved at higher preheating temperatures.

The bottom to top hardness ratio, which remained within clinically acceptable limits (≥ 80) in all groups in the present study, provides additional assurance of adequate depth of cure of 4mm increment used. Suhettia et al, ⁽⁸⁾ reported bottom to top ratios between 0.62 and 0.71 for several bulk-fill composites including Filtek Bulk-fill, values somewhat lower than those observed here. Not all studies report a uniformly positive effect of pre-heating on surface micro-hardness, and the divergence in the literature merits contextualization. Kahnamouei et al ⁽¹⁷⁾ evaluated X-tra fil and Opus Bulk-fill preheated to 68°C for 15 minutes, found no statistically significant difference in VH between preheated and non-preheated groups. ($p=0.719$), despite a significant increase in flexural strength. Perez et al ⁽¹⁸⁾ reported that in-fact preheating increased micro-hardness in Filtek One Bulk-fill and Aura bulk-fill but decreased it in Opus bulk-fill, underscoring the material dependence of the preheating response. Not all studies report a uniformly positive effect of pre-heating on surface micro-hardness, and the divergence in the literature merits contextualization. Kahnamouei et al ⁽¹⁷⁾ evaluating X-tra fil and Opus Bulk fill preheated to 68°C for 15 minutes, found no statistically significant difference in VH between preheated and non-preheated groups. ($p=0.719$), despite a significant increase in flexural strength.

From the clinical perspective, the temperatures investigated here 37°C and 54°C represent a clinically feasible range. Temperatures above 60°C risk premature polymerization during storage and potential pulpal irritation upon placement and have been associated in isolated reports with accelerated photo initiator degradation. Patussi et al ⁽¹⁹⁾ in a scoping review covering the breath of preheating research concluded that evidence favors preheating as a

technique to enhance polymerization efficiency and handling properties, while noting the heterogeneity that limits definitive clinical recommendations.

Limitation of the study

Several limitations of the present study should be acknowledged when interpreting these findings.

1. The use of single composite brand and a single curing protocol restrict the external validity of the results; performance across bulk fill formulations with varying filler contents monomer systems, and photo initiator packages cannot be inferred from the present data.
2. The specimen geometry (5mm diameter, 4mm depth) models are a simplified, idealized clinical increment without the geometric complexity, cavity walls or adhesive interface present in vivo.
3. No measures of polymerization shrinkage stress were obtained; which may partially off-set mechanical gains documented here.
4. Long term water sorption and the solubility assessments which degrade mechanical properties per se, were also beyond the scope of this investigation.

CONCLUSION

Preheating the bulk fill composite improved both degree of conversion and surface microhardness, with the best performance at 54°C. The top surface showed greater hardness than the bottom, while acceptable hardness ratios indicated adequate depth of cure and polymerization quality.

Authorship: The sole author designed the study, conducted and analyzed the research, interpreted the results, prepared the manuscript, and approved the final version, accepting full responsibility for its integrity.

Clinical Relevance

Scientific rationale of the study: Bulk fill composite exhibits reduced polymerization at greater depths, potentially compromising their mechanical properties and clinical longevity. Pre-heating has been proposed to enhance monomer conversion and material performance.

Principal findings: Pre-heating significantly improved the degree of conversion and surface micro-hardness at both top and bottom surfaces, with higher temperatures producing greater enhancements while maintaining adequate curing throughout the restoration.

Practical Implications: Pre-heating bulk-fill composite before placement may improve polymerization efficiency, optimize mechanical properties, and enhance the durability of deep posterior restorations, supporting its incorporation into routine restorative clinical practice where feasible.

Author's Contribution:

Concept & Design or acquisition of analysis or	Shahzad Ali Shah
--	------------------

interpretation of data:	
Drafting or Revising Critically:	Shahzad Ali Shah
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. 26-21-4 Dated 18.05.2026

REFERENCES

- Durr-E-Sadaf, Ahmad M, Gaikwad R, Arjumand B. Comparison of two different matrix band systems in restoring two surface cavities in posterior teeth done by senior undergraduate students at qassim university, saudi arabia: A randomized controlled clinical trial. *Ind j Dent Res* 2018;29(4):459–64. Jul 110.4103/ijdr.IJDR_26_1730127197
- Lehmann A, Barszcz B, Nijakowski K. Optimizing dental resin-based composite polymerization with distance and exposure variables: Preliminary study. *Materials* 2026;19(7):1390. Mar 3110.3390/ma1907139041976674
- Ibrahim I, Helal H, Ibrahim SH, Riad M. 24 months clinical prospective of proximal restorations with repeated preheating bulk fill composite up to ten cycles: Randomized controlled trial. *Sci Rep* 2024;14(1):23966–14. Oct 1410.1038/s41598-024-73200-639397060
- KHALID H, et al. Effect of nano-bioceramics on monomer leaching and degree of conversion of resin-based composites. *Dent Materials J* 2018;37(6):940–9.2710.4012/dmj.2017-33830135338
- Sorrentino E, et al. A mini-review on different polymerization protocols for resin-based dental composites. *Ital J Anat Embryol* 2023;127(2):61–4. 110.36253/ijae-14686
- Ibrahim II, Riad M, Ibrahim SH, Helal H. Effect of preheating on various properties of resin composite. A review article. *Delta Univ Scientific J* 2024;7(2):97–107. 110.21608/dusj.2024.323183.1093
- Mohammadi N, et al. Effect of pre-heating on the mechanical properties of silorane-based and methacrylate-based composites. *J Clin Experimental Dentist* 2016;8(4):e373–8. 110.4317/jced.5280727703604
- Sahetia AS, et al. Preheating effect on microhardness and depth of cure of three bulk-fill composite resins: An in vitro study. *Endodontol J Ind Endodontic Society* 2024;36(2):125–30. 110.4103/endo.endo_56_23
- El-Shazly RK, El-Zayat IMA, Mohsen MMA, Labib MEM. Clinical evaluation of Self-Adhesive Bulk-Fill composite versus conventional Nano-Hybrid composite in cervical Cavities—A randomized controlled clinical trial. *J Esthetic Restorative Dentistry* 2025;37(7):1907–19. 110.1111/jerd.1347240151057
- Srikumar G, Barde SP, Gajpal A, Kumari R, Phafat A, Patel P. An in vitro comparative evaluation of the effect of preheating of EverX posterior, bulk fill and G-aenial posterior composite resins on their microhardness and depth of cure. *Advances Human Biol* 2026;16(1):149–55.10.4103/aihb.aihb_138_25
- Bueno T, et al. Effects of pre-heating on physical-mechanical-chemical properties of contemporary resin composites. *Odontol* 2025;113(1):135–42. 110.1007/s10266-024-00953-x38797796
- Silva MVMd, et al. Surface analysis of a universal resin composite and effect of preheating on its physicochemical properties. *Brazilian Dent J* 2023;34(4):115–26. 110.1590/0103-644020230541137909634
- Keleş ZH, Tarım B. Effect of preheating on microhardness, degree of conversion, and depth of cure of various bulk-fill composites. *Selcuk Dent J* 2024;11(1):27–32. 2610.15311/selcukdentj.1219236
- Singha A, Dhanesha A, Nair S, Patel R, Behera S, Nanda S. Pre-heating effect on micro-hardness and depth of cure for three bulk-fill composite resins: An in vitro study. *Bio Information* 2024;20(9):1128–31. 3010.6026/973206300200112839917230
- Prasanna N, Pallavi Reddy Y, Kavitha S, Lakshmi Narayanan L. Degree of conversion and residual stress of preheated and room-temperature composites. *Ind J Dent Res* 2007;18(4):173–6. 110.4103/0970-9290.3582717938493
- Dias Theobaldo J, et al. Effect of preheating and light-curing unit on physicochemical properties of a bulk fill composite. *Clinical, cosmetic and investigational dentistry* 2017; 9:39–43. 110.2147/CCIDE.S13080328652817
- Abed Kahnamouei M, et al. The effect of preheating on microhardness and flexural strength of bulk-fill resin composites: An in-vitro study. *Eur Oral Res* 2024;58(3):133–8. 510.26650/eor.2023130201239588475
- Pérez-Pachas B, et al. Surface microhardness and depth of cure in bulk fill resin composites with and without preheating: An in vitro study. *J Clin Experimental Dentistry* 2025;17(7): e825–33. 110.4317/jced.6284440823109
- Patussi AFC, et al. Preheating of dental composite resins: A scoping review. *J Esthetic Restorative Dentist* 2023;35(4):646–56. 10.1111/jerd.1299136478368.

Enhancing Nurses' Knowledge through Meleis' Transition Theory-Based Educational Program for First-Time Mothers

Eman Tammuz Mahmood¹ and Salwa Hazim Al Mukhtar²

ABSTRACT

Objective: To evaluate the effectiveness and six-month retention of a Meleis' Transition Theory-based educational intervention in improving nurses' knowledge and self-efficacy

Study Design: A quasi-experimental, two-group, repeated-measures study

Place and Duration of Study: This study was conducted at the Al-Salam and Al-Khansaa Teaching Hospitals in Mosul, Iraq, from February 17, 2026 to April 17, 2026.

Methods: A total of 100 registered nurses were allocated to study and control groups (50 each). Knowledge and self-efficacy were assessed using a standardized questionnaire at three time points. Data was analyzed using descriptive statistics, repeated-measures ANOVA, between-group comparisons, and effect-size estimates.

Results: Significant time-by-group differences were observed for all outcomes ($p < .001$). In the study group, knowledge increased from 10.10 ± 2.20 at baseline to 31.50 ± 1.70 post-intervention and remained above baseline at follow-up (22.10 ± 2.40). Self-efficacy increased from 20.80 ± 1.80 to 44.50 ± 1.50 and remained elevated at follow-up (32.50 ± 1.70). Combined scores increased from 30.90 ± 2.50 to 76.00 ± 2.40 and remained higher at six months (54.60 ± 2.50). The control group showed minimal changes. Effect sizes were large ($\eta^2 = .893-.936$).

Conclusion: The Meleis' Transition Theory-based educational intervention significantly improved nurses' knowledge and self-efficacy, with substantial benefits retained at six months. Theory-based education may support sustained professional development in clinical nursing practice.

Key Words: Educational intervention; Self-efficacy; Knowledge; Nurses; Meleis' Transition Theory; Nursing education.

Citation of article: Mahmood ET, Al Mukhtar SH. Enhancing Nurses' Knowledge through Meleis' Transition Theory-Based Educational Program for First-Time Mothers. Med Forum 2026;37(9):21-25. doi:10.60110/medforum.370904.

INTRODUCTION

Clinical nursing requires a high level of proficiency and reliability from healthcare professionals to ensure safe and effective patient care. However, insufficient knowledge and limited confidence in performing clinical actions remain important barriers to competent and timely practice in clinical settings¹. Deficiencies in knowledge and self-efficacy may contribute to inadequate clinical performance, compromised patient safety, and reduced quality of care².

A substantial body of literature supports the use of educational interventions to address these challenges

and strengthen healthcare professionals' competencies. Structured and learner-centered educational programs have been shown to improve nurses' knowledge and practice-related behaviors. However, educational interventions often emphasize cognitive outcomes while giving less attention to psychosocial factors, particularly self-efficacy, which may influence the translation of acquired knowledge into clinical practice³.

Self-efficacy refers to an individual's perceived ability to successfully perform specific tasks. It is closely associated with motivation, confidence, persistence, and task performance. Studies involving practicing nurses have demonstrated that higher self-efficacy is associated with better clinical performance and decision-making abilities⁴. Therefore, educational interventions targeting both knowledge acquisition and self-efficacy may be more likely to produce clinically meaningful and sustained improvements in nursing practice⁵.

Meleis' Transition Theory provides a theoretical framework for understanding how individuals develop competence while adapting to new roles, responsibilities, and professional experiences. The theory emphasizes the importance of knowledge,

¹. M.Sc. Nursing Candidate / Professor², MCHN Nursing, College of Nursing, University of Mosul, Iraq

Correspondence: Dr. Salwa Hazim Al Mukhtar, Professor, PhD, MCHN Nursing, College of Nursing, University of Mosul, Iraq

Contact No: 9647510667405

Email: dr.salwa@uomosul.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

preparation, confidence, and mastery in facilitating healthy transitions. Applying its principles to structured educational experiences may support nurses in developing both the cognitive knowledge and confidence required for effective clinical practice⁶. Despite growing evidence supporting educational interventions for improving nurses' knowledge and self-efficacy, evidence regarding the long-term retention of these improvements remains limited. Therefore, this study aimed to determine whether a theory-based educational intervention grounded in Meleis' Transition Theory could improve practicing nurses' knowledge and self-efficacy and whether these improvements would be sustained over time.

METHODS

Research design and setting: The study employed a quasi-experimental, one-group repeated-measures design to evaluate the effectiveness of an educational intervention based on Meleis' Transition Theory in improving nurses' knowledge and self-efficacy. The study was conducted at Al-Salam and Al-Khansaa Teaching Hospitals in Mosul, Iraq, from February 17 to April 17, 2026. Participants were assessed at three time points: before the intervention (pre-test), immediately after the intervention (post-test I), and at follow-up (post-test II), allowing the evaluation of both the immediate and sustained effects of the intervention.

Research Sampling: Using a convenient sampling method, thirty registered nurses employed in various clinical departments participated as subjects in this study.

Interventional Program: Based on Meleis' Transition Theory, the educational intervention is intended to improve participants' knowledge, enhance their self-confidence and create participant competence. The educational intervention involved a combination of didactic instruction (instructional content) and interactive dialogue (participant participation), as well as hands-on practice experience (application). The design of this intervention addressed multiple areas of knowledge for participants; furthered participants' cognitive development and promoted participants' emotional/psychological growth.

Measuring instruments: Information was gathered utilizing a comprehensive survey instrument, which was divided into three distinct sections.

Section one: Demographic data sheet.

This section contained information about each subject such as age, educational degree earned, years of experience in the field of nursing, location where each worked, and if previous training or workshops had been completed.

Section two: assessment tool for knowledge.

This section contained a list of questions relating to the topic of educational intervention, and each question

represented either true or false format, and/or multiple-choice format.

Section three: Self-efficacy scale.

This section contained a Likert scale type format where participants could indicate their level of confidence in completing specific clinical activities. Response levels ranged from low to high.

Validity/reliability: All measuring instruments underwent an expert review process to ensure content validity. Internal reliability testing of the measuring tools produced satisfactory results, i.e., Cronbach alpha >.8.

Statistical analysis: Data were analyzed using SPSS version 28. Descriptive statistics included frequencies, percentages, means, and standard deviations. Changes across time points were assessed using repeated-measures ANOVA, with paired t-tests for pairwise comparisons. Effect sizes were estimated using partial eta squared (η^2p). Statistical significance was set at $p < 0.05$.

RESULTS

A total of 100 nurses participated, with 50 in each group. The groups were comparable in age, education, and experience at baseline ($p > .05$) (Table 1). Following the intervention, the study group showed marked improvements in knowledge (10.10 ± 2.20 to 31.50 ± 1.70) and self-efficacy (20.80 ± 1.80 to 44.50 ± 1.50), with scores remaining above baseline at follow-up (22.10 ± 2.40 and 32.50 ± 1.70 , respectively). The control group showed minimal changes (Table 2).

Significant Time $\times$ Group interactions were observed for knowledge ($F[2,196] = 820.45$, $p < .001$, $\eta^2p = .893$), self-efficacy ($F[2,196] = 1025.70$, $p < .001$, $\eta^2p = .912$), and combined scores ($F[2,196] = 1450.20$, $p < .001$, $\eta^2p = .936$), indicating large intervention effects (Table 3). Knowledge and self-efficacy improved from low to high levels after the intervention and remained moderate at follow-up (Table 4). Improvements were generally consistent across participant subgroups, with approximately 50–60% of the immediate gains retained at follow-up (Table 5).

Table No. 1: Demographic Characteristics of Participants by Group (N = 100)

Variable	Category	Study (n = 50) n (%)	Control (n = 50) n (%)
Age	≤ 30 years	30 (60.0)	29 (58.0)
	> 30 years	20 (40.0)	21 (42.0)
Education level	Diploma	28 (56.0)	27 (54.0)
	Bachelor's or higher	22 (44.0)	23 (46.0)
Years of experience	≤ 5 years	26 (52.0)	25 (50.0)
	> 5 years	24 (48.0)	25 (50.0)

Note. No significant differences between groups at baseline ($p > .05$).

Table No. 2: Knowledge, Self-Efficacy, and Combined Scores Across Time by Group

Outcome	Group	Pre-test (M ± SD)	Post-test I (M ± SD)	Post-test II (M ± SD)
Knowledge	Study	10.10 ± 2.20	31.50 ± 1.70	22.10 ± 2.40
	Control	10.00 ± 2.10	11.20 ± 2.00	10.80 ± 2.20
Self-efficacy	Study	20.80 ± 1.80	44.50 ± 1.50	32.50 ± 1.70
	Control	20.60 ± 1.70	21.40 ± 1.90	21.10 ± 1.80
Combined	Study	30.90 ± 2.50	76.00 ± 2.40	54.60 ± 2.50
	Control	30.60 ± 2.40	32.60 ± 2.60	31.90 ± 2.50

Note. Study group showed marked improvement; control group remained stable.

Table No. 3 Repeated-Measures ANOVA and Between-Group Comparisons

Outcome	Effect	F (df)	p	η^2
Knowledge	Time × Group	820.45 (2,196)	< .001	.893
Self-efficacy	Time × Group	1025.70 (2,196)	< .001	.912
Combined	Time × Group	1450.20 (2,196)	< .001	.936

Note. Significant interaction effects indicate strong intervention impact.

Table No. 4 Knowledge Domains and Self-Efficacy Trends by Group

Variable	Group	Pre-test	Post-test I	Post-test II
Knowledge domains	Study	Low	High	Moderate
	Control	Low	Low	Low
Correct responses (%)	Study	25–40%	85–95%	60–75%
	Control	25–40%	30–45%	30–40%
Self-efficacy	Study	Low	High	Moderate
	Control	Low	Low	Low

Note. Improvement observed only in the intervention group.

Table No. 5 Subgroup Analysis and Retention of Improvement (Study Group Only)

Variable	Immediate Improvement	Retained Improvement	p
Age	Similar	Similar	> .05
Education	Slight variation	Borderline	.048
Experience	Similar	Similar	> .05
Workplace	Similar	Similar	> .05
Overall retention	~50–60% retained	—	—

Note. No major subgroup differences; intervention effect consistent.

DISCUSSION

Comparison of the study and control groups across the three assessment points demonstrated clear improvements in knowledge, self-efficacy, and total scores among nurses who received the educational intervention (Table 2). In contrast, the control group showed only minimal changes over time. These findings indicate that the observed improvements were primarily associated with participation in the educational program rather than with the passage of time alone⁷⁻⁹.

This interpretation was further supported by the repeated-measures ANOVA (Table 3), which demonstrated significant Time × Group interactions for knowledge, self-efficacy, and total scores. The interaction indicates that changes over time differed significantly between the two groups, providing evidence of an intervention effect. The magnitude of these differences, together with the large effect sizes observed, suggests that the program produced substantial improvements in both cognitive and psychological outcomes¹⁰⁻¹².

The pattern of knowledge scores was particularly noteworthy. Participants in the study group had relatively low knowledge scores at baseline, followed by a marked increase immediately after the intervention. Although scores declined at the six-month follow-up, they remained substantially higher than baseline levels (Tables 2 and 5). This pattern suggests that the intervention produced strong immediate learning gains while also supporting meaningful longer-term retention. The partial decline at follow-up is consistent with the expected reduction in knowledge when educational content is not continuously reinforced¹³⁻¹⁴.

These findings are consistent with previous research demonstrating that simulation-based and interactive educational approaches can enhance knowledge acquisition, comprehension, and retention among nurses¹⁵. Simulation provides learners with opportunities to apply theoretical knowledge within realistic clinical scenarios without exposing patients to unnecessary risk. It also facilitates repeated practice, immediate feedback, reflection, and correction of

errors, thereby strengthening the integration of theoretical knowledge with clinical practice¹⁶.

Interactive learning strategies may have contributed further to the observed improvement. Approaches such as case-based discussions, problem-solving activities, guided clinical scenarios, and active participation encourage learners to engage with educational content rather than passively receive information. Previous evidence indicates that such approaches can promote deeper understanding and improve retention by encouraging active cognitive processing and practical application of knowledge¹⁷.

The present findings also support previous studies demonstrating the effectiveness of structured and learner-centered educational interventions in improving nurses' knowledge and professional competencies¹⁸. Educational programs based on clearly defined learning objectives and organized learning activities can provide nurses with a systematic framework for acquiring, applying, and reinforcing new knowledge. Such approaches may be particularly valuable in clinical environments where nurses must translate theoretical knowledge into timely and appropriate decisions.

An important finding of this study was the substantial improvement in self-efficacy following the intervention. Self-efficacy reflects an individual's confidence in their ability to successfully perform specific behaviors or tasks and is closely related to motivation, persistence, clinical performance, and decision-making. Previous research has similarly demonstrated that greater self-efficacy is associated with improved professional performance and clinical decision-making among nurses¹⁹. The sustained elevation in self-efficacy at follow-up therefore represents an important outcome because knowledge alone may be insufficient to influence practice when nurses lack confidence in applying what they have learned.

The simultaneous improvement in knowledge and self-efficacy supports the view that effective nursing education should address both cognitive and psychological dimensions of professional competence²⁰. This finding is particularly relevant to the theoretical basis of the present intervention. Meleis' Transition Theory emphasizes the processes through which individuals acquire knowledge, confidence, and mastery while adapting to professional roles and changing practice demands. From this perspective, the observed improvements may reflect not only acquisition of information but also greater confidence and readiness to apply newly acquired competencies in clinical practice.

Subgroup analyses within the study group revealed no statistically significant differences in intervention outcomes according to the examined demographic and professional characteristics (Table 5). This suggests that the intervention produced relatively consistent benefits

across participants of different ages, levels of professional experience, and workplace characteristics. Rather than demonstrating universal effectiveness, however, these findings indicate that no evidence of meaningful effect modification by the measured characteristics was detected in the present sample.

The comparability of the study and control groups at baseline further strengthens interpretation of the findings by reducing the likelihood that initial group differences accounted for the observed post-intervention outcomes. Moreover, inclusion of a comparison group and repeated assessments provided a stronger basis for distinguishing intervention-related changes from changes that might otherwise occur over time. Nevertheless, because the study employed a quasi-experimental rather than randomized design, causal interpretations should remain appropriately cautious.

The present results are broadly consistent with earlier literature supporting educational interventions as effective strategies for improving nurses' knowledge and self-efficacy. More recent evidence has similarly emphasized the value of structured, learner-centered, and theoretically informed educational approaches for strengthening professional competence and confidence²¹. The current study extends this evidence by demonstrating substantial differences between the intervention and control groups and by assessing whether improvements persisted over a six-month follow-up period.

CONCLUSION

This study demonstrates that a structured educational intervention based on Meleis' Transition Theory effectively improved nurses' knowledge and self-efficacy. Significant improvements were observed immediately after the intervention, with benefits remaining above baseline at follow-up despite some decline over time. The intervention showed consistent effects across demographic and professional characteristics. These findings support integrating theory-based educational programs into continuing nursing education, with periodic refresher sessions recommended to enhance long-term retention and sustain self-efficacy in clinical practice.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Eman Tammuz Mahmood, Salwa Hazim Al Mukhtar
Drafting or Revising Critically:	Eman Tammuz Mahmood, Salwa Hazim Al Mukhtar
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. 10(CCMRE-Nur-26-7)
Dated 16.02.2026.

REFERENCES

- Attia YK, Ibrahim RH. Difficulties experienced in clinical learning settings for nurses in Iraq: perspectives of nursing administrators and nursing instructors. *Informatics Med Unlocked* 2023;38:101236. doi:10.1016/j.imu.2023.101236.
- Essa BA, Ibrahim RH. Assessing patient safety culture among hospital nurses in Mosul: a comprehensive survey. *Malays J Nurs* 2024;16(1):251-264.
- Ibrahim RH, Yaas MH, Hamarash MQ, Al-Mukhtar SH, Abdulghani MF, Mushhadany OA. Adapting cognitive behavioral therapy for adolescents in Iraq via mobile apps: qualitative study of usability and outcomes. *JMIR Pediatr Parent* 2025;8:eXXXXX. doi:10.2196/XXXXX.
- Labrague LJ, De Los Santos JAA. Transition shock and newly graduated nurses' self-efficacy: a longitudinal study. *J Nurs Manag* 2024;32(2):145-153. doi:10.1111/jonm.137XX.
- Yaas MH, Hamarash MQ, Almushhadany OI, Ibrahim RH, Jassim RS. Assessing the effectiveness of clinical rotations in preparing undergraduate nursing students for practice: mixed study. *Malays J Nurs* 2023;15(2):87-99.
- Al-Waly LAM, Al-Wily MAS, Ibrahim RH. Nurses' knowledge regarding pneumonia in children under five years of age at pediatric wards in Kirkuk teaching hospitals. *Ind J Forensic Med Toxicol* 2020;14(2):1705-1710.
- Mohsen A, Ibrahim R, Hassan M, et al. Intradialytic muscle cramps among haemodialysis patients: prevalence, associated factors and clinical outcomes. *BMC Nephrol* 2025;26:118. doi:10.1186/s12882-025-03745-8.
- Abdullah MK, Ibrahim RH, Al-Wily MAS, et al. Nurses' attitudes, knowledge, and practices concerning evidence-based practice: a cross-sectional study. *Malays J Nurs* 2024;15(4):4-11.
- Habow HD, Ibrahim RH. Investigating the dynamics of women's health information on social media: nursing perspective. *Malays J Nurs* 2024;16(2):191-200.
- Hamid IZ, Ibrahim RH. Promoting sustainability healthcare practices: a quasi-experimental study on nursing education in governmental hospitals. *Malays J Nurs* 2025;16(4):218-228.
- Alneama RMJ, Ibrahim RH. An integration of Benner's theory to explore the learning styles and motivation as predictors of academic achievement among nursing students in Iraq. *J Holist Nurs Sci* 2025;12(1):95-104.
- Meleis AI. *Transitions Theory: Middle Range and Situation Specific Theories in Nursing Research and Practice*. Springer Publishing Company; New York, 2010. <https://www.scirp.org/reference/referencespapers?referenceid=1387493>
- Bradley J, Jayanna K, Shaw S, Cunningham T, Fischer E, Mony P, et al. Improving the knowledge of labour and delivery nurses in India: a randomized controlled trial of mentoring and case sheets in primary care centres. *BMC Health Serv Res* 2017;17(1). doi:10.1186/s12913-016-1933-1.
- Lin H, Liu G, Wang X, Xu Q, Guo S, Hu R. A virtual simulation-based training program on birthing positions: a randomized controlled trial. *BMC Nurs* 2023;22(1):318. doi:10.1186/s12912-023-01491-7.
- Kim S, Im M. Development and evaluation of nursing clinical practice education using m-learning. *Healthcare* 2024;12(2):206. doi:10.3390/healthcare12020206.
- Chatzea VE, Logothetis I, Kalogiannakis M, Rovithis M, Vidakis N. Digital educational tools for undergraduate nursing education: a review of serious games, gamified applications and non-gamified virtual reality simulations/tools for nursing students. *Information* 2024;15(7):410. doi:10.3390/info15070410.
- Hatem AA, Muhammad TR. Impact of an educational program on nurses' knowledge of poisoning management: a quasi-experimental study. *Al-Mustaqbal J Pharm Med Sci* 2024;2(3):Article 1. doi:10.62846/3006-5909.1016.
- Mohammed AK, Muhamed Nury LAH. Effectiveness of educational program on nurses/midwives performance regarding placenta examination at Maternity Teaching Hospital in Sulaimani/Iraq. *Mosul J Nurs* 2023;11(2):332-341.
- Hassan JM, Abdulkareem YZ, AlMukhtar SH. The impact of maternal obesity on newborn and maternal health status. *Obes Metab* 2021;18(4):417-424. doi:10.14341/omet12772.
- Abdullah MK, Abdulghani MF, Ibrahim RH, Mahmood HJ, Mohsen OA, Jassim RS. Nurses' attitudes, knowledge, and practices concerning evidence-based practice: a cross-sectional study. *Malays J Nurs* 2024;15(4):4-11. doi:10.31674/mjn.2024.v15i04.002.
- Muhammed Taher MI, Ibrahim RH. Knowledge, attitudes, and practice of university's employees about complementary and alternative medicine (CAM). *Inform Med Unlocked* 2023;37:101184. doi:10.1016/j.imu.2023.101184.

The Effect of Melatonin on Oxidative Biomarkers (MDA,SOD,GSH) in Patients with Stable Angina

Zahraa R. Hadi¹ and Layth Jabbar¹

Oxidative Stress
Biomarkers and
Clinical
Improvement in
Stable Angina

ABSTRACT

Objective: To investigate the relationship between change in oxidative stress markers and change in angina symptom burden and sleep quality in patients with stable angina given adjunctive melatonin therapy

Study Design: Randomized, quadruple blind, placebo-controlled Study

Place and Duration of Study: This study was conducted at the College of Pharmacy, University of Thi-Qar, Iraq from 1st February 2026 to 30th April 2026

Methods: One hundred patients were enrolled, among them 55 patients taking melatonin 5 mg once daily at night and 45 taking placebo, in addition to usual anti-anginal treatment. Oxidative stress markers were malondialdehyde, superoxide dismutase and glutathione. Clinical measures were the number of angina episodes per week, angina severity, short-acting nitrate use, and Pittsburgh Sleep Quality Index.

Results: In the melatonin group, malondialdehyde decreased from 4.82 ± 0.91 to 3.79 ± 0.81 $\mu\text{mol/L}$, while superoxide dismutase increased from 6.21 ± 1.15 to 7.15 ± 1.20 U/mL and glutathione from 38.7 ± 6.5 to 44.8 ± 7.1 $\mu\text{mol/L}$. Weekly angina episodes decreased from 3.4 ± 1.7 to 1.3 ± 1.1 , short acting nitrate use from 3.0 ± 1.8 to 1.0 ± 1.1 doses/week, and Pittsburgh Sleep Quality Index from 9.1 ± 3.0 to 6.2 ± 2.7 .

Conclusion: Favourable changes in oxidative stress biomarkers, particularly reduced malondialdehyde and increased glutathione, were associated with improved angina symptoms and sleep quality.

Key Words: Melatonin, Oxidative stress, Stable angina, Malondialdehyde, Superoxide dismutase, Glutathione, Angina symptoms, Pittsburgh Sleep Quality Index.

Citation of article: Hadi ZR, Jabbar L. The Effect of Melatonin on Oxidative Biomarkers (MDA,SOD,GSH) in Patients with Stable Angina. Med Forum 2026;37(9):26-30. doi:10.60110/medforum.370905.

INTRODUCTION

It has traditionally been explained by fixed atherosclerotic constriction of the epicardial coronary arteries but recent evidence has focused on a more complex pathophysiology, which involves stenosis, coronary microvascular dysfunction, vasomotor abnormalities, endothelial dysfunction, inflammation, and altered myocardial oxygen supply-demand balance^{1,2}. The view of oxidative stress as a biologically relevant pathway in coronary artery disease is gaining momentum since overproduction of reactive oxygen

species can limit the bioavailability of nitric oxide, enhance endothelial dysfunction, oxidize lipids and lipoproteins, increase vascular inflammation and promote atherosclerotic plaque development and destabilization^{3,4}. Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) are commonly employed to reflect different aspects of redox biology: lipid peroxidation, antioxidant enzymes and non-enzymatic antioxidant stores^{5,6}.

Melatonin may directly contribute to the reactive oxygen and nitrogen species scavenging, mitochondrial activity, endogenous antioxidant defenses, and inflammatory signaling pathways, which are pertinent to cardiovascular disease^{7,8}. Some altered melatonin physiology in patients with coronary artery disease and possible protective effect against ischemia have also been proposed by observational and clinical studies⁹.

Unstable angina, clinically it is important to know whether the change in the frequency of oxidative stress markers is similar to the change in the frequency of angina attack, use of nitrates, functional symptoms category, and quality of sleep¹⁰.

The hypothesis was that greater reduction in malondialdehyde and greater increases in superoxide dismutase and glutathione would be associated with larger reductions in weekly angina frequency, angina

¹. MSc Scholar / Assistant Professor², Department of Pharmacognosy, College of Pharmacy, University of Thi-Qar, Thi-Qar, Iraq

Correspondence: Layth Jabbar, Assistant Professor, Department of Pharmacognosy, College of Pharmacy, University of Thi-Qar, Thi-Qar, 64001, Iraq.
Contact No: 009647705512899
Email: layth.jabbar@utq.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

severity, short-acting nitrate use, and Pittsburgh Sleep Quality Index global score.

METHODS

This was a randomized, quadruple blind, placebo-controlled trial was conducted at College of Pharmacy, University of Thi-Qar, Iraq from 1st February 2026 to 30th April 2026 vide letter No. IQ.UTQ.PHARM.2025.005 dated 19th January 2026. One hundred patients were enrolled, among them 55 patients taking melatonin 5 mg once daily at night and 45 taking placebo, in addition to usual anti-anginal treatment. The administration at bedtime was selected based on the physiological functions of melatonin in circadian regulation and sleep-wake rhythm as well as allowing the assessment of its antioxidant and cardiometabolic significance. The biomarker panel included malondialdehyde, superoxide dismutase, and glutathione. MDA was measured in $\mu\text{mol/L}$ and was interpreted as a marker of lipid peroxidation. SOD was measured in U/mL and was interpreted as an enzymatic antioxidant defense marker. GSH was measured in $\mu\text{mol/L}$ and was interpreted as a non-enzymatic antioxidant marker. Fasting morning samples were preferred to reduce variability related to recent food intake and circadian effects. Samples were processed promptly and stored according to laboratory requirements for oxidative stress assays. Angina symptom burden was assessed using a standardized angina diary, while functional severity was summarized using Canadian Cardiovascular Society class. These approaches are consistent with commonly used symptom-based assessment in chronic stable angina. A participant with a large negative ΔMDA would be considered to have greater reduction in lipid peroxidation, while a participant with a large positive ΔGSH would be considered to have greater improvement in antioxidant reserve. This exploratory approach is biologically justified because oxidative stress, endothelial dysfunction, microvascular impairment, and sleep disturbance may interact in chronic coronary syndromes, but the analysis should be interpreted as hypothesis-generating rather than confirmatory.

RESULTS

There were 55 in melatonin arm and 45 in the placebo arm (Table 1). MDA decreased from $4.82 \pm 0.91 \mu\text{mol/L}$ at baseline to $3.79 \pm 0.81 \mu\text{mol/L}$ at week 8 in the melatonin group, giving a mean change of $-1.03 \pm 0.72 \mu\text{mol/L}$. In the placebo group, MDA decreased only slightly from $4.76 \pm 0.88 \mu\text{mol/L}$ to $4.55 \pm 0.90 \mu\text{mol/L}$, with a mean change of $-0.21 \pm 0.60 \mu\text{mol/L}$. The between-group difference in MDA change was statistically significant, indicating a greater reduction in lipid peroxidation among patients receiving melatonin. SOD activity increased from $6.21 \pm 1.15 \text{ U/mL}$ to

$7.15 \pm 1.20 \text{ U/mL}$ in the melatonin group, compared with a smaller increase from $6.18 \pm 1.09 \text{ U/mL}$ to $6.36 \pm 1.08 \text{ U/mL}$ in the placebo group. Similarly, GSH increased from $38.7 \pm 6.5 \mu\text{mol/L}$ to $44.8 \pm 7.1 \mu\text{mol/L}$ in the melatonin group, whereas the placebo group showed only a modest increase from $39.1 \pm 6.2 \mu\text{mol/L}$ to $40.2 \pm 6.6 \mu\text{mol/L}$ (Table 2).

Table No. 1: Baseline characteristics of the analyzed participants

Variable	Melatonin group (n = 55)	Placebo group (n = 45)
Age (years)	58.4 ± 7.3	57.9 ± 7.8
Male sex	33 (60%)	28 (62.2%)
BMI (kg/m^2)	29.4 ± 3.6	29.1 ± 3.9
Hypertension	38 (69.1%)	32 (71.1%)
Diabetes mellitus	23 (41.8%)	20 (44.4%)
Dyslipidemia	42 (76.4%)	35 (77.8%)
Current smoker	15 (27.3%)	11 (24.4%)
Beta-blocker use	44 (80%)	37 (82.2%)
Calcium channel blocker use	28 (50.9%)	22 (48.9%)
Long-acting nitrate use	31 (56.4%)	26 (57.8%)
Statin use	48 (87.3%)	40 (88.9%)
Weekly angina episodes	3.4 ± 1.7	3.3 ± 1.8
PSQI global score"	9.1 ± 3.0	9.3 ± 3.2

Table No. 2: Oxidative stress biomarkers at baseline, week 4, and week 8

Biomarker	Time point	Melatonin group (n=55)	Placebo group (n=45)	P value
MDA ($\mu\text{mol/L}$)	Baseline	4.82 ± 0.91	4.76 ± 0.88	0.74
	Week 4	4.21 ± 0.86	4.63 ± 0.87	0.018
	Week 8	3.79 ± 0.81	4.55 ± 0.90	<0.001
	Mean change	-1.03 ± 0.72	-0.21 ± 0.60	<0.001
SOD (U/mL)	Baseline	6.21 ± 1.15	6.18 ± 1.09	0.89
	Week 4	6.78 ± 1.18	6.29 ± 1.11	0.037
	Week 8	7.15 ± 1.20	6.36 ± 1.08	0.001
	Mean change	$+0.94 \pm 0.83$	$+0.18 \pm 0.69$	<0.001
GSH ($\mu\text{mol/L}$)	Baseline	38.7 ± 6.5	39.1 ± 6.2	0.76
	Week 4	41.9 ± 6.8	39.8 ± 6.4	0.12
	Week 8	44.8 ± 7.1	40.2 ± 6.6	0.001
	Mean change	$+6.1 \pm 5.4$	$+1.1 \pm 4.9$	<0.001

The clinical improvement indicated that improvement in oxidative biomarkers occurred alongside clinically meaningful improvement in angina symptom burden, rescue nitrate use, and subjective sleep quality (Table 3).

Correlation between biomarker changes and clinical changes: Increases in SOD and GSH were inversely associated with clinical change scores, because positive changes in antioxidant markers were associated with

negative changes in angina outcomes and PSQI (Table 4).

Regression analysis: In the model for change in weekly angina episodes, both Δ MDA and Δ GSH remained

independently associated with symptom improvement after adjustment for baseline angina frequency, age, sex, diabetes mellitus, hypertension, and treatment group (Table 5).

Table No. 3: Clinical outcome changes from baseline to week 8

Clinical outcome	Melatonin baseline	Melatonin week 8	Mean change	Placebo baseline	Placebo week 8	Mean change	Between-group p-value
Weekly angina episodes	3.4 $\pm$ 1.7	1.3 $\pm$ 1.1	-2.1 $\pm$ 1.4	3.3 $\pm$ 1.8	2.7 $\pm$ 1.6	-0.6 $\pm$ 1.2	<0.001
Angina severity score	6.1 $\pm$ 1.4	3.8 $\pm$ 1.6	-2.3 $\pm$ 1.5	6.0 $\pm$ 1.5	5.2 $\pm$ 1.7	-0.8 $\pm$ 1.3	<0.001
Nitrate use/week	3.0 $\pm$ 1.8	1.0 $\pm$ 1.1	-2.0 $\pm$ 1.3	2.9 $\pm$ 1.7	2.4 $\pm$ 1.5	-0.5 $\pm$ 1.1	<0.001
PSQI global score	9.1 $\pm$ 3.0	6.2 $\pm$ 2.7	-2.9 $\pm$ 2.4	9.3 $\pm$ 3.2	8.7 $\pm$ 3.0	-0.6 $\pm$ 2.1	<0.001

Table No. 4: Correlations between oxidative biomarker changes and clinical outcome changes

Biomarker change	Δ Weekly angina episodes	Δ Angina severity	Δ Nitrate use/week	Δ PSQI score
Δ MDA	r = 0.46, p<0.001	r = 0.39, p<0.001	r = 0.42, p<0.001	r = 0.44, p<0.001
Δ SOD	r = -0.28, p=0.005	r = -0.24, p=0.016	r = -0.26, p=0.009	r = -0.22, p=0.028
Δ GSH	r = -0.43, p<0.001	r = -0.36, p<0.001	r = -0.38, p<0.001	r = -0.41, p<0.001

Table No. 5: Multivariable regression models for predictors of clinical improvement

Dependent variable	Predictor	B coefficient	95% CI	Standardized β	p-value
Δ Weekly angina episodes	Δ MDA	0.42	0.15 to 0.69	0.31	0.003
	Δ GSH	-0.06	-0.11 to -0.02	-0.27	0.010
	Baseline angina frequency	-0.18	-0.32 to -0.04	-0.25	0.013
	Diabetes mellitus	0.21	-0.19 to 0.61	0.10	0.30
	Melatonin group	-0.78	-1.33 to -0.23	-0.29	0.006
Δ PSQI score	Δ MDA	0.72	0.27 to 1.17	0.34	0.002
	Δ GSH	-0.10	-0.18 to -0.02	-0.26	0.015
	Baseline PSQI	-0.23	-0.36 to -0.10	-0.33	0.001
	Diabetes mellitus	0.34	-0.38 to 1.06	0.09	0.35
	Melatonin group	-1.12	-2.02 to -0.22	-0.27	0.015

DISCUSSION

The contribution of oxidative stress to endothelial dysfunction, atherosclerosis, microvascular dysfunction and coronary symptom burden^{11,12}. Reduced MDA could be an indication of decreased lipid peroxidation and decreased oxidative damage of vascular and cell membranes, whereas increased GSH and SOD activity could reflect enhanced antioxidant protection and the redox balance^{5,6}. Since nitric oxide signaling and endothelial activity are vulnerable to oxidative stress, redox status can be improved, which can positively influence vascular tone and myocardial perfusion reserve^{13,14}.

Patients whose lipid peroxidation is reduced larger or whose antioxidant reserve is improved larger can be a subgroup that is more likely to benefit symptoms, but subgroup interpretation remains problematic, and such improvements should be confirmed prospectively^{15,16}.

Surveys of oxidative stress biomarkers in coronary artery disease underscore the notion that MDA and

other lipid peroxidation products, antioxidant enzymes and glutathione-related pathways can be used to complement information on vascular injury and antioxidant capacity¹⁷.

The results also correspond to the antioxidant biology of melatonin which is known. Recent reviews define melatonin as a direct free-radical scavenger and an indirect modulator of antioxidant defense mechanisms, such as mitochondrial functional and redox-sensitive signaling pathways^{18,19}.

A large percentage of cardiovascular melatonin studies has been devoted to ischemia-reperfusion injury, cardioprotection, metabolic disease or perioperative oxidative stress instead of symptom change instable angina, which supports the rationale of large-scale prospective studies in this particular population²⁰.

The strengths of this research are that it is a randomized placebo-controlled parent study, repeated biomarker measurements, multiple oxidative stress markers, and a measure of clinically significant outcomes. It has weaknesses such as exploratory secondary-analysis

design, small sample, potential residual confounding, use of selected biomarkers as opposed to a redox panel, and lack of causality inference based on biomarker outcome correlations²¹.

CONCLUSION

Greater reduction in MDA and greater increase in GSH were linked with larger reductions in angina frequency, angina severity, nitrate use, and PSQI score, while SOD showed weaker but consistent associations.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa R. Hadi, Layth Jabbar
Drafting or Revising Critically:	Zahraa R. Hadi, Layth Jabbar
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. IQ.UTQ.PHARM.2025.005
Dated 19.01.2026

REFERENCES

- Vrints C, Andreotti F, Koskinas KC, Rossello X, Adamo M, Ainslie J, et al. 2024 ESC Guidelines for the management of chronic coronary syndromes. *Eur Heart J* 2024;45(36):3415-37.
- Virani SS, Newby LK, Arnold SV, Bittner V, Brewer LC, Demeter SH, et al. 2023 AHA/ACC/ACCP/ASPC/NLA/PCNA guideline for the management of patients with chronic coronary disease. *Circulation* 2023;148(9):e9-e119.
- Alkufi HK, Kareem AT, Hasan SA, Najem NA. Protective and therapeutic role of Ajwa date extract in propionic acid-induced autism in rats. *J. Anim. Health Prod* 2026;14(1):199-204.
- Abu-Elfotuh K, Abbas AN, Najm MA, Qasim QA, Hamdan AM, Abdelrehim AB, et al. Neuroprotective effects of punicalagin and/or micronized zeolite clinoptilolite on manganese-induced Parkinson's disease in a rat model: Involvement of multiple pathways. *CNS Neurosci Therapeutics* 2024;30(10):e70008.
- Hashim ZR, Qasim QA, ALabbod MH. The association of serum calcium and vitamin D with insulin resistance and beta-cell dysfunction among people with type 2 diabetes. *Arch Razi Inst* 2022; 77(5):1593.
- Zamanian MY, Golmohammadi M, Nili-Ahmadabadi A, Alameri AA, Al-Hassan M, Alshahrani SH, et al. Targeting autophagy with tamoxifen in breast cancer: From molecular mechanisms to targeted therapy. *Fundam Clin Pharmacol* 2023;37(6):1092-108.
- Qasim Q. Antioxidants, lipid profiles, and glucose levels, as well as persistent inflammation, are central to the link between diabetes mellitus type ii and oxidative stress. *Georgian Med News* 2024; 1(351):158-61.
- Hasan SA, Abass AA, Jabbar L. The protective role of ethanolic extract of ginseng (*Panax ginseng*) against adverse physiological and histological alterations in the liver of female rabbits exposed to chlorine. *Advancements Life Sci* 2025;12(1):185-90.
- Mohammadi N, Alizadeh M, Akbarzadeh S, Rezaei M, Mahmoodi M, Netticadan T, et al. Melatonin administered postoperatively lowers oxidative stress and inflammation and significantly recovers heart function in patients undergoing CABG surgery. *Eur J Med Res* 2025;30:585.
- Margiana R, HamoudAlshahrani S, Kayumova D, HussienRadieAlawadi A, Hjazi A, Alsalamy A, et al. Association between maternal exposure to arsenic by drinking water during pregnancy and risk of preterm birth: a systematic review and meta-analysis. *Int J Environ Health Res* 2024;34(8):2947-56.
- Huldani H, Malviya J, Rodrigues P, Hjazi A, Deorari MM, Al-Hetty HR, et al. Discovering the strength of immunometabolism in cancer therapy: employing metabolic pathways to enhance immune responses. *Cell Biochem Function* 2024;42(2):e3934.
- Qasim QA, Al-Salman HN. The isolation characterization and assessment of bioactive flavonoid with special reference to anti-arthritis activity. *Materials Today: Proceedings* 2023;80:2567-72.
- Medina-Leyte DJ, Zepeda-García O, Domínguez-Pérez M, González-Garrido A, Villarreal-Molina T, Jacobo-Albavera L. Endothelial dysfunction, inflammation and coronary artery disease: potential biomarkers and promising therapeutical approaches. *Front Cardiovasc Med* 2021;8:644442.
- Abd-Alrassol KS, Qasim QA, Al-Salman HN, Hussein HH. The spectrophotometric determination of antiepileptic drug in standard and pharmaceutical formulations by diazotization coupling reaction and some metals complexes. *Sys Rev Pharmacy* 2020;11(3).
- Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. Melatonin: a pleiotropic, orchestrating regulator molecule. *Prog Neurobiol* 2011;93(3):350-84.
- Alshahrani SH, Alameri AA, Kahar F, Ramírez-Coronel AA, Obaid RF, Alsaikhan F, et al. Overview of the role and action mechanism of

- microRNA-128 in viral infections. *Microbial Pathogenesis* 2023;176:106020.
17. Ali RH, Hussein FK, Nasar DM, Abd Al salam Salem A. The relevance of mitochondrial DNA mutation in human diseases and forensic sciences. *Al-Nahrain J Sci* 2025;28(1):96-106.
 18. Al Alwan B, Alameri AA, Qasim QA, Vaqqosov S, Jawad Kadhim Z, Sivaraman R, et al. Evaluation of structural and thermodynamic parameters of dibenzothiophene desulfurization by carbon Nano-Filter. *Polycyclic Aromatic Compounds* 2023;43(9):8252-64.
 19. Jasim SA, Ramirez-Coronel AA, Alameri AA, Sapaev IB, Mustafa YF, Jalil AT, et al. Phenytoin drug detection study through the B24N24 and Al24N24 nano-clusters in gas and solvent phase: DFT, TD-DFT, and thermodynamic study. *Inorganic Chem Comm* 2023;153:110887.
 20. Yaprak M, Altun A, Vardar A, Aktoz M, Ciftci S, Ozbay G. Decreased nocturnal synthesis of melatonin in patients with coronary artery disease. *Int J Cardiol* 2003;89(1):103-7.
 21. Vickers AJ, Altman DG. Analysing controlled trials with baseline and follow up measurements. *BMJ* 2001;323(7321):1123-4.

The Effect of Empagliflozin on the Brain Toll Like Receptor-4 Gene Expression in the Experimental Models of Parkinson's Disease

Effect of
Empagliflozin on
the Brain Toll
Like Receptor-4
Gene Expression
in Parkinson's
Disease

Riham Fadhil Jawad Kadhim¹ and Selman Mohammed Selman²

ABSTRACT

Objective: To investigate the effect of empagliflozin on brain toll like receptor 4 gene expression in animal models of Parkinson and to assess the neuroprotective effect of this intervention

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq from 1st December 2025 to 30th April 2026.

Methods: Thirty healthy male albino rats were divided randomly in 5 experimental groups. The time spent on rotarod apparatus was used to assess anti-parkinsonism effect of levodopa/carbidopa and empagliflozin and the real time polymerase chain reaction was used to measure toll like receptor 4 gene expression.

Results: Rotenone administration leads to upregulation of toll like receptor 4 gene expression and significant decrease in body weight, also time spent on rotarod apparatus was considerably lower than the control group ($p \leq 0.05$). Conversely, therapeutic intervention with levodopa/carbidopa and empagliflozin in both doses showed a significant increase in locomotor activity and the body weight when compared with group 2. Groups 3, 4 and group 5 demonstrated significant down regulation of toll like receptor 4 gene expression when compared with rotenone group 2.

Conclusion: Empagliflozin exhibits promising anti-Parkinsonian like effects and it appears to attenuate the neurodegeneration effect induced by rotenone potentially through down-regulation of toll like receptor 4 in the mid brain tissue.

Key Words: Empagliflozin, Rotenone, Parkinson's disease, Neuroinflammation, Toll-like receptor 4.

Citation of article: Kadhim RFJ, Selman SM. The Effect of Empagliflozin on the Brain Toll Like Receptor-4 Gene Expression in the Experimental Models of Parkinson's Disease. Med Forum 2026;37(9):31-35. doi:10.60110/medforum.370906.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative condition marked by severe motor impairment due to dopamine depletion in the striatum and chronic degradation of dopaminergic neurons in the substantia nigra pars compacta. Despite the fact that the exact pathophysiology of PD is still unknown, mounting data indicates that neuroinflammation is essential to the development and course of the illness¹. One of the critical mediators of neuroinflammation is toll like receptor 4 (TLR4), a pattern recognition

receptor that is highly expressed in microglia, the primary immune cells of the central nervous system. Activation of TLR4 triggers intracellular signaling cascades leading to activation of transcription factors including nuclear factor-kappa B and the production of pro-inflammatory cytokines. In Parkinson's disease, these inflammatory mediators lead to oxidative stress, neuronal injury, and the gradual loss of dopaminergic neurons¹.

Several experimental studies have demonstrated that TLR4 expression is significantly increased in both human PD patients and animal models of the disease². Conversely, genetic deletion or inhibition of TLR4 has been shown to reduce neuroinflammation, improve dopaminergic neuron survival, and attenuate motor deficits in toxin-induced animal models of PD^{3,4}. TLR4 signaling is an important therapeutic target in the management of neurodegenerative conditions such as Parkinson's disease.

A common medication for type 2 diabetes is empagliflozin, a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2). Beyond its ability to decrease glucose, empagliflozin has shown neuroprotective qualities^{5,6}. Recent studies suggest that SGLT2

1. M.Sc. Scholar / Professor², Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq.

Correspondence: Riham Fadhil Jawad Kadhim, M.Sc. Scholar, Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq.

Contact No: 9647732945473

Email: riham.fadel@student.uobabylon.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

inhibitors may modulate inflammatory signaling pathways and reduce oxidative stress in various tissues, including the CNS. These pleiotropic effects raise the possibility that empagliflozin may influence neuroinflammatory mechanisms involved in neurodegenerative diseases.

Given the pivotal role of neuroinflammation in Parkinson's disease and the emerging anti-inflammatory properties of empagliflozin and investigating its potential effect on TLR4 signaling represent an important area of research.

METHODS

This experimental study was conducted at Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq from 1st December 2025 to 30th April 2026 vide letter No. 4-43 dated 6th November 2025 and 30 mature male albino rats were used. They weighed between 200 and 300 grams. The College of Medicine/University of Babylon's animal house kept the rats at 25°C with 10 hours of darkness and 14 hours of light, along with food and drink. Following two weeks of adaptation, the animals were divided into five groups at random in accordance with the experimental protocol. The weight of each rat was taken on day 0, 10 and 21 day and the rotarod test was performed at the day 22 to assess motor coordination and balance and to evaluate the effectiveness of treatments on animal models. The healthy, rotenone-free rats in group 1 are the negative control. For 21 days, every other day, rats in groups 2 (positive control), 3, 4, and 5 received rotenone. Group 2 rats were given 2.5 mg/kg of rotenone IP every other day without treatment⁷. Group 3 rats received 10 mg/kg/day of levodopa/carbidopa PO⁸. Each rat in groups 4 and 5 received 10 and 20 mg/kg/day of empagliflozin, respectively. The rotarod test was performed at the day 22 to assess motor coordination and balance and to evaluate the effectiveness of treatments on animal models.

The rotarod test is frequently employed to assess motor coordination and balance in animal models of PD. Each rat was placed on the rotarod apparatus for 3 minutes at speed 20rpm. The number of Rotation and the time

spent on the rotarod apparatus were recorded for each rat⁹.

On the twenty-second day, twenty-four hours after the last doses were given, the animals were beheaded. The brains were removed following the posterior foramen magnum incision in the skull. The olfactory bulbs and cerebellum were removed. Anatomical instruments were used to separate the midbrain tissue from the remainder of the brain. The separated midbrain tissue was sent to the lab for RNA extraction after being immediately stored in Trizol reagent Eppendorf.

Using the NCBI GeneBank database, primers for the target gene (TRL-4) and housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed by the online Primer3 design programme (Table 1).

Total RNA was isolated from midbrain using the AccuZol™ Total RNA Extraction Kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. NanoDrop Spectrophotometer was used to evaluate the quantity and purity of the isolated RNA. Treatment with a DNase I enzyme kit (Promega, Madison, USA) was performed to eliminate residual genomic DNA. Lastly, the cDNA for TLR-4 and GAPDH genes were created with the M-MLV Reverse Transcriptase kit, according to the manufacturer's protocol.

In a reverse transcriptase quantitative polymerase chain reaction (PCR), relative RNA expression was assessed using the SYBR green reporter dye. The TLR-4 gene and the housekeeping gene GAPDH were quantitatively expressed in template cDNAs from each sample using Bio-Rad iQ5 detection equipment (Bio-Rad, Richmond, Virginia)¹⁰.

GAPDH is a commonly used housekeeping gene, especially in gene expression studies. It encodes an enzyme of major regulatory importance that catalyzes oxidative phosphorylation of glyceraldehyde-3-phosphate in the glycolytic pathway. Importantly, its expression is relatively constant across multiple experimental conditions¹¹. The data was analyzed through SPSS-25. The one-way ANOVA followed by post hoc test was used.

Table No. 1: Primer of Toll like receptor 4 (TLR-4) and housekeeping gene (GADPH)

PRIMER	SEQUENCE (5'-3')	NCBI-Reference code
TLR4	TCCGCTGGTTGCAGAAAATG	NM-019178.2
qPCR primer	AGGCAGGAAAGGAACAATGC	
GAPDH	CAAATGGGGTGATGCTGGTG	NM-017008.4
qPCR primer	ATCACAAACATGGGGGCATC	

RESULTS

In comparison to the beginning of the trial on day 0, the control group 1's mean body weight increased significantly ($p=0.009$) on days 10 and 21. In contrast, the mean body weight of the rats in group 2 decreased

significantly ($p=0.025$) on days 10 and 21 in comparison to day 0. The mean body weight of the (L/C) group on day 21 was not significantly different from day 0 ($p=0.527$). The means of weight on days 10 and 21 did not differ significantly ($p>0.05$) between the groups of rats treated with EMPA concentrations of 10, 20 mg/kg and day 0. (Table 2, Fig. 1)

There was a significant decrease ($p=0.001$) in number of rotation in group 2 that were exposed to rotenone as compared to healthy group 1. Additionally, there were a significant increase ($p\leq 0.05$) in groups 3, 4 and group 5 as compared with group 2. Group 5 showed highly significant improvement ($p<0.05$) among all treated groups (Fig. 2)

Comparison of mean difference of time spent on rotarod among group showed that the group 2 had a significant ($p=0.001$) decrease on time spent on rotarod apparatus as compared to healthy group 1. Furthermore, there were a significant ($p\leq 0.05$) increase in groups 3, 4 and group 5 as compared to group 2 (Fig. 3).

Compared to the control group (Group 1), Group 2 treated 21 days with rotenone presented a statistically significant increase ($P < 0.05$) in gene fold change for TLR-4. By contrast, treatment with 10 mg/kg L/C

significantly decreased ($P < 0.05$) TLR-4 gene expression (group 3) figure 4 compared with that of animals exposed to rotenone alone for the same period of time. Likewise, 10 mg/kg empagliflozin-treated rats (Group 4) had a significant reduction ($P<0.05$) in TLR-4 gene fold change relative to Group 2. The most evident decrease ($P < 0.05$) was found in Group 5 (20 mg/kg empagliflozin $\times$ 21 days). Additionally, the fold change in TLR-4 gene in Group 5 was significantly lower ($P<0.05$) than Groups 3 and 4 (Fig. 4). For Ct values, lower Ct values were shown in Group 2, indicating an increase in TLR-4 gene expression. On the contrary, Groups 3, 4 and 5 had higher Ct values when compared to Group 2, indicating less expression of TLR-4. Stable GAPDH expression across experimental conditions was verified, as Ct values for each intracellular group were similar (Fig. 5).

Table No. 2: Mean of weight on day 0, 10 and 21 $\pm$ SEM

Weight	Group 1	Group 2	Group 3	Group 4	Group 5
Day 0	263.50 $\pm$ 7.80	244.00 $\pm$ 7.04	255.83 $\pm$ 11.39	257.83 $\pm$ 5.98	251.50 $\pm$ 7.77
Day 10	280.33 $\pm$ 8.48*	232.67 $\pm$ 6.63**	238.83 $\pm$ 10.54	249.67 $\pm$ 5.91	231.17 $\pm$ 7.08
Day 21	304.00 $\pm$ 7.61*	215.33 $\pm$ 6.09**	253.00 $\pm$ 11.44	268.50 $\pm$ 6.88	249.50 $\pm$ 7.61

*Progressive increase from day 0 $\rightarrow$ day 10 $\rightarrow$ day 21 **Significant decrease from day 0 $\rightarrow$ day 21

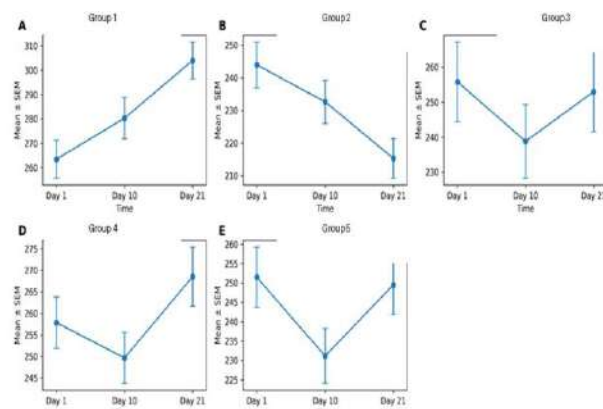


Figure No. 1: Mean of weight on day0, 10, and 21 $\pm$ SEM

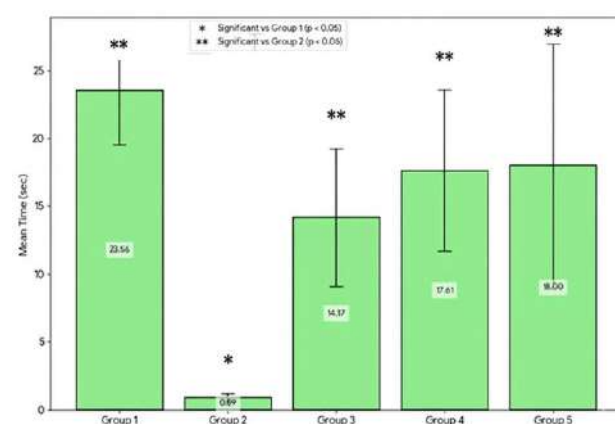


Figure No. 3: Mean of rotation (second) $\pm$ SEM in all rat groups

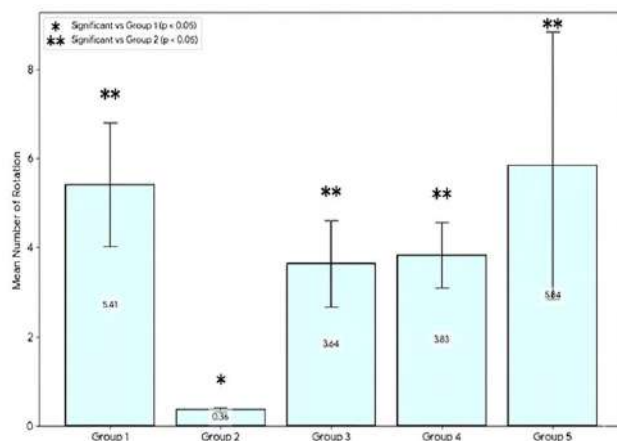


Figure No. 2: Mean of no. of rotations (revolution/minute) $\pm$ SEM

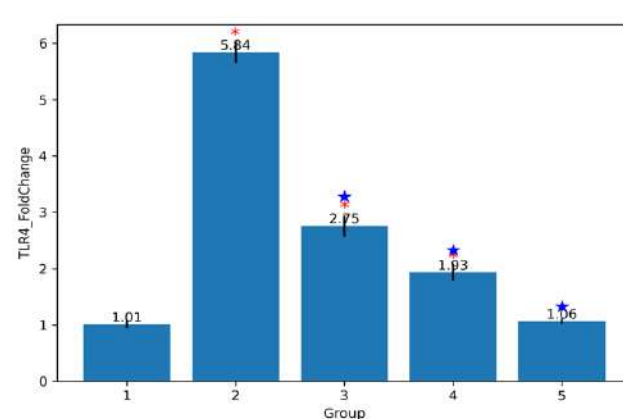


Figure No. 4: Mean of TLR4 fold change $\pm$ SEM

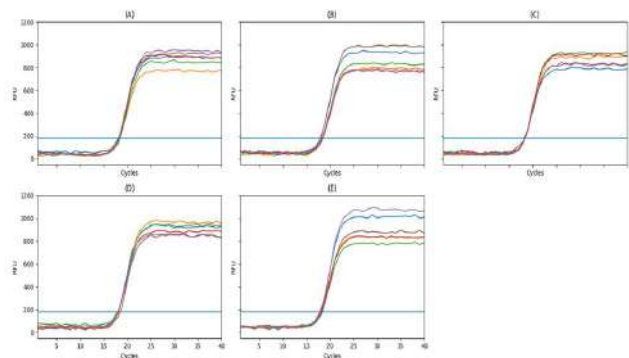


Figure No. 5: The Real Time plots of GAPDH gene expression

DISCUSSION

The changes in body weight are commonly reported in experimental models of PD and may reflect systemic metabolic disturbances and the disease progression. In the present study, rodents in the Parkinsonian group (group2) showed a significant reduction in body weight compared with the control group (group 1). This observation is consistent with previous studies demonstrating that neurodegeneration and metabolic dysregulation contribute to weight loss in Parkinsonian models¹². On the other hand, Treatment with empagliflozin partially ameliorated this reduction in body weight of rotenone exposed rats suggesting that empagliflozin may improve metabolic balance and overall physiological status in affected animals and this finding is consistent with Motawi et al¹³. Empagliflozin has been shown to exert pleiotropic effects beyond glycemic control, including anti-inflammatory and neuroprotective actions within the CNS¹⁴.

Motor impairment is a cardinal feature of PD and reflects the progressive degeneration of dopaminergic neurons in the substantia nigra. In the present study, the induction group demonstrated impaired motor coordination as indicated by decreased latency to fall from the Rotarod apparatus and decrease the number of Rotation. These findings are consistent with previous studies of Parkinson's disease models showing significant deficits in motor performance¹⁵. Additionally, treatment with empagliflozin significantly improved motor performance compared with untreated Parkinsonian rats and this finding is not contradict with the improvement in motor function that have been reported by Mohammed et al¹⁶ investigating the effect of SGLT2 inhibitors in neurodegenerative disorders suggesting that these agents may attenuate neuronal dysfunction and protect dopaminergic pathways. Neuroinflammation is increasingly recognized as a crucial contributor to the progression of PD. TLR4 plays a key role in activating innate immune responses and promoting the release of pro-inflammatory cytokines. In the present study, the induction group exhibited significant upregulation of TLR4 gene

expression compared with the control group and this finding supporting the involvement of inflammatory signaling pathways in PD pathology and this is consistent with Zaho et al¹⁷. Activation of the TLR4 signaling cascade can trigger the downstream inflammatory pathways which ultimately contribute to microglial activation and neuronal damage.

Additionally, when compared to the untreated Parkinsonian group, empagliflozin treatment significantly decreased TLR4 expression levels, especially at high doses. Research on rats revealed that empagliflozin, at a dose of 10 mg/kg/day, decreases TLR-4 gene expression in kidney tissues, demonstrating its capacity to inhibit TLR-4 gene expression¹⁸. This finding suggests that empagliflozin may exert anti-inflammatory effects by modulating TLR4 signaling pathways and was consistent with previous studies which demonstrated that suppression of TLR4 related inflammatory pathways can alleviate neuroinflammation and improve neuronal survival in experimental models of PD^{19,20}. However, to our knowledge, this is the first studies to provide direct evidence of empagliflozin's ability to downregulate TLR4 gene expression in neurodegenerative model of PD. This novel finding highlights the translation potential of Empagliflozin in modulation crucial inflammatory pathways relevant to pathogenesis of PD. Limitations: First off, even though the rotenone-induced Parkinson's disease model in rats is well-established, it might not accurately reflect the complexity of the human condition, which would restrict how broadly the findings can be applied. Second, the study's brief duration may have limited its ability to show the long-term impacts of empagliflozin medication or the chronic course of Parkinson's disease. Thirdly, since changes in mRNA expression do not always accurately reflect protein level or activity, future research using immunohistochemistry to assess the amount of TLR4 protein in the brain tissue would offer more convincing evidence. The present study indicated that empagliflozin may exert neuroprotective effects in Parkinson's disease through multiple mechanisms including improvement of systemic metabolic status, enhancement of motor performance, and suppression of TLR4-mediated neuroinflammatory responses.

CONCLUSION

Empagliflozin ameliorated Parkinsonism like behavioral deficit induced by rotenone and also showed anti-inflammatory effect in the Parkinsonism induced model, it down-regulated TLR4 gene expression and its neuroprotective potential in the experimental model.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Riham Fadhil Jawad Kadhim, Selman Mohammed Selman
--	---

Drafting or Revising Critically:	Riham Fadhil Jawad Kadhim, Selman Mohammed Selman
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.4-43 Dated 06.11.2025

REFERENCES

- Heidari A, Yazdanpanah N, Rezaei N. The role of Toll-like receptors and neuroinflammation in Parkinson's disease. *J Neuroinflammation* 2022; 19(1):135.
- Conte C, Ingrassia A, Breve J, Bol JJ, Timmermans-Huisman E, van Dam AM, et al. Toll-like receptor 4 is upregulated in Parkinson's disease patients and co-localizes with pSer129 α Syn: a possible link with the pathology. *Cells* 2023;12(10):1368.
- Campolo M, Paterniti I, Siracusa R, Filippone A, Esposito E, Cuzzocrea S. TLR4 absence reduces neuroinflammation and inflammasome activation in Parkinson's diseases in vivo model. *Brain Behav Immun* 2019;76:236-47.
- Shao Q hang, Chen Y, Li F fang, Wang S, Zhang X ling, Yuan Y he, et al. TLR4 deficiency has a protective effect in the MPTP/probenecid mouse model of Parkinson's disease. *Acta Pharmacol Sin* 2019;40(12):1503-12.
- Amin EF, Rifaai RA, Abdel-latif RG. Empagliflozin attenuates transient cerebral ischemia/reperfusion injury in hyperglycemic rats via repressing oxidative-inflammatory-apoptotic pathway. *Fundam Clin Pharmacol* 2020;34(5):548-58.
- Bezdoode MF, Kalhori Z, Razlansari R, Jamshidi A. Empagliflozin improves follicular development by attenuating oxidative stress and inflammation in a mouse model of polycystic ovary syndrome. *Cell Tissue Biol* 2025;19(6):550-8.
- Alabi A, Ajayi A, Benneth BA, Omorogbe O, Solomon U. Methyl jasmonate ameliorates rotenone-induced motor deficits in rats through its neuroprotective activity and increased expression of tyrosine hydroxylase immunopositive cells. *Metab Brain Dis* 2019;34.
- Srimathi PK, Vijayalashmi K, Selvaraj R. Behavioral studies of wistar rats in rotenone induced model of parkinson's disease. *Int J Pharm Pharmaceutical Sci* 2017;9:159.
- Rao SV, Hemalatha P, Yetish S, Muralidhara M, Rajini PS. Prophylactic neuroprotective propensity of Crocin, a carotenoid against rotenone induced neurotoxicity in mice: Behavioural and biochemical evidence. *Metab Brain Dis* 2019;34(5):1341-53.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 2001; 25(4):402-8.
- Wang S, Zhang K, Song X, Huang Q, Lin S, Deng S, et al. TLR4 overexpression aggravates bacterial lipopolysaccharide-induced apoptosis via excessive autophagy and NF- κ B/MAPK signaling in transgenic mammal models. *Cells* 2023;12(13): 1769.
- Lawana V, Cannon JR. Rotenone neurotoxicity: Relevance to Parkinson's disease. In: *Advances in neurotoxicology*. Elsevier; 2020.p.209-54.
- Motawi TK, Al-Kady RH, Abdelraouf SM, Senousy MA. Empagliflozin alleviates endoplasmic reticulum stress and augments autophagy in rotenone-induced Parkinson's disease in rats: Targeting the GRP78/PERK/eIF2 α /CHOP pathway and miR-211-5p. *Chem Biol Interact* 2022;362: 110002.
- Dai W, Alavi R, Li J, Carreno J, Pahlevan NM, Kloner RA. Empagliflozin demonstrates neuroprotective and cardioprotective effects by reducing ischemia/reperfusion damage in rat models of ischemic stroke and myocardial infarction. *Sci Rep* 2025;15(1):8986.
- Prasad EM, Hung SY. Behavioral tests in neurotoxin-induced animal models of Parkinson's disease - antioxidants. *MDPI* 2020; 1-50.
- Mohammed NN, Tadros MG, George MY. Empagliflozin repurposing in Parkinson's disease; modulation of oxidative stress, neuroinflammation, AMPK/SIRT-1/PGC-1 α , and wnt/ β -catenin pathways: *Inflammopharmacol* 2024;32(1):777-94.
- Zhao Z, Li F, Ning J, Peng R, Shang J, Liu H, et al. Novel compound FLZ alleviates rotenone-induced PD mouse model by suppressing TLR4/MyD88/NF- κ B pathway through microbiota-gut-brain axis. *Acta Pharm Sin B* 2021;11(9):2859-79.
- Ravindran S, Munusamy S. Renoprotective mechanisms of sodium-glucose co-transporter 2 (SGLT2) inhibitors against the progression of diabetic kidney disease. *J Cell Physiol* 2022; 237(2): 1182-205.
- Yang L, Huang H, Fan Y, Xu L, Jin X, Xiao B, et al. Astragaloside IV ameliorates Parkinson's disease by inhibiting TLR4/NF- κ B-dependent neuro inflammation. *Int Immunopharmacol* 2025; 160:114972.
- Hua Y hai, Huang X yue, Ma J jing, Wu J, Zhou Z hui. Ezetimibe mitigates microglial activation in Parkinson's disease via TLR4/JNK pathway inhibition: evidence from network pharmacology and experimental validation. *Brain Res* 2025; 150116.

Effectiveness of Diaphragmatic Breathing Technique on Stress Reduction among Patients with Acute Heart Failure: A Quasi-Experimental Study

Effect Of
Diaphragmatic
Breathing
Techniques On
Stress With
Acute Heart
Failure

Amer J. Kadush¹, Daa F. Jrood², Malak Hassan Sallal³, Maath Ahmed Khalaf⁴ and
Maher Abdulameer Atiyah⁵

ABSTRACT

Objective: To evaluate the effect of diaphragmatic breathing techniques on stress levels in patients with acute heart failure.

Study Design: Quasi-experimental study

Place and Duration of Study: This study was conducted at the Al-Muthanna Governorate-Iraq from 15th January 2026 to 30th June 2026.

Methods: Fifty patients diagnosed with acute heart failure in selected before and during the use of diaphragmatic breathing, Perceived Stress Questionnaire evaluated stress levels.

Results: The high (36%) and moderate (52%) levels of stress were present before intervention, with a mean score of 2.24 ± 0.657 . The diaphragmatic breathing intervention, most individuals were classified as low stress (70%) and high levels of stress were eliminated (0%), leading to a lower mean score of 1.30 ± 0.463 . A statistically significant ($P < 0.05$) correlation between the sociodemographic factors and stress levels after posttest was not found.

Conclusion: The diaphragmatic breathing method was found to have a significant, positive impact on stress levels related to patients with acute heart failure. The intervention was effective regardless of socio-demographic characteristics.

Key Words: Effectiveness, Breathing technique, Stress reduction, Acute heart failure

Citation of article: Kadush AJ, Jrood DE, Sallal MH, Khalaf MA, Atiyah MA. Effectiveness of Diaphragmatic Breathing Technique on Stress Reduction among Patients with Acute Heart Failure: A Quasi-Experimental Study. Med Forum 2026;37(9):36-41. doi:10.60110/medforum.370907.

INTRODUCTION

Acute heart failure (AHF) is one of the most serious cardiovascular diseases and also constitutes a major global health concern due to its high prevalence, rehospitalisation rates and mortality. Heart failure is a clinical syndrome defined as the inability of the heart to

pump enough blood to meet metabolic needs leading to symptoms of congestive dyspnoea; fatigue; oedema and exercise intolerance.¹ Acute heart failure is often accompanied by psychological distress characterized mainly by worry and anxiety that aggravates the patients' physiological condition thereby impairing recovery outcomes and quality of life.²

Psychological stress is the feeling that environmental or internal demands exceed one's adaptive capacity. Stress can activate both the sympathetic nervous system and hypothalamic-pituitary-adrenal axis of cardiac patients, resulting in increased heart rate and blood pressure as well excessive secretion of hormones including cortisol and catecholamines.³ Untreated stress in patients with heart failure may lead to nonadherence, sleep disorders, sadness and an unstable emotional state negatively impacts the cardiovascular performance.⁴

The significance of non-pharmacological interventions on the physiological and psychosocial outcomes of cardiac patients has been reaffirmed by recent advancements in nursing and alternative therapies. Diaphragmatic breathing techniques have been the focus of widespread interest as effective, simple and inexpensive methods for reducing stress and improving relaxation.⁵ Diaphragmatic or deep abdominal (belly) breathing is a type of controlled respiration that

¹ Instructor, Department of Adult Health Nursing, College of Nursing, Al-Muthanna University, Iraq

² Instructor, Department of Psychiatric & Mental Health Nursing, College of Nursing, Al-Muthanna University, Iraq

³ Assistant Instructor, Department of Adult Health Nursing, College of Nursing, Al-Muthanna University, Iraq

⁴ Assistant Lecturer, Public Health Nursing, University of Al-Maarif College of Nursing, Iraq

⁵ Instructor, Department of Fundamental of Nursing, College of Nursing, University of Basrah, Iraq

Correspondence: Amer J. Kadush, Instructor, Adult Health Nursing, College of Nursing, Al-Muthanna University Iraq.
Contact No: +9647810727033
Email: amer.kadoosh@mu.edu.iq

Received: July, 2026

Reviewed: August, 2026

Accepted: August, 2026

involves using the diaphragm to help you take more relaxed and steady breaths, rather than shallow chest breathing. This technique helps in the inhalation of oxygen into our lungs as it decreases respiratory load, induces characteristics that are parasympathetic and elicits a relaxation response.⁶

The diaphragmatic breathing helps with stress relief and emotional regulation, participants with depression considered diaphragmatic breathing successful, and there were significant decreases in negative affect and perceived stress.⁵ Improved deep breathing exercise resulted in a reduced salivary cortisol, as well as changes to autonomic nervous system regulation. In terms of heart health.⁷ The breathing exercises can significantly reduce stress and negative emotions such as anxiety, tension or worry in heart failure patients. The relaxation-oriented respiratory practices improve anxiety, fatigue, sleep quality and health-related quality of life among patients with chronic illness.⁸

The purposes were to assess the effect of deep breathing technique on stress in patients with heart failure and to test the association of socio-demographic characteristics of patients with stress levels after practicing diaphragmatic breathing.

METHODS

Quasi-experimental study was carried out at Al-Muthanna Governorate-Iraq from 15th January 2026 to 30th June 2026 vide letter 3459/Approval/SDFB Date January 7, 2026. Fifty patients diagnosed with acute heart failure in selected before and during the use of diaphragmatic breathing. All patients with an acute heart failure diagnosis, 8 years of age or older, conscious and able to speak and provide consent were included. Individuals with severe psychological or cognitive issues, acute respiratory distress that hinders them from performing breathing exercises and critical instability were excluded. The demographic information related to age, sex, education, place of

living and income per month was noted. The level of stress was assessed with the Perceived Stress Questionnaire (PSQ) designed by Harald Fliege and colleagues. It is a standardized instrument designed to measure perceived psychological stress with various emotional and cognitive stress-related problems in mind. The scale included measures of tension, anxiety, weariness (boredom), stress, and emotional depletion. Before and during the application of the diaphragmatic breathing technique, participants' answers were evaluated.⁹

Training sessions were conducted through organized meetings by the researcher on diaphragmatic breathing technique. Participants were instructed to sit comfortably and perform slow deep input through the nose while expanding their abdomen, retain 1-2s, and then slowly exhale through the mouth. Seven sessions were conducted on a bi-daily basis, with duration of approximately 15 to 20 minutes each. The approach was ensured to be implemented correctly by observing participants in the sessions. Data were collected from the participants directly in a pre-test (before the diaphragmatic breathing) and post-test (after a complete intervention). Data were analyzed using SPSS-26. The Chi-square test was utilized to determine associations between socio-demographics and stress. Statistical significance was defined by $P < 0.05$.

RESULTS

There were mostly males (62%), between 51-62 years of age (42%) who lived in cities (56%), with more than half obtained a primary educational level (64%) and reported a fairly sufficient monthly income (80%) [Table 1]. The stress scale items showed that mean values ranged from 0.82 to 2.08. The dominant frequencies for most questions were between "Often" and "Usually," indicating that individuals had higher self-reported stress levels prior to the intervention.

Table No. 1: Distribution of sociodemographic characteristics (n=50)

Variable	No.	%
Age (years)	≤50	8.0
	51-62	42.0
	63-74	28.0
	≥75	22.0
Sex	Male	62.0
	Female	38.0
Education level	No education	18.0
	Primary	64.0
	Secondary	14.0
	Bachelor degree	4.0
Residence	City	56.0
	Rural	44.0
Monthly income	Sufficient	8.0
	Somewhat sufficient	80.0
	Not sufficient	12.0

Table No. 2: The stress levels before and after practicing diaphragmatic breathing techniques

Symptoms of Stress	Scoring	Before			After		
		F	%	Mean	F	%	Mean
You feel rested	Almost never	10	20	1.94	20	40	1.00
	Some-times	6	12		10	20	
	Often	11	22		20	40	
	Usually	23	46		-	-	
You feel that too many demands are being made on you	Almost never	15	30	1.68	21	42	0.90
	Some-times	2	4		13	26	
	Often	17	34		16	32	
	Usually	16	32		-	-	
You have too many things to do	Almost never	9	18	1.62	14	28	1.02
	Some-times	12	24		21	42	
	Often	18	36		15	30	
	Usually	11	22		-	-	
You feel you're doing things you really like	Almost never	19	38	1.26	21	42	0.92
	Some-times	10	20		13	26	
	Often	10	20		15	30	
	Usually	11	22		1	2	
You fear you may not manage to attain your goals	Almost never	16	32	1.72	28	56	0.70
	Some-times	4	8		9	18	
	Often	8	16		13	26	
	Usually	22	44		-	-	
You feel calm	Almost never	16	32	1.58	21	42	1.14
	Some-times	8	16		5	10	
	Often	7	14		20	40	
	Usually	19	38		4	8	
You feel frustrated	Almost never	12	24	1.70	20	40	0.86
	Some-times	3	6		17	34	
	Often	23	46		13	26	
	Usually	12	24		-	-	
You are full of energy	Almost never	19	38	1.36	28	56	0.86
	Some-times	8	16		4	8	
	Often	9	18		15	30	
	Usually	14	28		3	6	
You feel tense	Almost never	7	14	2.08	16	32	1.10
	Some-times	6	12		13	26	
	Often	13	26		21	42	
	Usually	24	48		-	-	
Your problems seem to be piling up	Almost never	12	24	1.80	24	48	0.88
	Some-times	6	12		9	18	
	Often	12	24		16	32	
	Usually	20	40		1	2	
You feel you're in a hurry	Almost never	21	42	1.32	22	44	0.92
	Some-times	8	16		10	20	
	Often	5	10		18	36	
	Usually	16	32		-	-	
You feel safe and protected	Almost never	8	16	1.92	14	28	1.18
	Some-times	3	6		15	30	
	Often	24	48		19	38	
	Usually	15	30		2	4	
You have many worries	Almost never	19	38	1.16	20	40	1.00
	Some-times	10	20		15	30	
	Often	15	30		10	20	
	Usually	6	12		5	10	
You enjoy yourself	Almost never	14	28	1.58	21	42	1.02
	Some-times	7	14		10	20	
	Often	15	30		16	32	

	Usually	14	28		3	6	
	Almost never	16	32		20	40	
	Some-times	11	22		13	26	
	Often	10	20		16	32	
	Usually	13	26		1	2	
	Almost never	22	44		23	46	
	Some-times	13	26		20	40	
	Often	12	24		6	12	
	Usually	3	6		1	2	
	Almost never	13	26		27	54	
	Some-times	9	18		13	26	
	Often	15	30		10	20	
	Usually	13	26		-	-	
	Almost never	23	46		33	66	
	Some-times	15	30		15	30	
	Often	10	20		2	4	
	Usually	2	4		-	-	
	Almost never	14	28		29	58	
	Some-times	7	14		20	40	
	Often	16	32		1	2	
	Usually	13	26		-	-	
	Almost never	9	18		38	76	
	Some-times	8	16		9	18	
	Often	19	38		2	4	
	Usually	14	28		1	2	

Table No.3: Pre-distribution of total stress levels in the study sample before applying diaphragmatic breathing technique

Variables	Classification	F	%	Mean	Std. Deviation
Stress Level	Low	6	12	2.24	0.657
	Moderate	26	52		
	High	18	36		

Table No. 4: Distribution of total stress levels among the study sample after diaphragmatic breathing technique

Variables	Classification	F	%	Mean	Std. Deviation
Stress Level	Low	35	70	1.30	0.463
	Moderate	15	30		
	High	0	0		

Table No. 5: Bivariate correlation between sample socio-demographic characteristics and stress after executing the diaphragmatic breathing techniques

Sociodemographic Characteristics	Significance
Age	Chi-square=3.659, df=3, P.=0.301 (Non-significant)
Sex	Chi-square=2.138, df=1, P=0.144 (Non-significant)
Residence	Chi-square=.758, df=1, P=0.384 (Non-significant)
Monthly income	Chi-square=1.310, df=4, P=0.520 (Non-Significant)
Education levels	Chi-square=0.935, df=3, P=0.817 (Non-significant)

The post-intervention showed a significant improvement in stress management scores, with mean differences from 0.32 to 1.18. They mostly moved to the 'Almost never', and 'Sometimes' answer categories, indicating that less stress was experienced after diaphragmatic breathing treatment (Table 2).

The pre-intervention stress levels show that more than half of the study group (52%) is classified as moderate stress and 36% are high stress. The sample presents an

overall mean score 2.24 ± 0.657 , suggesting that the vast majority of people endured an exceptional amount of psychological stress (Table 3).

The overall level of stress after the intervention showed an excellent distribution, representing a clear improvement for crude (70%) from the 'low stress' group. Notably, extreme stress was entirely eliminated (0%) and moderate stress decreased to 30%. The overall mean score decreased significantly to

1.30±0.463 which indicates that the intervention proved effective in reducing perceived stress among study participants (Table 4).

Stress is not significantly associated with sociodemographic variables and was performed using univariate analysis. No statistically significant correlation was observed for any of the variables: age ($P=0.301$), gender ($P=0.244$), residence ($P=0.384$), monthly income ($P=0.520$) and educational level ($P=0.817$) and showed that the decrease in stress was consistent across all demographic groups within the study population (Table 5)

DISCUSSION

In the current study, the largest portion of all patients was aged 51-62 years. Coronary artery disease more commonly occurs in elderly patients. The extent and severity of coronary artery calcification are predictive markers for coronary atherosclerosis, which is known to progress with age especially in middle aged and elderly.¹⁰

The metropolitan population seems to be more at risk because of the stress of urbanization and lifestyle changes that add up as sedentary, fast food consumption and all changes in diet due to transition from rural populations to urban living, all predisposing factors for coronary artery diseases. This is similar to the current study in which males comprised 62% of our sample and 63.3% of all participants. The results are in line with study which found a much larger percentage of men with cardiac disease have large coronary arteries.¹¹ This is due to a larger number of CAD in men, resulting from higher exposure rates to the major risk factors (smoking, work-related stress and unhealthy lifestyle). Cardiac hormones might have a protective effect against coronary artery disease in premenopausal women.

In the current study, 64% of respondents had a primary education (6% uneducated, 27% secondary education, and 6% at least bachelor's degree. These results were confirmed by research showing that worse education attainment is associated with poor cardiovascular prognosis and a higher event rate in stable coronary artery disease patients.¹² This effect might be attributed to lower levels of education, resulting in poor health literacy that makes individuals less aware about cardiovascular risk factors, the prevention of disease and adherence with healthy living and treatment guidelines.

In this study, 56% owned urban region and 44% rural area. Another study from the North-Eastern Zone of India supports this result, it clearly shows that prevalence of coronary artery disease with risk factors was much higher (urban: 68.83% and rural: 31.17%). This indicates that urban way of life and environment in mega cities act as a risk promoting drug for elevated CAD risk.¹³ The very high rate among urban dwellers

due to high prevalence associated with stress, less physical activity and increased ultra-processed foods intake in cities causes an upsurge in cardiovascular diseases particularly coronary artery disease.

Most participants earned a decent monthly income (80%) whereas fewer reported having a sufficient or inadequate income. Socioeconomic status, risk of arteriosclerotic cardiovascular disease and cause-specific and all-cause mortality: National Health and Nutrition Examination Survey 2005-2018.¹⁴ This study indicate that economic conditions impact access to preventive measures including both effective health care and healthy eating behaviours, which are associated with risk of developing CVD or also known as cardiovascular disease management.

This study showed that diaphragm breathing application reduced post-stress levels in patients. Before the intervention, most had moderate to high stress scores; after the intervention, more moved to a lower stress level, and the mean stress score was also significantly lower. Diaphragmatic breathing was effective for reducing perceived psychological stress in acute heart failure. The physiological and psychological effect of diaphragmatic breathing through its facilitation of parasympathetic nervous system activity, promotion of relaxation, inhibition of sympathetic arousal and increased oxygenation underlie this improvement. Besides, controlled respiration could minimize adrenal fatigue and emotional pressure which can help patients reach greater psychological balance and also manage anxiety more effectively. In addition, another non-invasive and easy in application method is called diaphragmatic breathing which helps to improve mental health related conditions and coping ability on patients with cardiovascular diseases. The present results are consistent with previous studies, relaxation methods (diaphragmatic breathing exercises) might relieve stress and anxiety in people with cardiac diseases or chronic illnesses.⁵ Diaphragmatic breathing significantly reduced stress and negative emotions in adults.⁷ Moreover, deep diaphragmatic breathing leads to decreased cortisol release and enhanced autonomic modulation, mechanisms multi-faceted functions in stress relief.³

The current research did not show a statistically significant association between patient stress and their age, gender, education or monthly income ($p>0.05$). Close correlation of patient anxiety on the basis of residential area ($p<0.05$) showed that rural persons were more anxious than urban persons. Study documented analogous results. This is one of the main reasons behind the lack of access to health care and nutrition education in rural areas.^{15,16}

This study demonstrated the role of nurses providing non-pharmacological therapies to promote mental well-being among cardiac patients. Diaphragmatic breathing may serve as a simple nursing intervention for possible

incorporation into ADL standard cardiac care to reduce stress and improve comfort in patients. Diaphragmatic breathing may be a simple, safe and inexpensive nursing intervention which is able to be added to routine cardiac care thus far to lower anxiety and improve comfort for patients.

CONCLUSION

The diaphragmatic breathing approach successfully reduced stress in patients with acute heart failure. Before intervention, majority of the participants belonged to moderate or high stress levels while after utilizing this specific method of diaphragmatic breathing most shifted to low categories in stress level and complete suppression of higher categories.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Amer J. Kadush, Diaa F. Jrood, Malak Hassan Sallal
Drafting or Revising Critically:	Maath Ahmed Khalaf, Maher Abdulameer Atiyah
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. 3459/Approval/SDFB Dated 07.01.2026

REFERENCES

- Ponikowski P, Voors AA, Anker SD, Bueno H, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;37(27):2129-2200.
- McDonagh TA, Metra M, Adamo M, Gardner RS, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J* 2021;42(36):3599-3726.
- Perciavalle V, Blandini M, Fecarotta P, Buscemi A, et al. The role of deep breathing on stress. *Neurol Sci* 2017;38(3):451-8.
- Celano CM, Villegas AC, Albanese AM, Gaggin HK, Huffman JC. Depression and anxiety in heart failure: a review. *Harv Rev Psychiatr* 2018; 26(4): 175-84.
- Ma X, Yue ZQ, Gong ZQ, Zhang H, et al. The effect of diaphragmatic breathing on attention, negative affect and stress in healthy adults. *Front Psychol* 2017;8:874.
- Jerath R, Crawford MW, Barnes VA, Harden K. Self-regulation of breathing as a primary treatment for anxiety. *Appl Psychophysiol Biofeedback* 2015; 40(2):107-15.
- Johnson MJ, Close L, Gillon SC, Molassiotis A, et al. Use of breathing exercises for patients with heart failure to reduce stress and dyspnea. *Eur J Cardiovasc Nurs* 2020; 19(7): 625-32.
- Chen YF, Huang XY, Chien CH, Cheng JF. The Effectiveness of Diaphragmatic Breathing Relaxation Training for Reducing Anxiety. *Perspect Psychiatr Care* 2017;53(4):329-36.
- Fliege H, Rose M, Arck P, Levenstein S, et al. PSQ. Perceived stress questionnaire. *Leibniz Institute for Psychol (ZPID)* 2009;45.
- Gerke O, Lindholt JS, Abdo BH, Lambrechtsen J, et al. Prevalence and extent of coronary artery calcification in the middle-aged and elderly population. *Eur J Prev Cardiol* 2022;28(18): 2048-55.
- Jarrah MI, Hweidi IM, Al-Dolat SA, Alhawtmeh HN, et al. The effect of slow deep breathing relaxation exercise on pain levels during and post chest tube removal after coronary artery bypass graft surgery. *Int J Nurs Sci* 2022;9(2):155-61.
- Kelli HM. Educational attainment and coronary artery disease outcomes. *J Am Heart Assoc* 2019;8(17): e013165.
- Akhtar N, Paul P, Kumar T, Paul UK. Prevalence of coronary artery disease and the associated risk factors among the patients attending the medicine department in a tertiary care teaching hospital in the North Eastern Zone in India. *Int J Adva Med* 2023;10(12): 823-29.
- Shen R. Socioeconomic status and cardiovascular disease risk. *Front Public Health* 2022; 10: 1017271.
- Yu Y, Liu X, Wang LJ, Wang S, Ao H. History of cardiopulmonary resuscitation in ancient China: a narrative review. *J Cardiothoracic Surg* 2020; 15(1):1-7.
- Chen C, Zeng H. Curcumin attenuates myocardial ischemia and reperfusion-induced pro-inflammatory response through activation of the Nrf-2/HO-1 signaling pathway. *Muthanna Med J* 2022;9:41-56.

Advancing Respectful Maternity Care: Evaluating Nursing Perspectives and Clinical Practices in Safeguarding Birthing Rights during Complex Labors

Evaluate the
Obstacles to
Providing
Respectful
Maternity
Services in Labor
Room

Doaa Sami Rashash¹, Layla Oun AlWassiti² and Fatima Jaber Shinjar³

ABSTRACT

Objective: To evaluate the obstacles to providing respectful maternity services in the standard labor room

Study Design: Descriptive cross-sectional study

Place and Duration of Study: This study was conducted at the Nasiriyah City, Southern Iraq, from 1st September 2025 to 31st December 2025.

Methods: One hundred and fifty mothers having a child between the ages of 0 and 1 year and answering every question were enrolled.

Results: Empathize giving women-focused care, and protecting rights. Exhibiting empathy include interacting with women and cultivating a cordial relationship and decision-making and women's safety were essential elements of women-centered care. The protection of rights represented the need to uphold dignity, treat everyone equally, and establish a proper environment.

Conclusion: Iraqi midwives saw courteous maternal care as a more all-encompassing concept rather than concentrating on preventing abuse. Being polite and encouraging, the first step towards providing respectful maternity care was to women. Providing safe, treatment based on evidence including women in the care they provide, and establishing a setting that is appropriate for women, family members, and caregivers are all important aspects of promoting respectful care.

Key Words: Maternity care, Clinical practices, Perspective.

Citation of article: Rashash DS, Al Wassiti LO, Shinjar FJ. Advancing Respectful Maternity Care: Evaluating Nursing Perspectives and Clinical Practices in Safeguarding Birthing Rights during Complex Labors. Med Forum 2026;37(9):42-46. doi:10.60110/medforum.370908.

INTRODUCTION

Freedom from harm, privacy and confidentiality protection, informed consent, independence and dignity, family support, effective communication, and continuity of care are all components of respectful maternity care¹. Obstetric aggression, contempt, and abuse can occur throughout pregnancy and labor for women and those giving birth worldwide, despite the

well-established advantages of compassionate maternity care². The words dehumanized care, disrespect and abuse will be used throughout this document, mistreatment, and obstetric violence are frequently and interchangeably used in the literature, but there is no agreement on the vocabulary or typology concerning obstetric violence³. Despite this lack of agreement, instances of obstetric violence are highlighted by gender inequity, over medicalization, the possibility of injury, and an underlying danger to women's rights⁴.

Protecting women's reproductive rights and providing high-quality maternal care are crucial elements of both the global development plan and public health. As the premier UN organization for sexual and reproductive health, UNFPA is crucial to achieving these goals because of its dedication to protecting women's rights and dignity and guaranteeing widespread access to maternal health care services. This vision is reflected in UNFPA's declared mission, to deliver an atmosphere where any pregnancy is desirable⁵.

Every single childbirth is safe and every young person's capacity is fulfilled, highlighting the need of high-quality maternity care and respect for women's rights worldwide. A woman should receive quality medical care throughout her pregnancy in order to maximize her satisfaction during gestation, childbirth, and the

¹. Instructor, Maternity & Neonatal Health Nursing, College of Nursing, University of Thi-Qar

². Instructor, Maternity & Neonatal Health Nursing, University of Sumer, College of Medicine, Nasiriyah City, Iraq.

³. Instructor, Adult Nursing Department, Faculty of Nursing, University of Thi-Qar, Nasiriyah City, Iraq

Correspondence: Doaa Sami Rashash, Instructor, Maternity & Neonatal Health Nursing, College of Nursing, University of Thi-Qar, Nasiriyah City, Iraq.

Contact No: 9647812807085

Email: doaa-s@utq.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

postpartum period⁶. The Indian government is trying very hard to develop a number of programs that support institutional delivery for low-income pregnant women by ensuring accessible, skilled medical professionals in order to reduce maternal and newborn mortality and morbidities⁷. These efforts did not have the intended effects; numerous maternal deaths, particularly in underperforming states, underscore the necessity of raising the standard of care for institution births⁸.

Despite the international rules on respectful mother care (RMC) of pregnant women, pregnant women are allegedly abused, mistreated, and neglected during the whole pregnancy cycle, especially in low- and middle-income countries (LMIC). This jeopardizes women's rights to life, health, physical integrity, and freedom from discrimination, as well as their rights to RMC and evidence-based institutional care⁹. At least one instance of abuse is found in a comprehensive analysis of obstetric violence in India¹⁰.

Respectful mother care in LMICs reveals that constraints including cleanliness, equipment availability or quality, provider competency, or conduct cause women to be dissatisfied with health facilities. Adaptation to parenthood and competent caregiving behaviors depend heavily on maternal parental self-efficacy, which is especially important for primiparous women¹¹. Women's confidence can be damaged by unpleasant and disrespectful labor experiences, which may result in reduced maternal-infant wellbeing and long-term emotional pain¹². Conversely, mothers' self-efficacy and coping skills are linked to positive and polite care experiences. Although respectful maternity care has gained more attention recently, educational contacts with women about childbirth may not always reflect actual care practices¹³.

METHODS

This descriptive cross-sectional study was conducted at Nasiriyah city, Southern Iraq from 1st September to 31st December 2025 vide letter No. 7 dated 7th June 2025. One hundred and fifty mothers having a child between the ages of 0 and 1 year, consenting to take part in the study, and answering every question on the questionnaire were the requirements for inclusion. Due to the possibility of introducing confounding variables and the distinct clinical and emotional processes involved in cesarean delivery, they were eliminated. To find participants, snowball sampling was employed. Initially, the researchers used private communication networks to distribute the survey link to mothers they knew. These women were asked to fill out. Only mothers who met the requirements to be included in the survey received the link and the survey. The sample size was calculated using a correlation-based power analysis with an effect size of 0.50, an alpha level of 0.05, and a confidence level of 99% using the G*Power 3.1 software was employed to ascertain the minimal

sample size required before data collection, resulting in 200 mothers. Because the survey was done online, the sampling process was deliberately prolonged to increase representativeness and guard against potential data loss due to incomplete or inaccurate responses. As a result, 150 mothers who met the inclusion criteria and filled out the survey completely were included. A post-hoc based power analysis utilizing the final sample size revealed that the statistical significance was larger than 0.99, suggesting a high degree of accuracy and dependability in the study results¹⁴. The SPSS-17 was used to analyze the data. Chi square was calculated to determine the relationship between thoughtful maternity care and demographic characteristics. Statistical significance was considered as $P < 0.05$.

RESULTS

Thirty six (24%) had completed senior or high school, 37 (25%) lived in rural areas, 80 (53%) worked for the government, and 60 (40%) were employed by the government. The majority of women had one child 60 (40%), two children 50 (33%), and more than two children 40 (27%). Female healthcare workers performed 80 (53%) of the spontaneous vaginal births (Table 1).

Table No. 1: The demographic information of the females (N = 150)

Variable	No.	%
Age (years)		
15-19	22	15.0
20-24	28	18.5
25-29	29	19.5
30-34	35	23.0
>35	36	24.0
Education		
Absence of formal schooling	20	13.0
elementary school	30	20.0
Secondary education	28	19.0
High school or senior	37	25.0
A diploma or higher	35	23.0
Residence		
Urban	70	47.0
Rural	80	53.0
Occupation		
Homemaker	50	33.0
Private employment	40	27.0
Government employment	60	40.0
Number of children		
1	60	40.0
2	50	33.0
>2	40	27.0
Current mode of delivery		
Spontaneous vaginal delivery	80	53.0
Vacuum/Forceps	70	47.0

Forty one (28%) of the mothers who were given considerate maternity care stated it was comfortable, and 80 (53%) said keeps the space between beds intact.

Of the 80 (53%) RMC domains, the majority of women reported Records will be kept secure and safe. Conversely, just 10 (7%) HCWs who identified themselves reported care was not consented (Table 2).

Table No. 2: Prevalence of compassionate maternity care n=150)

Respectful Maternity Care	No.	%
Abuse of the body		
Considers comfort	41	28.0
Limit drinking	38	25.0
Limit eating	36	24.0
Injure physically	35	23.0
Resources and the physical surroundings		
Create a serene and peaceful atmosphere	70	47.0
Maintains distance between beds	80	53.0
Non-confidential care		
A curtain was used to protect privacy during the inspection	70	47.0
Records will be kept secure and safe.	80	53.0
Non-consented care		
HCWs introduced themselves	10	7.0
Encouraged to ask doubts	20	13.0
Explained prior to every practice or activity	25	17.0
Prior to taking any action or practicing	20	13
Allowed to move	16	11.0
Permitted to select the provision method	14	9.0
Describe the advantages & disadvantages of the delivery method	20	13.0
Monitored delivery progress	25	17.0

DISCUSSION

Women's perceptions of RMC were high, according to the study's findings, which were assessed in the context of the body of current research. Recognized as an essential part of high-quality obstetric care, RMC is based on universal human rights and aims to protect women from abuse while guaranteeing their autonomy, dignity, ability to make informed decisions, emotional support, and the development of mother-infant bonds¹⁵. Miller et al¹⁶ emphasized that without RMC, clinical care cannot be considered superior. The women have high perceptions of RMC (70.2-90%)^{17,18} which is consistent with the current study. However, a different study found a significantly lower average perception score (44.99)¹⁹, and a recent Turkish study found that nearly three out of four women are mistreated after giving birth²⁰.

The subjective and dynamic character of delivery experiences, differences in care environments, and women's expectations shaped by cultural norms could all contribute to these conflicting findings. This

circumstance emphasizes the ongoing need to strengthen standards for compassionate maternity care practices and promote understanding. The results of our survey show that 16.60% of victims had been physically abused. An Indian systematic review reports a comparable percentage of 16.96% physical abuse²¹. A different survey found that 2.7% of victims experienced physical abuse²². Physical abuse was reported at a greater proportion than ours, 23.2%, in a community-based research conducted in Ethiopia²³. However, only 2-2.3% of verbal or physical abuse was found in other studies conducted in the same nation²⁴. Inadequate reporting in some African studies could be due to ignorance or the perception that abuse is widespread²⁵. In this study, 70.55% of women reported continuity of care which is consistent with Ethiopian study results²⁶. The severe staffing scarcity seen in LMICs is likely the cause of the similarities. The majority of respondents (99.20%) reported an adequate environment and resources, which is encouraging according to our study's great environment and resources findings. According to our research, demonstrating empathy for women by interacting with them and building cordial relationships is a crucial component of RMC. In fact, the midwives thought that the primary factor influencing RMC was having a close-knit and encouraging relationship with women. Several studies from Australia, New Zealand, Norway, Sweden, and the UK have documented the development of cordial and pleasant interactions with women²⁷. Additionally, Bradfield and his colleagues defined "being with women" as the core of the midwifery philosophy and practice²⁸. Positive interpersonal and emotional treatment experiences are equally valuable to clinical and contextual environmental elements, according to the experiences of Nigerian and Ugandan women^{29,30}. The midwives in our study also emphasized RMC's capacity to deliver timely, current care with sufficient knowledge to enable a woman to take part in decision-making. A midwife also highlighted the importance of birth centers that might help RMC by providing women with midwifery-style care. Several studies have shown that when informed permission is gained, prompt, safe, effective, and efficient care may be provided. Additionally, several studies have shown that birth centers result in positive birth experiences^{31,32}. The RMC expressed respect for women's customs, religion, nationality, autonomy, and privacy, according to Iranian midwives³³. A thorough analysis revealed that women's delivery experiences were influenced by culture. The quality of birth experiences for women and their families will be enhanced by culturally competent maternal health care³⁴. According to midwives, Behruzi et al³⁵ emphasized the significance of honoring women's traditions, values, and beliefs.

CONCLUSION

During childbirth, the RMC may be helpful measurement tool to assist stop the abuse. Every domain is highlighted in the current study. The RMC from the women and its health-related barriers, the caregivers, improving medical staff's institutional policies, training, and resources were found. Women's rights would be strengthened during childbirth. Quality of care in a way that guarantees women has a good delivery experience and receive evidence-based therapy. The necessity of supply-side RMC barriers, which should be in place to be considered into account in order to support and improve institutional execution help enhance the health of mothers and infants.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Doaa Sami Rashash, Layla oun AlWassiti
Drafting or Revising Critically:	Doaa Sami Rashash, Fatima Jaber Shinjar
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.7 Dated 07.06.2025

REFERENCES

- Adinew YM, Kelly J, Marshal A, Smith M. Care providers' perspectives on disrespect and abuse of women during facility-based childbirth in Ethiopia: a qualitative study. *Int J Women's Health* 2021;13:1181-95.
- Adinew YM, Kelly J, Smith M, Marshall A. Women's perspectives on disrespect and abuse during facility-based childbirth in Ethiopia: a qualitative study. *BMC Preg Childbirth* 2023;23(1):444.
- Adu-Bonsaffoh K, Tamma E, Maya E, Vogel JP, Tunçalp Ö, Bohren MA. Health workers' and hospital administrators' perspectives on mistreatment of women during facility-based childbirth: a multicenter qualitative study in Ghana. *Reprod Health* 2022;19(1): 1-11.
- Afulani PA, Kelly AM, Buback L, Asunka J, Kirumbi L, Lyndon A. Providers' perceptions of disrespect and abuse during childbirth: a mixed-methods study in Kenya. *Health Policy Planning* 2020;35(5):577-86.
- Agyenim-Boateng A, Cameron H, Bemah A. Health professionals' perception of disrespectful and abusive intrapartum care during facility-based childbirth in LMIC: a qualitative systematic review and thematic synthesis. *Int J Afr Nurs Sci* 2021;15:100326.
- Sendo EG, Chauke ME, Ganga-Limando M. Women's perspectives on the measures that need to be taken to increase the use of healthcare facility delivery service among slums women, Addis Ababa, Ethiopia: A qualitative study. *Reprod Health* 2021;18:174.
- Saha R, Paul P. Institutional deliveries in India's nine low performing states: levels, determinants and accessibility. *Glob Health Action* 2021;14:2001145.
- Sharma G, Penn-Kekana L, Halder K, Filippi V. An investigation into mistreatment of women during labour and childbirth in maternity care facilities in Uttar Pradesh, India: a mixed methods study. *Reprod Health* 2019;16:7.
- Patabendige M, Agampodi SB, Jayawardane A, Wickramasooriya DJ, Agampodi TC. Perceptions on respectful maternity care in Sri Lanka: Study protocol for a mixed-methods study of patients and providers. *PLoS One* 2021;16:e0250920.
- Ansari H, Yeravdekar R. Respectful maternity care during childbirth in India: A systematic review and meta-analysis. *J Postgrad Med* 2020;66:133-40.
- Shrivastava S, Sivakami M. Evidence of 'obstetric violence' in India: An integrative review. *J Biosoc Sci* 2020;52:610-28.
- Lusambili A, Wisofsch S, Shumba C, Obure J, Mulama K, Nyaga L, et al. Health care workers' perspectives of the influences of disrespectful maternity care in rural Kenya. *Int J Environ Res Public Health* 2020;17:8218.
- Ishola F, Owolabi O, Filippi V. Disrespect and abuse of women during childbirth in Nigeria: A systematic review. *PLoS One* 2017;12:e0174084.
- Cantor AG, Jungbauer RM, Skelly AC, Hart EL, Jorda K, Davis-O'Reilly C, et al. Respectful maternity care: A systematic review. *Ann Intern Med* 2024;177(1):50-64.
- Mira-Catalá P, Hernández-Aguado I, Chilet-Rosell E. Respectful maternity care interventions to address women mistreatment in childbirth: what has been done? *BMC Pregnancy Childbirth* 2024;24(1):322.
- Miller S, Abalos E, Chamillard M, Ciapponi A, Colaci D, Comandé D, et al. Beyond too little, too late and too much, too soon: A pathway towards evidencebased, respectful maternity care worldwide. *Lancet* 2019;388(10056):2176-92.
- Muhayimana A, Kearns I, Darius G, Olive T, Thierry UC. Reported respectful maternity care received during childbirth at health facilities: A cross sectional survey in Eastern Province. *Rwanda Midwifery* 2024;133:103996.
- Dabhi S, Dabhi T. Perspective of post-natal mothers regarding respectful maternity care during

- facility-based child birth. *Nurs J Ind* 2023; 45.
19. Asci O, Bal MD. The prevalence of obstetric violence experienced by women during childbirth care and its associated factors in Türkiye: a cross-sectional study. *Midwifery* 2023;124:103766.
 20. Avcı N, Kaydirak MM. A qualitative study of women's experiences with obstetric violence during childbirth in Turkey. *Midwifery* 2023;121:103658.
 21. Ansari H, Yeravdekar R. Respectful maternity care during childbirth in India: A systematic review and meta-analysis. *J Postgrad Med* 2020;66:133-40.
 22. Bhattacharya S, Sundari-Ravindran TK. Silent voices: Institutional disrespect and abuse during delivery among women of Varanasi district, northern India. *BMC Pregnancy Childbirth* 2018;18:338.
 23. Wassihun B, Deribe L, Worede N, Gultie T. Prevalence of disrespect and abuse of women during child birth and associated factors in Bahir Dar town, Ethiopia. *Epidemiol Health* 2018;40:e2018029.
 24. Sheferaw ED, Kim YM, van den Akker T, Stekelenburg J. Mistreatment of women in public health facilities of Ethiopia. *Reprod Health* 2019;16:130.
 25. Bulto GA, Demissie DB, Tulu AS. Respectful maternity care during labor and childbirth and associated factors among women who gave birth at health institutions in the West Shewa zone, Oromia region, Central Ethiopia. *BMC Pregnant Childbirth* 2020;20:443.
 26. Ishola F, Owolabi O, Filippi V. Disrespect and abuse of women during childbirth in Nigeria: A systematic review. *PLoS One* 2017;12:e0174084.
 27. Guilliland K. The midwifery partnership: a model for practice/Karen Guilliland and Sally Pairman. 2nd ed. New Zealand College of Midwives: Wellington; 2010.p.65.
 28. Aune I, Amundsen HH, SkagetAas LC. Is a midwife's continuous presence during childbirth a matter of course? Midwives' experiences and thoughts about factors that may influence their continuous support of women during labor. *Midwifery* 2014;30(1):89-95.
 29. Lundgren I, Berg M, Lindmark G. Is the childbirth experience improved by a birth plan? *J Midwifery Womens Health* 2003;48(5):322-8.
 30. Warwick C. Delivering high quality midwifery care: the priorities, opportunities and challenges for midwives. *Midwifery* 2010;26(1):9-12.
 31. Bohren MA, Titiloye MA, Kyaddondo D, et al. Defining quality of care during childbirth from the perspectives of Nigerian and Ugandan women: A qualitative study. *Int J Gynaecol Obstet* 2017;139:4-16.
 32. Hitzert M, Hermus MA, Scheerhagen M, et al. Experiences of women who planned birth in a birth centre compared to alternative planned places of birth. Results of the Dutch Birth Centre Study. *Midwifery* 2016;40:70-8.
 33. Jamas MT, Hoga LA, Tanaka AC. Mothers' birth care experiences in a Brazilian birth centre. *Midwifery* 2011;27:693-9.
 34. Withers M, Kharazmi N, Lim E. Traditional beliefs and practices in pregnancy, childbirth and postpartum: A review of the evidence from Asian countries. *Midwifery* 2018;56:158-70.
 35. Behruzi R, Hatem M, Goulet L, Fraser WD. Perception of humanization of birth in a highly specialized hospital: let's think differently. *Health Care Women Int* 2014;35(2):127-48.

Prevalence, Biofilm-Forming Capacity and Expression of Matrix-Associated Genes in Clinical *Pseudomonas Aeruginosa* Isolates from Babylon, Iraq

Manar Fadhil Obaid¹ and Ali Hmood Al-Saadi²

Identify the Number of *Pseudomonas Aeruginosa* from Different Clinical Specimens and Quantify Biofilm Production

ABSTRACT

Objective: To identify the number of *Pseudomonas aeruginosa* from different clinical specimens and quantify biofilm production and to compare gene expression of *pslA*, *pelA* and *fliC* among biofilm and non-biofilm phenotypes by using reverse transcription-quantitative polymerase chain reaction

Study Design: Prospective cross-sectional study

Place and Duration of Study: This study was conducted at the Department of Life Sciences, College of Science, University of Babylon, Iraq from 1st November 2025 to 30th April 2026.

Methods: Fifty samples from laboratory confirmed *Pseudomonas aeruginosa* were collected from 50 patients; 87 isolates were also cultured on Cetrimide agar and confirmed as *Pseudomonas aeruginosa* via standard polymerase chain reaction utilizing the *azu* gene (476 bp). Biofilm development was determined based on OD₅₉₀ readings from stained crystal violet biofilms formed following 24 hr of static culture in 96 well plates. Gene expression was measured via reverse transcription-quantitative polymerase chain reaction on an Mx3005P with SYBR Green utilizing *rpoD* as a normalizing reference gene and the 2^{-ΔΔC_T} method for data analysis.

Results: Seventy six (87.4%) were confirmed as *Pseudomonas aeruginosa*; wound sample comprised 30% of total samples; overall confirmatory increase across each type ranged from 81.8% (urine) to 100% (blood). Biofilms were created by 80.3% of confirmed isolates; of these, strongly biofilm-producing (vs weak) isolates constituted the majority (42.1%). Gene expression of *pslA* (13.5-62.5-fold; F=121,038; P<0.0001) and *pelA* (1,116-5,181-fold; F=903,770,772; P<0.0001) were significantly downregulated in all biofilm-producing phenotypes relative to non-biofilm-producing phenotypes; *fliC* was significantly upregulated (4.2-19.6-fold; F=497.9; P<0.0001).

Conclusion: *P. aeruginosa* clinical specimens from Babylon are highly capable of biofilm formation; however, the unexpected expression profile of biofilm producing specimens suggests that there is a significant dissociation between phenotypic expression of biofilm components and the mechanism of biofilm persistence.

Key Words: *Pseudomonas aeruginosa*, Biofilm, Exopolysaccharide, Prevalence, Nosocomial infection.

Citation of article: Obaid MF, Al-Saadi AH. Prevalence, Biofilm-Forming Capacity and Expression of Matrix-Associated Genes in Clinical *Pseudomonas Aeruginosa* Isolates from Babylon, Iraq. Med Forum 2026;37(9):47-51. doi:10.60110/medforum.370909.

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacillus that can grow in a wide range of different environments and survive in harsh and damaging environments of the human body. *P. aeruginosa*, one of the leading causes of health-care associated infection in the world, causes significant morbidity and mortality around the world and is

associated with a large volume of cases in patients who are immune compromised or require prolonged hospitalization, or patients with an indwelling medical device^{1,2}. The clinical importance of *P. aeruginosa* increases due to its inherent resistance to many antibiotic classes (e.g., β-lactams, aminoglycosides, and fluoroquinolones) in addition to its ability to acquire new resistance factors through horizontal gene transfer or mutation³.

The virulence determinants of *P. aeruginosa* are extremely complex and include surface structures used for adhesion to the host and evasion of the immune response, an extensive number of secreted toxins and proteases, and sophisticated regulatory networks that allow for coordinated behavioral changes at the population level in response to environmental or host-derived signals^{4,5}. A central regulatory system governing the virulence of *P. aeruginosa* is the process of quorum sensing. Quorum sensing allows for the interaction of multiple *P. aeruginosa* cells by utilizing a set of chemical molecules, such as the N-acyl

¹. M.Sc. Student / Professor² / Department of Life Sciences, College of Sciences, University of Babylon, Iraq.

Correspondence: Manar Fadhil Obaid, M. Sc. Student, Department of Life Sciences, College of Sciences, University of Babylon, Iraq.

Contact No: 07843718428

Email: sci712.mannar.f@student.uobabylon.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

homoserine lactones or the *Pseudomonas* quinolone signal, to activate the expression of several different virulence genes, including those encoding exotoxin A, the LasB elastase, alkaline protease, pyocyanin, and rhamnolipids, which all contribute to tissue damage, immune evasion, and ultimately the success of the bacterium in establishing an infection⁶. In addition to these planktonic virulence mechanisms, *P. aeruginosa* has the ability to form biofilms as a different way to survive as compared to free-living bacteria and significantly enhances the ability of bacteria to survive in the environment⁷. Biofilm formation occurs in *P. aeruginosa*. The developmental stages of *Pseudomonas aeruginosa* biofilms include reversible attachment and irreversible attachment (adhesion), microcolony formation, maturation into three-dimensional structures (tower), and dispersal of motile cells that can set up a new infection site at other locations^{8,9}.

METHODS

This prospective cross-sectional study was conducted at Department of Life Sciences, College of Science, University of Babylon, Iraq 1st November 2025 to 30th April 2026 vide letter 8251008 date 8-10-2025. Fifty samples from laboratory confirmed *Pseudomonas aeruginosa* were collected from 50 patients. The types of specimens collected were 15 wound swabs; 12 mid-stream urine specimens from patients with suspected urinary tract infections; 10 expectorated sputum specimens from patients with respiratory infections; 8 peripheral blood cultures; and 5 cerebrospinal fluid specimens obtained through lumbar punctures. All specimens were transported to the laboratory within 2 hours after collection using sterile transport media (i.e., Stuart's medium for wound swabs; sterile containers for liquid specimens). Using the streak plate method, all samples were plated onto Cetrimide agar plates (Liofilchem, Italy; Cat. No. 610041) and placed in a 37°C incubator for 24–48 hours of aerobic incubation. Colonial morphology, colour (pyocyanin blue-green, pyoverdine yellow-green fluorescent) and pattern of growth were noted. To maximise the amount of intra-specimen diversity captured, two morphologically different types of colonies were picked from plates with phenotypically diverse morphological could yield 87 total bacterial isolates from 50 specimens. Preliminary phenotypic identification was performed by Gram staining, KOH testing (3% KOH), and catalase (3% H₂O₂) and oxidase tests (1% tetramethyl-p-phenylenediamine dihydrochloride) following the protocols established by the authors.

Extracting genomic DNA from cultures grown 18-24 hours utilized the boiling method. One colony was diluted in 100 µl sterile distilled water, vortexed, boiled for 10-15 minutes, cooled quickly (on ice), centrifuged (10,000 rpm, 5 minutes); the supernatant was the template for PCR. The *azu* gene was amplified from

genomic DNA using species-specific PCR primers that produced 476 bp products (F: 5'-CAGTCGACGCCATCTTC-3'; R: 5'-AACCAGTTGTTCTTGCCG-3'). PCR reactions (25 µl) used Hot FirePol Master Mix (Solis BioDyne, Estonia) and were subjected to the following thermal cycling: 95°C × 5 mins; 35 × (95°C × 30 s, 55°C × 30 s, 72°C × 45 s); 72°C × 10 min. PCR products were resolved on 1.5% agarose gels containing RedSafe dye (Intron, Korea) at 75 V for 45 minutes using UV transillumination.

The standard crystal violet microtiter plate assay was used to quantify biofilm production 18 from overnight cultures.

A total of twelve biofilm representative isolates (three per category) were selected for transcriptional profiling according to their median OD₅₉₀ values in each category. Once the biofilm isolates were prepared in mid-log-phase cultures (OD₆₀₀ ≈ 0.5), total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) with on-column DNase I digestion to eliminate any genomic DNA contamination from the biofilm isolates. RNA quality was verified on a Quantus Fluorometer (Promega, USA) using A₂₆₀/A₂₈₀ ratios (1.8-2.1), and only those samples with RNA integrity numbers (RIN) ≥ 7.0 were used for downstream applications. Synthesis of complementary DNA (cDNA) was performed using 500 ng of total RNA and the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) following manufacturer's recommendations to use random hexamer primers to create cDNA.

Quantifying mRNA levels of *pslA*, *pelA* and *fliC* was accomplished using Mx3005P Real-Time PCR instruments (Stratagene, USA) which utilized the SYBR Green Master Mix (Qiagen, Germany) for detection. For each reaction (20 µl total volume), 10 µl of the SYBR Green Master Mix, 1 µl of the forward and reverse primers (10 pmol/µl), 1 µl of cDNA, and 7 µl of nuclease-free water were added to an optical well plate and the following thermocycler program was used: (1) 95°C for 10 min, (2) 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, and (3) final step of melt curve analysis from 60°C to 95°C (increments of 0.5°C). The housekeeping gene used as a reference for relative expression was the sigma factor gene *rpoD*. Each gene was quantified in technical duplicates and three biological replicates per treatment group were included. Relative mRNA expression was determined by the 2^{-ΔΔC_T} (Livak and Schmittgen) method where non-biofilm-producing strains were the calibrator strain for calculating relative expression. The data was entered and analyzed through SPSS-26.

RESULTS

Clinical samples were obtained from 50 patients with five different kinds of infections. Selective culture on Cetrimide agar yielded 87 bacterial isolates. Of these, the overall confirmation rate of *P. aeruginosa* by conventional PCR amplification of the *azu* gene was 76

out of 87 (an 87.4% confirmation rate). Confirmation rates were highest in samples from cerebrospinal fluid (100%) and lowest in urinary tract specimens (81.8%). The largest absolute number of confirmed *P. aeruginosa* isolates (23 isolates or 26.5%) were identified in wounds due to the high volume of specimens and the high prevalence of *P. aeruginosa* in cutaneous infections. Eleven isolates were excluded from all future analyses because they did not amplify the *azu* gene (Table 1). Figure 1 showed the agarose gel electrophoresis (1.5%, RedSafe stain, 75 V, 45 min) showing characteristic 476 bp *azu* gene amplicons from confirmed *P. aeruginosa* clinical isolates. Lanes 1-2: negative controls; positive control; M: 100 bp DNA ladder.

Of the 76 confirmed isolates, biofilm formation was found at an occurrence rate of 80.3% (61 of 76). Strong (maximum) biofilm producers represented the largest proportion of the overall number of isolates as 32 of 76

(42.1%), moderate biofilm producers represented the next largest proportion of the total number of isolates: 29 of 76 (38.2%), weak (minimum) biofilm producers 6 of 76 (7.9%) and non-producing biofilms 9 of 76 (11.8%). As for OD_{590} values, the strong biofilm producers are scored with a wide range from 0.404 to 2.173. Two wound specimen biofilm producers had an OD_{590} value greater than 1.4, yielding an approximate three to five times strong producer level hyper-biofilm phenotype. The vast majority of strong biofilm producers were found within the respiratory (43.8%) or the wound (43.5%) isolates. The overwhelming majority of non-producing biofilm isolates were found within the CSF isolates (28.6%); however, only a small number of CSF isolates (Table 2). The RT-qPCR Gene Expression of *pslA*, *pelA*, and *fliC* were shown in Tables 3-4.

Table No. 1: Distribution of clinical specimens, bacterial isolates, and confirmed *P. aeruginosa* across infection site categories

Specimen Type	Total Collected	Bacterial Isolates	Confirmed <i>P. aeruginosa</i>	Confirmation Rate (%)
Wound infections	15	26	23	88.5
Urinary tract infections	12	22	18	81.8
Respiratory (sputum)	10	18	16	88.9
Blood cultures	8	14	12	85.7
Cerebrospinal fluid	5	7	7	100.0
Total	50	87	76	87.4

Table No. 2: Biofilm-forming capacity of confirmed *P. aeruginosa* isolates (n=76) stratified by infection source

Infection Source	N	Strong No. (%)	Moderate No. (%)	Weak No. (%)	None No. (%)
Wound	23	10 (43.5)	9 (39.1)	2 (8.7)	2 (8.7)
Urinary tract	18	7 (38.9)	7 (38.9)	2 (11.1)	2 (11.1)
Respiratory	16	7 (43.8)	7 (43.8)	1 (6.2)	1 (6.2)
Blood	12	5 (41.7)	4 (33.3)	1 (8.3)	2 (16.7)
CSF	7	3 (42.9)	2 (28.6)	0 (0.0)	2 (28.6)
Total (n=76)	76	32 (42.1)	29 (38.2)	6 (7.9)	9 (11.8)

Table No. 3: Mean CT values ($\pm$ SD) for *pslA*, *pelA*, *fliC* and reference gene *rpoD* across biofilm phenotype categories, with ANOVA F-statistics

Gene	Non-producers (CT $\pm$ SD)	Weak producers (CT $\pm$ SD)	Moderate producers (CT $\pm$ SD)	Strong producers (CT $\pm$ SD)	F-value (ANOVA)
<i>pslA</i>	28.31 $\pm$ 0.17	29.55 $\pm$ 0.25	26.61 $\pm$ 0.21	29.60 $\pm$ 0.36	121,038*
<i>pelA</i>	34.68 $\pm$ 0.23	33.48 $\pm$ 0.34	34.23 $\pm$ 0.06	35.37 $\pm$ 0.23	903,770,772*
<i>fliC</i>	20.27 $\pm$ 0.34	29.55 $\pm$ 0.10	19.53 $\pm$ 0.44	21.69 $\pm$ 0.10	497.9*
<i>rpoD</i>	23.58 $\pm$ 0.22	24.56 $\pm$ 0.09	22.34 $\pm$ 0.08	23.24 $\pm$ 0.03	Reference

Table No. 4: Relative fold-change expression ($2^{-\Delta\Delta C_T}$ method, non-producers as calibrator) of *pslA*, *pelA*, and *fliC* in biofilm-producing isolate categories

Gene	Weak producers (fold-change $\pm$ SD)	Moderate producers (fold-change $\pm$ SD)	Strong producers (fold-change $\pm$ SD)	Regulation Direction
<i>pslA</i>	0.074 $\pm$ 0.005	0.016 $\pm$ 0.001	0.030 $\pm$ 0.001	↓ Downregulated
<i>pelA</i>	0.0009 $\pm$ 0.0001	0.0002 $\pm$ 0.00001	0.0004 $\pm$ 0.00001	↓↓ Strongly down
<i>fliC</i>	19.59 $\pm$ 1.23	4.22 $\pm$ 0.22	7.87 $\pm$ 0.17	↑ Upregulated

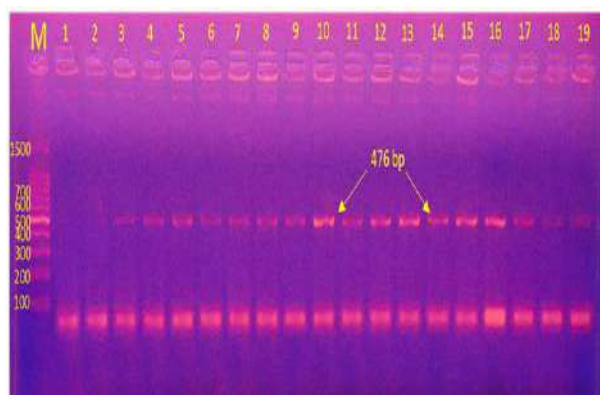


Figure No. 1: Gel electrophoresis image: *azu* gene PCR amplicons (476 bp) confirming *P. aeruginosa* from diverse clinical specimens. M = 100 bp ladder; NC = negative control (*E. coli*); PC = positive control (PAO1 reference strain)

DISCUSSION

We found a significant and consistent prevalence of *P. aeruginosa* in multiple types of clinical specimens. Second, we demonstrated the ability of the extracted *P. aeruginosa* isolates to produce biofilm, with strong producer isolates being a dominant phenotype among confirmed samples. Finally, we identified a very interesting profile of gene expression in the *P. aeruginosa* isolates that was marked by a down-regulation of biosynthesis-related genes, such as *pslA* and *pelA*, that are critical to biofilm formation and a concurrent up-regulation of the motility gene *fliC*. Overall, this study provides the most thorough molecular epidemiologic description of biofilm formation by *P. aeruginosa* in Babylon Province, Iraq and serves to challenge many fundamental concepts that are intrinsic to current biofilm formation theories^{10,11}.

The overall molecular confirmation rate (i.e., proportion of total isolates that were confirmed by molecular means) of all isolates from all specimen types (n=68) was 87.4%. The percentage of confirmed isolates is important to highlight the EPC of *P. aeruginosa* as a nosocomial pathogen in Babylon Province and is similar to the confirmation percentages reported in comparable studies from other provinces in Iraq. The prevalence of *P. aeruginosa* among Gram negative organisms isolated from clinically multi-drug resistant clinical collections in central Iraq by Al-Dahmoshi et al¹² was between 83-90%, while Hassan et al¹³ confirmed that their isolates of *P. aeruginosa* were also present at very high rates among patients in Baghdad area hospitals. The specimen-specific confirmation rates for the *P. aeruginosa* samples in the current study ranged from 81.8% for the urine specimens to 100% for the cerebrospinal fluid specimens, and reflect demonstrated trends of *P. aeruginosa* in regards to their ability to be pathogenic to different anatomical sites. It is particularly interesting to note that the high rate of confirmation from CSF specimens is high (though based on a small number of

specimens) and indicates the severe immunocompromised state of patients from whom lumbar puncture specimens are collected; this is an environment where biosphere organisms can colonize^{14,15}. The use of the *azu* gene as a molecular target for species-specific confirmation is methodologically validated; azurin is the copper-binding electron transfer protein encoded only in *P. aeruginosa*, without corresponding genomic equivalents in related *Pseudomonas* species, giving it exceptional specificity for diagnostic purposes^{16,17}.

The 80.3% prevalence of biofilm formation among confirmed strains is consistent with biofilm forming stats reported in the literature; for example, Al-Shammaree et al¹⁸ reported 82.4% of *P. aeruginosa* isolates from Najaf and Hassan et al¹³ reported 78.9% of isolates from Baghdad. It has been reported that biofilm formation among *P. aeruginosa* from clinical settings globally has fallen in a range between approximately 70%-90% depending on specimen type and methodology¹⁹. The predominance of highly biofilm-producing strains (42.1% of this cohort) is clinically significant due to the well-established positive correlation between biofilm forming strength, minimum inhibitory concentrations for antibiotics, failure rate and length of stay in a hospital. Hyper-biofilm-producing strains identified in this study were characterized by OD₅₉₀ measurements of >1.4, substantially above the strong producer threshold, and are especially worthy of further study because there is mechanistic linkage between hyper-biofilm phenotype and permanent activation of the RetS-GacS-GacA regulatory cascade leading to a chronic infection lifestyle characterized by high levels of matrix production, excessive alginate production and down-regulation of acute virulence factors²⁰. These strains may represent particularly hard to treat strains associated with persistent relapsing infections. The transcriptional results presented show that *pslA* and *pelA* were down-regulated in the biofilm-forming isolates. This is the opposite of what is predicted under traditional biofilm regulatory theory. Traditional beliefs about how biofilms form come from studies on the laboratory reference strains, *Pseudomonas aeruginosa* PAIO and PA14, which suggest that the transition from planktonic to biofilm form occurs when the c-di-GMP signal is elevated, which triggers the expression of both the *psl* and *pel* gene clusters and inhibits the expression of genes involved in flagellar motility (such as *fliC*)^{10,11}. These results suggest that this traditional model does not hold true for clinical isolates of *P. aeruginosa* and points to other mechanisms by which this may occur¹⁴.

CONCLUSION

87.4% of bacterial isolates from humans are *Pseudomonas aeruginosa*. Wound infections were the most prevalent source of infection, and 80.3% of confirmed isolates had evidence of biofilm formation.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Manar Fadhil Obaid, Ali Hmood Al-Saadi
Drafting or Revising Critically:	Manar Fadhil Obaid, Ali Hmood Al-Saadi
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. 8251008 Dated 08.10.2025

REFERENCES

- Jurado-Martín I, Sainz-Mejías M, McClean S. *Pseudomonas aeruginosa*: An audacious pathogen with an adaptable arsenal of virulence factors. *Int J Mol Sci* 2021;22(6):3128.
- Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: An update. *Drugs* 2021; 81(18):2117-31.
- Botelho J, Grosso F, Peixe L. Antibiotic resistance in *Pseudomonas aeruginosa* - Mechanisms, epidemiology and evolution. *Drug Resist Updat* 2020; 44:100640.
- Javed H, Srivastava A, Yadav A, Yadav N, Tyagi RK, Pandey LM. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther* 2024; 9(1):63.
- Moradali MF, Ghods S, Rehm BH. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. *Front Cell Infect Microbiol* 2021;7:39.
- Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, et al. *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct Target Ther* 2022;7(1):199.
- Maurice NM, Bedi B, Sadikot RT. *Pseudomonas aeruginosa* biofilms: Host response and clinical implications in lung infections. *Am J Respir Cell Mol Biol* 2020;58(4):428-39.
- Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, et al. Bacterial biofilm and associated infections. *J Chin Med Assoc* 2021;81(1):7-11.
- Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. *Pathog Dis* 2020;67(3):159-73.
- Ahmed MN, Abdelsamad A, Wassermann T, Porse A, Becker J, Sommer MOA, et al. The evolutionary trajectories of *P. aeruginosa* in biofilm and planktonic growth modes exposed to ciprofloxacin: Beyond selection of antibiotic resistance. *npj Biofilms Microbiomes* 2023;9(1):28.
- Silby MW, Winstanley C, Godfrey SA, Levy SB, Jackson RW. *Pseudomonas* genomes: Diverse and adaptable. *FEMS Microbiol Rev* 2021;35(4):652-80.
- Al-Dahmoshi HOM, Al-Khafaji NS, Jeyad AH, et al. Molecular characterization of virulence factors in multidrug resistant *Pseudomonas aeruginosa* isolates from Iraqi hospitals. *J Pure Appl Microbiol* 2020;14(2):1383-92.
- Hassan RH, Al-Marjani MF, Kadhim HS. Molecular detection of virulence and biofilm formation genes in multidrug-resistant *Pseudomonas aeruginosa* isolates collected from Baghdad hospitals. *J Pure Appl Microbiol* 2021;15(3):1484-95.
- Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage *Pseudomonas aeruginosa* infections. *Drugs Context* 2021;7:212527.
- Freschi L, Vincent AT, Jeukens J, Emond-Rheault JG, Kukavica-Ibrulj I, Dupont MJ, et al. The *Pseudomonas aeruginosa* pan-genome provides new insights on its population structure, horizontal gene transfer, and pathogenicity. *Genome Biol Evol* 2020;11(1):109-20.
- Mohammed SH, Saleh IY, Abdul-Hassan IA. Prevalence and antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* isolated from burn patients in Baghdad, Iraq. *Burns* 2023;49(4):892-9.
- Olewi MH, Jasim MH, Al-Halbosi MMF. Ventilator-associated pneumonia caused by multidrug-resistant *Pseudomonas aeruginosa* in intensive care units: A cross-sectional study. *Med J Babylon* 2021;18(3):320-26.
- Al-Shammaree SA, Al-Ghanimi MA, Sadiq SK. Biofilm formation and antimicrobial resistance patterns of *Pseudomonas aeruginosa* isolated from patients in Najaf province, Iraq. *J Pure Appl Microbiol* 2023;17(1):543-53.
- Al-Rubaye AF, Tariq ZN, Alrubaiy L. Molecular characterization and phylogenetic analysis of multidrug resistant *Pseudomonas aeruginosa* isolated from Iraqi patients. *Gene Rep* 2022;26:101481.
- Khalil MA, Al-Jebory KN, Alzubaidy TH. Virulence genes and their association with clinical outcomes in *Pseudomonas aeruginosa* bacteremia in Iraq. *Microb Pathog* 2021;160:105173.

Role of Anisotropic Analytical Algorithm and Acura's XB Algorithm in the Treatment of Breast Cancer using Volumetric Modulated Arc Therapy Technique

Anisotropic Analytical Algorithm and Acura's XB Algorithm in Breast Cancer

Reem Aref Abd Allateef¹ and Numan Salman Dawood¹

ABSTRACT

Objective: To compare and analyses the dosimetric parameters between the anisotropic analytical algorithm and acuros XB (AXB) algorithm in volumetric modulated arc therapy plans for breast cancers

Study Design: Retrospective study

Place and Duration of Study: This study was conducted at the Warith International Cancer Institute, Al Thaqalyn Cancer Hospital Iraq, from 1st January 2025 to 30th June 2025.

Methods: 30 women with left-sided breast cancer were enrolled. All the patients underwent for treatment by volumetric modulated arc therapy technique, where planning target volume dose calculation was using anisotropic analytical algorithm and acuros XB algorithms of the same optimisation parameters. The prescription doses to the target ranged between 4000 cGy and 5000 cGy.

Results: There is a highly significant difference between anisotropic analytical algorithm and acuros XB calculations, where P-value was less than 0.05. For planning target volume 95% (breast), the calculation of the mean doses with anisotropic analytical algorithm and acuros XB were 93.58% and 94.83%, respectively (P=0.001). As well as, For planning target volume 90% (breast), the calculation of the mean doses with anisotropic analytical algorithm and acuros XB were 97.52% and 98.02%, respectively (P=0.01). At the same time, the mean dose of the PTV 10.7% (breast) was 9.33% and 13.36% for anisotropic analytical algorithm and acuros XB respectively (P=0.003). There are no significant differences between anisotropic analytical algorithm and acuros XB of the Boost cases and of the organ at risk.

Conclusion: There is significant difference between anisotropic analytical algorithm and acuros XB calculation for breast cancer therapy. The dose delivered using acuros XB was higher than the anisotropic analytical algorithm while no significant difference was between anisotropic analytical algorithm and acuros XB analyses calculated using Boost. At the same time, no significant difference was between anisotropic analytical algorithm and acuros XB analyses calculated using volumetric modulated arc therapy with regard to lymph nodes and organs at risk (heart, lung, and spinal cord).

Key Words: Volumetric modulated arc therapy, Acuros XB, Anisotropic analytical algorithm, Breast cancer.

Citation of article: Allateef RAA, Dawood NS. Role of Anisotropic Analytical Algorithm and Acura's XB Algorithm in the Treatment of Breast Cancer using Volumetric Modulated Arc Therapy Technique. Med Forum 2026;37(9):52-56. doi:10.60110/medforum.370910.

INTRODUCTION

Recent advancements in radiation therapy, particularly volumetric modulated arc therapy (VMAT) and anisotropic analytical algorithm (AAA), have

significantly improved the treatment of larynx, rectum, and breast cancers¹. VMAT improved precision and efficiency by optimizing dose administration while safeguarding healthy tissue². The AAA enhanced its dose calculation using sophisticated modelling techniques^{3,4}. This comparative study, supported by patient data, provides insights into calculation algorithms, with the objective of enhancing therapy results for patients with laryngeal, rectum, and breast cancers the optimization of VMAT and the selection of appropriate dosage^{5,6}. The AAA method, renowned for its precision and widespread application in radiation treatment planning, exhibits limitations due to its exclusive reliance on tissue density parameters, thereby overlooking the critical factor of elemental composition^{7,8}. This impacts its accuracy in regions with differing tissue densities and implanted materials possessing high atomic numbers⁹. Acuros XB (AXB) is

1. PG Student / Professor², Department of Physiology, College of Medicine, University of Baghdad, Baghdad, Iraq.

Correspondence: Numan Salman Dawood, Professor, Department of Physiology, College of Medicine, University of Baghdad, Baghdad, Iraq.

Contact No: 9647705389735

Email: numans@comed.uobaghdad.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

a sophisticated method that directly addresses the linear Boltzmann transport equation, providing Monte Carlo-level precision with greater efficiency¹⁰. AXB enhances AAA by dividing dose calculation into two stages: modelling the radiation beam within the accelerator head and subsequently calculating the dose distribution in the patient^{11,12}. AXB's distinctive characteristic is its comprehensive analysis of the elemental composition of tissues, integrating voxel geometry with mass density and material composition derived from CT scans. This guarantees elevated precision in varied density settings¹³. AXB primarily computes dose-to-medium but can convert it to dose-to-water, hence introducing some uncertainty^{14,15}. Nonetheless, dose-to-medium continues to be favored for treatment assessment and outcome analysis, with ongoing investigations into optimal clinical dosage reporting methodologies¹⁶.

METHODS

This retrospective study was conducted at Warith International Cancer Institute, Al Thaqalyn Cancer Hospital Iraq from 1st January 2025 to 30th June 2025 vide letter No. 32525/QM/Approval/54FG4 dated 14th December 2024. It was as part of the assessment of the. Thirty patients with left breast cancer represent the number of female samples between 35 to 55 years were enrolled. The heights were between 155 and 170 cm, as well as having weights from 50 to 85 kg. The left cancer patients with chemotherapy were included. The right breast and lung tumors, patients less than 35 years of age, large peripheral tumors located far from the heart and spinal cord, and tumors invading other organs such as the heart or vertebrae were excluded. All the patients underwent VMAT treatments with dual arc using AAA calculation, and then the dose plans of the same patients was calculated using AXB of the same optimisation parameters. This assessment utilized the institutional protocol's dose constraints, addressing both PTV coverage criteria and doses to Organs At Risk (OAR). The Prescription doses were 4000 cGy to 5000cGy for 30 patients in general most tumors detected early are between 1-3 cm (approximately 1-15 cm³), in advanced cases (before surgery or chemotherapy) the tumor may reach 50-200 cm³ or more. The volume of the PTV varied; the doses to OAR such as the maximum dose to the spinal cord PRV and total lung (V20 and V16 were the relative volumes of total lung receiving 20 Gy and 16 Gy, respectively). The dose calculations between AAA and AXB techniques depends on the treatment covered to the target area, it has closely matched to the shape of the target that was determined by CT-scan, and on the distribution of the dose within target area. At the same time, it depends on the doses received by sensitive areas like the spinal cord, heart and lung. The data was analyzed through SPSS-25. The significant difference depends on the evaluation using the paired t-test for the

difference between paired observations. The p-value was considered statistically significant when it was less than 0.05.

RESULTS

The mean averages of age was 42.25±4.5 years, height was 159.65±4cm and weight of 65.5±4.75kg. There is a highly significant (P<0.05) difference between AAA and AXB. For PTV 95% (breast), the mean doses with AAA and AXB were 93.58% and 94.83%, respectively (P=0.001). As well as, For PTV 90% (breast), the mean doses with AAA and AXB were 97.52% and 98.02%, respectively (P=0.01). At the same time, the mean dose of the PTV 107% (breast) was 9.33% and 13.36% for AAA and AXB, respectively (P=0.003) [Table 1, Fig. 1].

It was showing that there is no significant (P>0.05) differences AAA and AXB. For PTV 95%, the calculation of the mean doses with AAA and AXB were 95.25% and 95.1% respectively (P=0.312). For PTV 90%, the mean doses with AAA and AXB were 99.03% and 98.69% respectively (P=0.658). At the same time, the mean dose of the PTV 107% was 2.4% and 11.23% for AAA and AXB, respectively (P=0.295) [Table 2, Fig. 2].

Table 3 compared radiation doses to OAR from VMAT plans using AAA and AXB, showing no significant (P<0.05) differences for lung, heart and spinal cord maximum. The lung V20 maximum doses with AAA and AXB averaging 24.38% and 24.38% respectively (P=1). Lung V16 doses also showed negligible difference, with a mean of 29.28% for AAA and 29.26% for AXB (P=0.091). At the same time, the dose of the lung mean was 12.56% and 12.3% for AAA and AXB, respectively (P=0.57). Similarly, the dose of the heart mean was 2.27% and 2.69% for AAA and AXB, respectively (P-value= 0.133) while the dose of the spinal cord maximum was 12.48% and 12.12% for AAA and AXB calculations, respectively (P-value= 0.876) [Fig. 3].

Table No. 1: Comparison between AAA and AXB algorithms calculation for breast and lymph node within 15 fractions

Organ ID	Dose (cGy) AAA%	Dose (cGy) AXB	P-value
PTV95% (breast)	93.58±2.373	94.83±1.75	0.001
PTV 90% (breast)	97.52±2.16	98.02±1.76	0.01
PTV 107 (breast)	13.36±0.842	9.33±1.123	0.003
PTV 95% (LN)	95.48±3.89	94.1±5.55	0.616
PTV 90% (LN)	98.31±2.36	98.1±2.893	0.356
PTV 107 (LN)	6.28±0.562	3.42±0.894	0.42

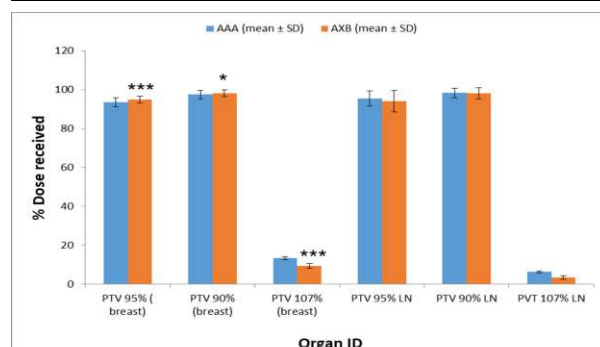


Figure No. 1: Comparison of the dose received by breast and lymph nodes (Dose 4000 cGy) of the breast cancer therapy between the two calculations algorithms (AAA and AXB). *Significant difference at p-value level equal to or less than 0.05.

Table No. 2: comparison between AAA and AXB algorithms calculation for breast within 5 fractions (Boos) dose1000 cGy; after 15 fractions (for breast and lymph node)

Organ ID	Dose (cGy) AAA%	Dose (cGy) AXB	P-value
PTV 95% (Boost)	95.25±2.426	95.1±2.992	0.312
PTV 90% (Boost)	99.03±1.304	98.69±2.218	0.658
PTV 107% (Boost)	2.4±0.658	1.23±0.314	0.295

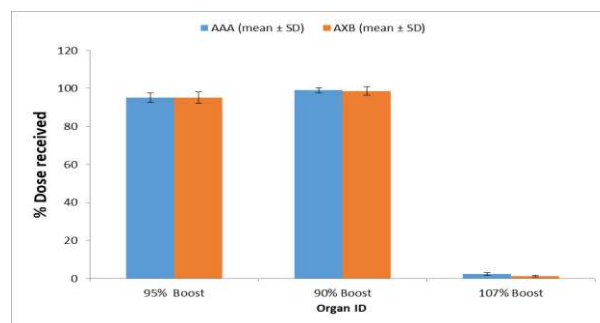


Figure No. 2: Comparison of the dose received by Boost case from 1000 cGy of the breast cancer therapy between the two calculations algorithms (AAA and AXB).

Table No. 3: Comparison between AAA and AXB algorithms of the critical organs at risk dose at the end of radiotherapy course

Organ ID	Dose (cGy) AAA%	Dose (cGy) AXB	P-value
Lung V20	24.38±3.02	24.38±3.07	1
Lung V16	29.28±3.056	29.26±2.97	0.91
Lung mean	12.56±1.337	12.3±1.404	0.57
Heart mean	2.27±1.051	2.69±1.008	0.133
Spinal Cord max.	12.48±2.72	12.12±2.76	0.876

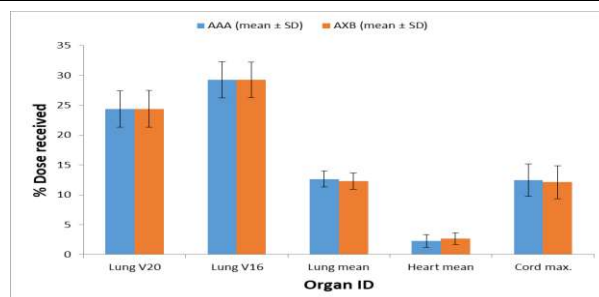


Figure No. 3: Comparison of the dose received by risk at organ (OARs) from total dose (5000 cGy) of the breast cancer therapy between the two calculations algorithms (AAA and AXB).

DISCUSSION

Dose calculation using the AAA and AXB algorithms is critical to determining the dose received by the target from VMAT. One study demonstrated that the clinical dose effects of both AAA and AXB were analyzed by VMAT plans in prostate cancer patients, confirming a slight difference in PTV D95% values (ranging from 0.21% to 0.67%).^{17,18} Additionally, another study noted that AXB estimations for PTV70 in nasopharyngeal cancer were inferior to those for aortic aneurysms, revealing considerable disparities in bone content dosages.^{19,20}

Some research explained that comparing the PTV dosage statistics of AAA and AXB, providing the mean doses with standard deviations, For D 99%, AAA has a little higher average dose than AXB, while AXB has a higher maximum recorded dosage. The average dosages are similar, with no significant differences as indicated by the p-values, showing that the two techniques have identical dosage distributions.²¹

Multiple studies validate that there are nuanced differences in dose of the volume dose estimation for cancer between the AXB and AAA algorithms, with p-values demonstrating the statistical importance of the variations in computations for breast cancer treatment using VMAT plans.^{22,23} These studies agree with the results of our study, where our study was a high significant difference between AAA and AXB calculations in PTV 90, PTV 95 and PTV 107 cases, the p-values were 0.001, 0.01, and 0.003, respectively for breast, and the p-values were 0.616, 0.356, and 0.42, respectively for LN (Table 1, Fig. 1). At the same time, for the Boost cases of PTV 95%, PTV 90%, and PTV 107%, the p-values were 0.31, 0.65, and 0.295, respectively, the results indicated that there are no statistically significant changes existed in the administered dose, although a slight difference in mean for AXB compared to AAA (Table 2, Fig. 2). As well as, our study, which compared dose statistics for OAR (lung, spinal cord, and heart) cases using two different calculation algorithms, AAA and AXB, the mean dose differences are minimal, where the mean dose showing

a slightly lower mean for AXB compared to AAA. However, this difference is not statistically significant, as evidenced by the p-values that are all well above 0.05 (Table 3, Fig. 3).

The findings correspond with the studies by Aamir et al²⁴ and Sandercock et al²⁴, indicating a p-value of 0.579, which signifies the statistical insignificance of the difference between the two sets of point dosage data. The computed point doses from the AAA and AXB methodologies aligned with the measured point dose. Their analysis revealed that both approaches exhibited similar accuracy in point dose measurement when compared to the dosage computed by the TPS.²⁵ Subsequent studies relied on the gamma analysis outcomes, which demonstrated a significant degree of concordance between the AAA and AXB dosimetry systems, with both satisfactorily fulfilling the gamma requirements. The p-value was above 0.05, signifying that the performance disparity was not statistically significant.²⁶

CONCLUSION

The significant differences in breast cancer treatment outcomes between VMAT plans calculated using AAA and AXB where the dose received using AXB was superior to AAA. There was no significant difference between AAA and AXB calculated using Boost. At the same time, there were no significant differences between AAA and AXB calculated using VMAT for lymph nodes and organs at risk (heart, lung, and spinal cord). This highlights the importance of selecting a suitable calculation algorithm for areas with high contrast in the surrounding tissue and could developed by performing in vitro dosimetry in heterogeneous phantoms.

Recommendation: Based on our research results, we recommend using the AXB technique instead of AAA to calculate the dose received by the breast tumor. We also recommend comparing these techniques for other body organs to determine which is better for calculating the dose received by the affected organ.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Reem Aref Abd Allateef, Numan Salman Dawood
Drafting or Revising Critically:	Reem Aref Abd Allateef, Numan Salman Dawood
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. 32525/QM/Approval/54FG4
Dated 14.12.2024

REFERENCES

1. Srinivasan K, Sekaran SC, Thangavelu S, Belliappa MS. Dosimetric impact of AAA and AXB dose calculation algorithm in VMAT treatment planning for rectal tumors. *J Med Phys* 2025; 50(1):86-92.
2. Hunte SO, Clark CH, Zyuzikov N, Nisbet A. Volumetric modulated arc therapy (VMAT): a review of clinical outcomes-what is the clinical evidence for the most effective implementation? *Br J Radiol* 2022; 95(1136): 20201289.
3. Raheem HM, Dawood NS, Al-khalisy MH. The role of Modulation Complexity Score (MCS) of the VMAT and IMRT techniques in the treatment planning of left non-small lung cancer. *Oncol Radiotherapy* 2023;17(5):137-42.
4. Padmanaban S, Warren S, Walsh A, Partridge M, Hawkins MA. Comparison of Acuros (AXB) and Anisotropic Analytical Algorithm (AAA) for dose calculation in treatment of oesophageal cancer: effects on modelling tumour control probability. *Radiat Oncol* 2014; 9:286.
5. Shi J, Yang F, Ju X, Huang Y, Jiang F. Comparative study on dosimetry of VMAT and IMRT in assisted radiotherapy after radical resection of rectal cancer. *Oncol Lett* 2017; 13(5):2971-2974
6. Shams W, Khalid, Ahlam T. Mohammed and Asmaa T. Uthman. Effect of Salivary Melatonin on Ionizing Radiation Worker and its Effect on Periodontal Disease. *J Fac Med Baghdad* 2024; 66 (1), 1-6.
7. Bufacchi A, Caspiani O, Rambaldi G, Marmioli L, Giovinazzo G, Polsoni M. Clinical implication in the use of the AAA algorithm versus the AXB in nasopharyngeal carcinomas by comparison of TCP and NTCP values. *Radiat Oncol* 2020;15(1):150.
8. Abdel Aziz ZS, Dawood NS, Al-khalisy MH. Evaluation of the effect of type II diabetes mellitus on bone mineral density of upper and lower limbs by dual-energy X-ray absorptiometry. *J Fac Med Baghdad* 2023; 65(1):27-33.
9. Singh VP, Badiger NM. Effective atomic numbers of some tissue substitutes by different methods: A comparative study. *J Med Phys* 2014;39(1):24-31.
10. Rana S, Rogers K. Dosimetric evaluation of Acuros XB dose calculation algorithm with measurements in predicting doses beyond different air gap thickness for smaller and larger field sizes. *J Med Phys* 2013; 38(1):9-14.
11. Kargar N, Zeinali A, Molazadeh M. Impact of Dose Calculation Algorithms and Radiobiological Parameters on Prediction of Cardiopulmonary

- Complications in Left Breast Radiation Therapy. *J Biomed Phys Eng* 2024; 14(2):129-40.
12. Fadhil NK, Dawood NS, Al-Khalisy MH. The impact of different nanoparticle concentrations combined with gamma rays on breast cancer cells. *J Fac Med Baghdad* 2025; 67(2):182-8.
 13. Musstaf RA, Al-Sahlanee MHR, Dawood NS. Photobiomodulation Effect of 850 nm Near-infrared Diode Laser on the Release of M1-related Cytokines Inmonocytic THP-1 Cells. *Int J Bioautomation* 2023, 27(1), 5-14.
 14. Delbaere A, Younes T, Vieilleveigne L. On the conversion from dose-to-medium to dose-to-water in heterogeneous phantoms with Acuros XB and Monte Carlo calculations. *Phys Med Biol* 2019; 64(19):195016.
 15. Al-Aubaidi TI, Ahmed M. Correlation between the histopathological grade and size of breast cancer with axillary lymph node involvement. *J Fac Med Baghdad* 2017; 59 (4), 1-6.
 16. Kry SF, Lye J, Clark CH, Andratschke N, Dimitriadis A, Followill D, Howell R, et al. Report dose-to-medium in clinical trials where available; a consensus from the Global Harmonisation Group to maximize consistency. *Radiother Oncol* 2021; 159:106-11.
 17. Dawood NS, Abdel Aziz ZS, Alkhaales HM. Evaluation of the relationship between osteoporosis and body fat mass of the upper and lower extremities by dual-energy X-ray absorptiometry. *Al-Rafidain J Med Sci* 2024; 6(1):34-8.
 18. Gopalakrishnan Z, Bhasi S, P R, Menon SV, B S, Thayil AG, Nair RK. Dosimetric comparison of analytical anisotropic algorithm and the two dose reporting modes of Acuros XB dose calculation algorithm in volumetric modulated arc therapy of carcinoma lung and carcinoma prostate. *Med Dosim* 2022; 47(3):280-87.
 19. Lui JCF, Lee FKH, Law CC, Chow JCH. Does dose calculation algorithm affect the dosimetric accuracy of synthetic CT for MR-only radiotherapy planning in brain tumors? *J Appl Clin Med Phys* 2025; 26(5):e70030.
 20. Dawood NS, Musstaf RA, AL-Sahlanee MHR. Model for prediction of the weight and height measurements of patients with disabilities for diagnosis and therapy. *Int J Bioautomation* 2021; 25(4):343-352
 21. Tsimpoukelli M, Patatoukas G, Chalkia M, Kollaros N, Kougioumtzopoulou A, Michaletou D, et al. Dosimetric comparison and evaluation of two computational algorithms in VMAT treatment plans. *J Appl Clin Med Phys* 2023; 24(9):e14051.
 22. Guebert A, Conroy L, Weppeler S, Alghamdi M, Conway J, Harper L, et al. Clinical implementation of AXB from AAA for breast: Plan quality and subvolume analysis. *J Appl Clin Med Phys* 2018;19(3):243-50.
 23. Hussein EA, Al-Rawaq KJ. Assessment of early side effects of radiotherapy in breast cancer patients. *J Fac Med Baghdad* 2016;58(3):202-7.
 24. Sandercock G. The Standard Error/Standard Deviation Mix-Up: Potential Impacts on Meta-Analyses in Sports Medicine. *Sports Med* 2024; 54(6):1723-32.
 25. Katake A, Kumar L, Singh B, Mishra N, Gurjar P, Vashistha R, Basandrai D. Dosimetric Evaluation of different algorithms on heterogeneous slab phantom using CMS XiO and MONACO treatment planning system for 4MV, 6MV and 15MV beam energy: an institutional study. *Asian Pac J Cancer Prev* 2024; 25(12): 4381-9.
 26. Guerrero Urbano MT, enrys AJ, Adams EJ, Norman AR, Bedford JL, Harrington KJ, Nutting CM, et al. Intensity-modulated radiotherapy in patients with locally advanced rectal cancer reduces volume of bowel treated to high dose levels. *Int J Radiat Oncol Biol Phys* 2006; 65(3): 907-16.

Salivary Cortisol and the Reproductive Endocrine Profile in Infertile Iraqi Men, with Preserved Sperm Chromatin Maturity: A Cross-Sectional Study

Salivary Cortisol
and the
Reproductive
Endocrine Profile
in Infertile

Layla Kadhun Radeef¹, Manal Nasih Ahmed Hamdan², Thulfiqar Ibrahim Raheem³,
Amina Abbas Alwan¹ and Ali Ibrahim Al-Dulaimi⁴

ABSTRACT

Objective: To examine the associations of salivary cortisol with the reproductive hormone profiles and with sperm chromatin maturity across semen-quality phenotypes in infertile men.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Andrology Clinic, Najaf Iraq from 1st September 2025 to 30th November 2025.

Methods: One hundred infertile men were classified by WHO criteria into normozoospermia, oligozoospermia, asthenozoospermia and oligoasthenoteratozoospermia were enrolled. Salivary cortisol, serum luteinizing hormone, follicle-stimulating hormone, total testosterone, prolactin and estradiol, and sperm chromatin maturity (assessed by aniline blue staining) were measured.

Results: Salivary cortisol was inversely associated with follicle-stimulating hormone ($\rho = -0.28$, $p=0.005$; false discovery rate $q=0.026$; 95% CI -0.45 to -0.09) and this association remained independent in multivariable regression (standardized beta $= -0.20$, $p=0.029$). Cortisol showed a weaker positive association with estradiol ($\rho = +0.22$, $p=0.029$) that did not survive false discovery rate correction, and no significant association with luteinizing hormone, testosterone or prolactin. Follicle-stimulating hormone and testosterone differed across phenotypes ($p<0.001$), being highest in normozoospermia. Sperm chromatin immaturity was low (median 6% aniline-blue-positive), did not differ across phenotypes ($p=0.60$), and was not associated with cortisol or the endocrine profile, apart from a weak correlation with prolactin ($\rho = +0.28$, $p=0.011$).

Conclusion: In infertile men, higher salivary cortisol is independently associated with lower follicle-stimulating hormone, consistent with an inhibitory influence of hypothalamic-pituitary-adrenal activity on the gonadotropic axis. Sperm chromatin maturity was largely preserved and unrelated to stress or the endocrine profile.

Key Words: Salivary cortisol, Psychological stress, Follicle-stimulating hormone, Reproductive hormones, Sperm chromatin maturity, Aniline blue, Male infertility

Citation of article: Radeef LK, Hamdan MNA, Raheem TI, Alwan AA, Al-Dulaimi AI. Salivary Cortisol and the Reproductive Endocrine Profile in Infertile Iraqi Men, with Preserved Sperm Chromatin Maturity: A Cross-Sectional Study. Med Forum 2026;37(9):57-61. doi:10.60110/medforum.370911.

INTRODUCTION

¹. Assistant Lecturer, Department of Medical Laboratory, College of Health & Medical Technologies, University of Al-Kafeel, Najaf, Iraq

². Assistant Professor, Department of Obstet & Gynecol, College of Medicine, University of Kerbala, Karbala, Iraq

³. Assistant Lecturer, Department of Urology, Iraqi Board of Medical Specializations, Al-Sadr Medical City, Najaf, Iraq

⁴. Professor, Department of Anatomy, Faculty of Medicine, University of Kufa, Najaf, Iraq

Correspondence: Ali Ibrahim Al-Dulaimi, Professor, Department of Anatomy, Faculty of Medicine, University of Kufa, Najaf, Iraq.

Contact No: +9647801282226

Email: aliir.abbas@uokufa.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

Male infertility is a major global health concern, contributing to about half of all cases of couple infertility.¹ Semen analysis remains the cornerstone of evaluation², but it does not capture the hormonal and molecular factors that shape testicular function, and there is growing interest in additional markers.

The hypothalamic-pituitary-gonadal (HPG) axis directs spermatogenesis, with circulating FSH, LH and testosterone serving as recognised determinants of semen quality.³ Stress perturbs this system: poorer male fertility has been tied to psychological stress⁴, and the hypothalamic-pituitary-adrenal (HPA) axis restrains the HPG axis through both central and peripheral actions.⁵ HPA activity can be tracked non-invasively through salivary cortisol⁶, whereas salivary alpha-amylase indexes sympathetic tone.⁷ Mechanistically, glucocorticoids suppress gonadotropin secretion and testicular steroidogenesis and can trigger Leydig-cell apoptosis^{8,9}, and clinical studies have associated stress

with altered reproductive hormones and reduced semen quality.^{10,11} FSH in particular drives Sertoli-cell function and the early stages of spermatogenesis.¹²

Sperm chromatin maturity, established during spermiogenesis through the replacement of histones by protamines that compacts and protects the paternal genome, is a recognised marker of sperm quality, and defective protamination detectable by aniline blue staining has been linked to reduced fertilisation and pregnancy outcomes.^{13,14} Poorly condensed, protamine-deficient chromatin is also more vulnerable to oxidative injury, and impaired chromatin condensation predicts assisted-reproduction outcomes.¹⁵ Because systemic stress can raise oxidative load and perturb spermiogenesis, a relationship between HPA activity and sperm chromatin maturity is biologically plausible but has rarely been tested directly in men.

We therefore measured salivary cortisol, the reproductive hormone profile and sperm chromatin maturity in infertile men, and examined how cortisol relates to the endocrine profile and to chromatin maturity, and how these markers vary across semen-quality phenotypes. The study characterises associations within an infertile population; it does not include a fertile control group and makes no claim that the measured concentrations are abnormal.

METHODS

This cross-sectional study was conducted at Andrology Clinic, Najaf Iraq 1st September 2025 to 30th November 2025 No. 159/UOA dated 15-8-2025 and 100 men of infertility care were enrolled. Each contributed a semen sample, a venous blood draw and a saliva sample on the same day, together with his recorded clinical and demographic details. Infertility was defined at the couple level as failure to achieve a pregnancy after at least 12 months of regular unprotected intercourse; men with a normal semen analysis (the normozoospermic group) were therefore included as the male partners of infertile couples, in whom female or unexplained factors may contribute, since a normal ejaculate does not exclude a male partner from an infertile couple.

Semen was assessed following WHO protocols.¹⁸ Using the WHO 2010 thresholds (concentration 15 million per millilitre; progressive motility 32%), each man was placed in one of four groups: normozoospermia, oligozoospermia, asthenozoospermia or OAT. Sperm chromatin maturity was assessed by aniline blue staining, which discriminates lysine-rich histones (retained in immature chromatin, stained) from arginine-rich protamines (mature chromatin, unstained), and expressed as the percentage of aniline-blue-positive (immature-chromatin) spermatozoa

Salivary cortisol was measured by a competitive-inhibition ELISA for which the manufacturer validates saliva (ELK Biotechnology, ELK8526; working range 3.13 to 200 ng/mL; sensitivity 1.02 ng/mL; intra- and

inter-assay CV under 8% and 10%). Serum LH, FSH, total testosterone, prolactin and estradiol were each run on matched ELISA kits per the manufacturers' instructions, with values read from the relevant plate standard curve. Sampling of saliva and blood was confined to a fixed evening window (17:00 to 21:00; median 18:00) to dampen diurnal variation. Salivary alpha-amylase was additionally assayed but discarded as unreliable, the recorded values (observed range approximately 12 to 244 U/mL) falling outside the manufacturer's validated range for the kit

All computations were carried out in Python using SciPy, stats models and scikit-learn. Group differences were tested by the Kruskal-Wallis test, and associations by the Spearman rank-correlation coefficient, with magnitudes interpreted by conventional thresholds as weak (absolute rho below 0.3), moderate (0.3 to 0.5) or strong (above 0.5). For the family of cortisol-hormone correlations, p values were adjusted by the Benjamini-Hochberg procedure to control the false discovery rate (FDR), and 95% confidence intervals were obtained by nonparametric bootstrapping (percentile method, 5000 resamples). To assess whether the cortisol-FSH association was independent of demographic and gonadal factors, a rank-based multiple regression was fitted with FSH as the dependent variable and cortisol, age, body-mass index, testosterone and LH as predictors. The post-hoc sensitivity analysis indicated 80% power (alpha = 0.05) to detect a correlation of absolute rho of at least 0.28, so the study was only borderline-powered for the observed effect and weaker associations would not have been detectable.

RESULTS

Among the 100 men, the four groups numbered 26 (normozoospermia), 25 (oligozoospermia), 22 (asthenozoospermia) and 27 (OAT). Group-mean age fell between 27.7 and 33.5 years and group-mean body-mass index between 26.2 and 28.5 kg/m² (Table 1).

Table No. 1: Demographic characteristics by semen-quality group

Group	No.	Age (years)	BMI (kg/m ²)
Normozoospermia	26	33.5±9.3	28.2±7.7
Oligozoospermia	25	27.7±4.4	27.2±3.0
Asthenozoospermia	22	32.0±5.6	26.2±4.2
OAT	27	30.2±6.6	28.5±6.7

Salivary cortisol was inversely associated with FSH at a weak-to-moderate magnitude (Spearman rho = -0.28, p=0.005), the only association to survive FDR correction (q=0.026; bootstrap 95% CI -0.45 to -0.09) (Table 2, Fig. 1). A weaker positive association with estradiol (rho=+0.22, p=0.029) did not survive correction (q=0.072), and cortisol was not significantly associated with LH, total testosterone or prolactin.

FSH and total testosterone differed significantly across phenotypes (both Kruskal-Wallis $p < 0.001$), being highest in normozoospermia and lower in the abnormal

groups, and estradiol also differed ($p = 0.005$). LH and prolactin did not differ significantly across phenotypes (Table 3).

Table No. 2: Spearman correlations of salivary cortisol with reproductive hormones

Hormone	Rho	P	q (FDR)	95% CI
FSH	-0.28	0.005	0.026	-0.45 to -0.09
Estradiol	+0.22	0.029	0.072	+0.01 to +0.42
Testosterone	-0.14	0.175	0.291	-0.34 to +0.07
LH	-0.07	0.514	0.514	-0.25 to +0.12
Prolactin	+0.08	0.410	0.513	-0.12 to +0.26

Table No. 3: Reproductive hormones by semen-quality group, median (interquartile range)

Marker	Normo	Oligo	Asthen	OAT
FSH (mIU/mL)	1.70 (1.57-1.95)	0.77 (0.47-1.07)	1.16 (0.62-1.37)	0.97 (0.70-1.40)
Testosterone (ng/mL)	3.75 (2.70-4.83)	1.80 (1.60-2.10)	2.30 (2.10-2.30)	2.10 (1.70-2.35)
LH (mIU/mL)	7.15 (4.78-10.5)	7.50 (5.70-10.5)	9.65 (6.22-14.4)	7.50 (5.75-10.0)
Prolactin (ng/mL)	46.2 (29.5-70.4)	39.5 (20.0-61.1)	59.9 (38.4-81.5)	47.2 (39.5-68.8)
Estradiol (pg/mL)	237 (73-387)	257 (188-350)	190 (103-253)	123 (65-197)

Kruskal-Wallis p: FSH < 0.001; Testosterone < 0.001; LH 0.320; Prolactin 0.119; Estradiol 0.005

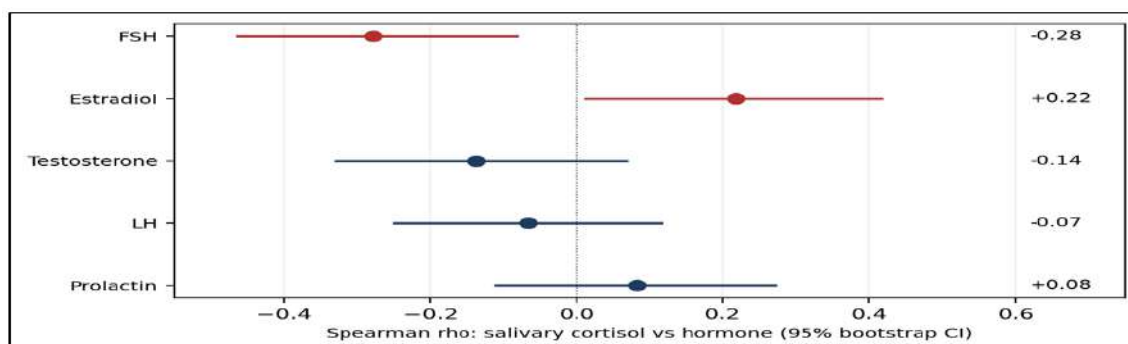


Figure No. 1: Spearman correlations (with 95% bootstrap confidence intervals) of salivary cortisol with the reproductive hormones. Only the inverse association with FSH excludes zero. Cool colour, negative; warm colour, positive

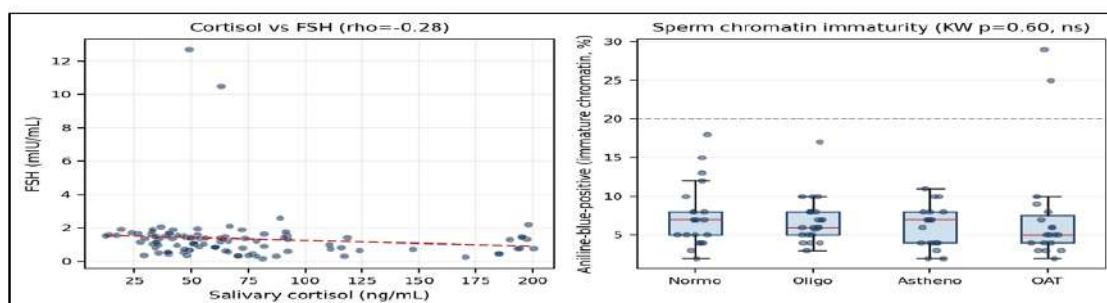


Figure No. 2: Left: Salivary cortisol (ng/mL) versus FSH (mIU/mL); the dashed line shows the linear trend for visualisation only. Right: sperm chromatin immaturity (aniline-blue-positive spermatozoa, %) across semen-quality phenotypes (n = 81); boxes show median and interquartile range. Chromatin immaturity did not differ across phenotypes (Kruskal-Wallis $p = 0.60$, not significant)

Table No. 4: Rank-based regression model predicting FSH

Predictor	Beta	SE	P value
Testosterone	+0.48	0.090	<0.001
Cortisol	-0.02	0.089	0.029
BMI	+0.10	0.090	0.247
LH	+0.06	0.088	0.472
Age	-0.02	0.090	0.840

To test whether the cortisol-FSH association was independent of demographic and gonadal factors, a rank-based multiple regression was fitted with FSH as the dependent variable (Table 4). The model was significant (adjusted R-squared = 0.26, F-test $p < 0.001$). Salivary cortisol remained an independent inverse predictor of FSH (standardized beta = -0.20, $p = 0.029$) after adjustment for age, BMI, testosterone and LH;

testosterone was the strongest predictor ($\beta = +0.48$, $p < 0.001$).

Sperm chromatin immaturity (aniline-blue positivity, available for 81 of 100 men) was low (median 6%, range 2 to 29%), with only 2 men exceeding 20% and none exceeding 30% immature spermatozoa, indicating predominantly mature chromatin. It did not differ across semen-quality phenotypes (Kruskal-Wallis $p = 0.60$) and was not significantly associated with salivary cortisol ($\rho = +0.14$, $p = 0.22$), LH, FSH, testosterone or estradiol. The only nominal association was a weak positive correlation with prolactin ($\rho = +0.28$, $p = 0.011$), which was not corrected for multiple testing and should be regarded as exploratory (Fig. 2). Overall, sperm chromatin maturity was preserved in this cohort and was independent of stress and the endocrine profile.

DISCUSSION

In this cohort of infertile males, higher salivary cortisol was associated with lower FSH, and this inverse relationship was the only cortisol-hormone association to survive correction for multiple testing and remained independent after adjustment for age, BMI, testosterone and LH. By contrast, sperm chromatin immaturity was low and showed no relationship with cortisol or the endocrine profile. The principal message is therefore an independent inverse cortisol-FSH association set against preserved, stress-independent sperm chromatin maturity. The inverse cortisol-FSH association is biologically coherent. The HPA axis exerts an inhibitory influence on the HPG axis⁵, and glucocorticoids can suppress gonadotropin-releasing hormone and gonadotropin secretion.⁹ A relative reduction in FSH in men with higher cortisol is consistent with this central inhibition, and because FSH is the principal driver of Sertoli-cell function and early spermatogenesis¹², such an effect could provide one route by which stress relates to impaired spermatogenesis.^{11,12} The expected inverse cortisol-testosterone relationship was present in direction but did not reach significance⁸, which may reflect the modest sample, single-sample cortisol measurement, or the low testosterone concentrations characteristic of this infertile cohort. The cortisol-FSH association is of weak-to-moderate magnitude ($\rho = -0.28$; 95% CI -0.45 to -0.09); it is statistically robust but accounts for only a limited share of the variance in FSH, and is therefore best read as one contributory influence on gonadotropic output rather than a dominant determinant.

Sperm chromatin maturity, which reflects the histone-to-protamine transition of spermiogenesis rather than oxidative DNA strand breakage showed no association with salivary cortisol or the reproductive hormones and did not differ across phenotypes.¹⁵ Two features help explain this. First, immaturity values were low (median 6%, only 2 men above 20% and none above 30%), so

the cohort had largely mature chromatin and limited variance, a range-restriction effect that mechanically constrains any correlation involving chromatin immaturity and means the null findings cannot exclude a relationship in populations with greater chromatin defects. Second, HPA-mediated stress, acting largely through the gonadotropic axis, need not perturb the protamination programme of spermiogenesis.¹⁶ The isolated weak correlation with prolactin did not withstand the multiplicity of testing and is best regarded as a chance finding pending replication. These observations indicate that, in this population, sperm chromatin maturity was preserved and largely independent of stress-axis activity, a negative result that is itself informative.¹⁷

Strengths include the use of a salivary stress marker, a full reproductive hormone panel, a direct measure of sperm chromatin maturity, phenotype stratification, rank-based statistics with FDR control and bootstrap confidence intervals, and a multivariable model.

Limitations: First, the study included only infertile men and no fertile control group, so it characterises associations within an infertile population and cannot determine whether the measured concentrations are abnormal. Second, the design is cross-sectional and cannot establish causality. Third, the cohort was modest ($n = 100$; chromatin maturity available for 81) and drew on a pre-existing sample without an a priori power calculation, so weaker associations may have been missed; in particular, sperm chromatin immaturity showed marked range restriction (median 6%, no value above the 30% threshold), which limits the power to detect any chromatin-maturity association and means the negative chromatin findings should be read with that constraint in mind. Fourth, salivary cortisol was sampled once within an evening window, which coincides with the diurnal nadir; a single evening measurement likely attenuates the true association magnitude, and repeated or diurnal sampling, or the cortisol awakening response, would better capture HPA activity and probably yield stronger estimates. Fifth, salivary alpha-amylase was measured but excluded because its values lay outside the validated assay range, and several of the serum endocrine measurements sit well outside commonly cited male reference intervals: estradiol exceeded the typical upper limit of about 40 pg/mL in 92 of 100 men (28 above 300 pg/mL), prolactin exceeded about 25 ng/mL in 81 of 100 men (10 values recorded as right-censored at the assay ceiling of 100 ng/mL), and FSH (median 1.2 mIU/mL) and total testosterone (median 2.3 ng/mL) lay at or below the lower reference limits.

CONCLUSION

In infertile males, higher salivary cortisol was independently associated with lower FSH, supporting an inhibitory link between HPA activity and the

gonadotropic axis, whereas sperm chromatin maturity was largely preserved and unrelated to stress or the endocrine profile.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Layla Kadhum Radeef, Manal Nasih Ahmed Hamdan, Thulfiqar Ibrahim Raheem
Drafting or Revising Critically:	Amina Abbas Alwan, Ali Ibrahim Al-Dulaimi
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. 159/UOA dated 15-8-2025

REFERENCES

- Liu Y, Zhang F, Wang J. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: an analysis of the Global Burden of Disease Study 2019. *Reprod Biol Endocrinol* 2023;21:109.
- Meccariello R, Fasano S, Pierantoni R, Cobellis G. Modulators of the hypothalamic-pituitary-gonadal axis for the control of spermatogenesis and sperm quality in vertebrates. *Front Endocrinol (Lausanne)* 2014;5:135.
- Dutta S, Sengupta P, Bagchi S. Reproductive hormones and semen quality. *Asian Pac J Reprod* 2019;8(4):151-9.
- Nargund VH. Effects of psychological stress on male fertility. *Nat Rev Urol* 2015;12(7):373-82.
- Schneid-Kofman N, Sheiner E. Does stress effect male infertility? A debate. *Med Sci Monit* 2005; 11(8):SR11-13.
- Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinol* 2009;34(2):163-71.
- Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinol* 2009;34(4):486-96.
- Hardy MP, Gao HB, Dong Q, et al. Stress hormone and male reproductive function. *Cell Tissue Res* 2005; 322(1):147-53.
- Whirledge S, Cidlowski JA. Glucocorticoids, stress, and fertility. *Minerva Endocrinol* 2010;35(2):109-25.
- Nordkap L, Jensen TK, Hansen AM, Lassen TH, Bang AK, Joensen UN, et al. Psychological stress and testicular function: a cross-sectional study of 1215 Danish men. *Fertil Steril* 2016;105(1): 174-87.
- Bhongade MB, Prasad S, Jiloha RC, Ray PC, Mohapatra S, Koner BC. Effect of psychological stress on fertility hormones and seminal quality in male partners of infertile couples. *Andrologia* 2015;47(3):336-42.
- Wang JM, Li ZF, Yang WX, Tan FQ. Follicle-stimulating hormone signaling in Sertoli cells: a licence to the early stages of spermatogenesis. *Reprod Biol Endocrinol* 2022;20:97.
- Sellami A, Chakroun N, Ben Zarrouk S, Sellami H, Kebaili S, Rebai T, et al. Assessment of chromatin maturity in human spermatozoa: useful aniline blue assay for routine diagnosis of male infertility. *Adv Urol* 2013; 2013:578631.
- Irez T, Dayioglu N, Alagoz M, Karatas S, Guralp O. The use of aniline blue chromatin condensation test on prediction of pregnancy in mild male factor and unexplained male infertility. *Andrologia* 2018; 50(10): e13111.
- Agarwal A, Parekh N, Panner Selvam MK, Henkel R, Shah R, Homa ST, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *World J Mens Health* 2019; 37(3): 296-312.
- World Health Organization. WHO laboratory manual for the examination and processing of human semen. 6th ed. Geneva: WHO; 2021.p.55.
- Hammadeh ME, Zeginiadov T, Rosenbaum P, Georg T, Schmidt W, Strehler E. Predictive value of sperm chromatin condensation (aniline blue staining) in the assessment of male fertility. *Arch Androl* 2001; 46(2):99-104.

The Role of TP53 Genetic Mutation in Breast Cancer Pathogenesis

Role of TP53
Genetic Mutation
in Breast Cancer

Soura Alaa Hussein¹, Ahmed Mudher Al-Khaykanee² and Tiba A.H. Al-Rubaye³

ABSTRACT

Objective: To review the role of TP53 genetic mutations in breast cancer

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Marjan Teaching Hospital in Babylon, from 1st December 2023 to 30th June 2025.

Methods: This case-control was conducted at Marjan Teaching Hospital in Babylon and 200 subject were enrolled, 100 patients with breast cancer and 100 healthy undivided were recruited. Subjects were selected at after the extraction of DNA the genotype of rs17878362 C/T and rs1042522 G/C was done using TaqMan-based Real-time PCR technique.

Results: The significant was noted in cancer antigen 15-3 level between the two group where it was 45.22±4.32U/ml in cases and 22.24±2.10U/ml in control (p=0.001).as for the polymorphism rs17878362. There was a statically significant difference in the frequency of CC 29% and CT 58% genotypes as well as in T alleles between cases and control (p=0.001), also similar result were obtained for the polymorphism rs1042522 .the frequencies of GG 38%; GC 62% genotypes and C alleles were statistically significantly more in the cancer group (P=0.001). The highest risk was found for TT genotype of rs17878362 which had the OR equal to 3.11. Discrepancies from Hardy-Weinberg Equilibrium were detected in the cases of breast cancer group in both genetic polymorphism (p=0.001).

Conclusion: The strong correlation between TP53 gene polymorphism rs17878362 and rs1042522 and high cancer incidence. These variants seem to affect the function of TP53 protein in terms of programmed cell death, causing genomic instability which is associated with developing of tumors. Thus, they might serve as important markers for cancer predicant and treatment.

Key Words: Breast cancer, Polymorphism, TP53 Genetic Mutation.

Citation of article: Hussein SA, Al-Khaykanee AM, Al-Rubaye TAH. The Role of TP53 Genetic Mutation in Breast Cancer Pathogenesis. Med Forum 2026;37(9):62-66. doi:10.60110/medforum.370912.

INTRODUCTION

According to the WHO data, breast cancer (BC) is currently the leading cause of death and morbidity due to its high incidence in females. for instance, about 2.3million new cases of BC are expected in 2022 while the forecasts predict that in 2025, BC will comprise almost 30% of newly diagnosed cancer among females in developed regions¹. Although 5 years several rates for locally diagnosed BC are relatively high and exceed

90% the mortality to incidence ratio continues to be significantly high in underdeveloped countries due to late stage presentation of the disease². Epidemiological evidence indicates including the gradual increase in BC incidence among postmenopausal females and a considerable 1.4% yearly increase in cases of early-onset BC among those under fifty years old¹. Both trends are believed to be driven by complicated interaction between lifestyle factors, such as increased obesity and late childbearing and genetic predisposition³. It has also been forecasted that there will be around 3.2 million new BC cases per year in 2050 if current tendencies continue⁴. Today's clinical management of BC relies heavily on molecular subtypes characterized by the status of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor2 (HER2) expression⁵. Two new terms HER2-low and HER2 ultralow were introduced into the BC classification system to make a wider range of patients eligible for the use of drugs formerly prescribed exclusively for HER2 positive patents⁶. The TP53 gene is situated at 17p13.1 location and is responsible for p53 protein production, which sever as a transcription activator in cellular processes following different stimuli^{7,8}. As a wild type p53 usually has low levels and is under control of the negative inhibitor of

¹. Assistant Professor, Department of Technical Nursing, Technical Institute-Baghdad, Middle Technical University, Iraq.

². Lecturer, General Directorate of Education of Babylon Governorate, Iraq.

³. Lecturer, Department of Animal Production Technique, Al-Furat Al-Awsat Technical University, College of Health & Medical Technology, Babylon, Iraq.

Correspondence: Ahmed Mudher Al-Khaykanee, Lecturer, General Directorate of Education of Babylon Governorate, Iraq.

Contact No: 009647811602922

Email: ahmedmudher14@gmail.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

the TP53 in case of any changes in genomic integrity or activation of oncogenes TP53 becomes stable and gets activated⁹. Activated TP53 associates with certain DNA response elements to activate 500 target genes and triggers either cell cycle arrest via P21 expression or repair processes or causes programmed cell death if damage cannot be repaired¹⁰. Differently from other tumor suppressor genes which are often subject to deletion more than 70% of TP53 alterations are missense motions in the region of DNA binding domain¹¹. The mutant forms of TP53 possess a gain of function.

METHODS

This case-control was conducted at Marjan Teaching Hospital in Babylon in the period from 1st December 2023 to 30th June 2025 letter No. FTU-1401/Approval/JSDJNEHU dated November 25, 2023 and 200 subject were enrolled, 100 patients with breast cancer and 100 healthy undivided were recruited. The diagnosis of the BC was done through ultrasound magnetic resonance imaging, mammography on biopsy together with confirmatory test the CA15-3 marker test. Four milliliters of peripheral blood were taken from each subject two milliliters of the blood where then used of genotype TP53 whereas the other two milliliters where use of CA15-3 analysis

Genomic DNA was executed using the pure link Genomic DNA Excretion Kit USA based on the protocol provide by the manufacturer Qiagen DNA sample where type using TaqMan genotyping assay kit with the applied biosystem 7500 real time PCR system (Foster City CA, USA) TaqMan probes with VIC and FAM fluorophore markers were used for the TP53C/T RS17878362 and G/C RS1042522 primer sequences respectively the reference sequences (gene bank NC-000017.11:7668421-7687490) is shown below:

TP53 C/T rs17878362

Forward (5→3): CTGGGGACCTGGAGGGCTG

Reverse (5→3): TCACCCAGCCCTCCAGGT

TP53 G/C rs1042522

Forward (5→3): TCCCCCTTGCCGTCCCAAGCA

Reverse (5-3): CTGGTGCAGGGGCCACGCGT

The component of the 25 µl reaction mixture includes 6.25 µl of water nucleus 3 1.25 µl of snip test and 12.5 µl of Tuchman Master mix the reaction was performed in individual PCR tube containing 5 µl of Genomic DNA template 10 mg on microliter and 5 µl of DNA free water as the negative control the solution was mixed on beer fairly centrifuge before the reaction sample were edited to the thermal cycle and amplified genotype was analyzed by the applied biosystem. The CA15-3 tumor marker analysis was done by employing the Cubase E411 machine Machine an equipment that is used in testing tumor marker. Statistical analysis was done using the SPSS-18. For the control group Hardy-Weinberg equilibrium was tested for the genotype

frequencies odds ratio OR and their 95% confidence intervals (CI) were stimulated by the Chi square testing the relationship between the risk of BC and the genotype of the SNP of TP53 gene RS17878362 and RS1042522. Also, linkage disequilibrium between the two SNPs was stimulated by Halo view A p-value of less than 0.005 was considered significant.

RESULTS

There was no significant in age and gender while significant in tumor marker CA15.3U/ml (Table 1). It can be observed from table two that there exists a highly significant genetic correlation P=0.001 between the study samples and particular mutations of TP53. in the case of RS17878362, the control sample displays the predominance of the CC genotype 72% while BC sample indicates a greater number of individuals with the CT genotype 58% as well as with the T allele. Similarly, it is evident that in the control sample GG genotype has the greatest frequency 62% while in BC sample it is the GC genotype 62% as well as C allele (Table 2).

Table No. 1: Demographic and laboratory results of patients and controls (n=200)

Group	Control (n=100)	Breast cancer (n=100)	P value
Age	55.22±6.32	56.44±3.33	2.32
CA15.3U/ml	22.24±2.1	45.22±4.32	0.001

Table No. 2: Frequency of genotypes and alleles for TP53C/T rs17878362 and G/C rs1042522

Variable	Control		Breast Cancer		X ²	P value
	No.	%	No.	%		
Genotypes TP53 Rs17878362						
CC	72	72.0	30	30.0	60.33	0.001
CT	20	20.0	58	58.0		0.001
TT	8	8.0	12	12.0		0.001
Alleles						
C	164	82.0	118	40.0	61.54	0.001
T	36	18.0	82	60.0		0.001
TP53 Rs1042522						
GG	62	62.0	25	25.0	52.41	0.001
GC	30	60.0	62	62.0		0.001
CC	8	8.0	13	13.0		0.0014
Alleles						
G	154	77.0	112	56.0	64.37	0.001
C	46	23.0	88	44.0		0.001

The statistical assessment shows that rs104522 is statistically significantly different from HWE and the BC cohort P=0.014, while the controller group is in

agreement with HWE $P = 0.07$. The rs17878362SNP is found to be statically significantly different from HWE in both the case and control groups Case group $P = 0.003$, control group $P = 0.004$ the difference, mark with asterisks indicate that the observed frequency of alleles such as GG, GC, and CC may differ from the expected frequency in these populations (Table 3).

Table No. 3: Genotype frequencies observed and their agreement with the Hardy Weinberg Equilibrium

	Observed	Expected	χ^2	P value
TP53rs1042522				
Case (n = 100)				
GG	25	21.8	4.795	0.014
GC	62	52.2		
CC	13	28.0		
Control (n = 100)				
GG	62	53	2.68	0.07
GC	30	25.1		
CC	8	4		
TP53 rs17878362				
Case (n = 100)				
CC	30	26.0	5.153	0.003
CT	58	53.0		
TT	12	20.0		
Control (n = 100)				
CC	72	66.0	4.027	0.004
CT	20	24.0		
TT	8	5.0		

Table No. 4: Associations of TP53rs1042522 and TP53 rs17878362 polymorphisms

Genotype	OR (CI.95%)	P
TP53 rs1042522		
GG®	1	
GC	3.08 (1.99 – 6.87)	<0.001
CC	2.72 (0.63 – 8.04)	<0.001
GC+CC	2.03 (2.25-7.00)	<0.001
Alleles		
G®	1	
C	2.55 (1.78–5.03)	<0.001
TP53 rs17878362		
CC	1	
CT	2.92 (2.00 – 7.23)	<0.001
TT	3.11 (2.01 – 6.77)	<0.001
CT+TT	2.55 (2.43-5.77)	<0.001
Alleles		
C®	1	
T	2.88 (1.57–1.95)	<0.001

There is a strong correlation in statistics between the occurrence of mutations in TP53 RS1042522 and RS17878362 and an enhanced likelihood of developing BC with regard to R 1042522. It is clear that the presence of C allele and the genotype GG leads to an

increase in BC risk, with P value less than 0.001 Regarding RS 17878362, the risk of developing BC is higher for the genotype TT compared to the genotype CC, resulting in odds ratio of 3.11. It is worth noting that there is a clear tendency in the combined genotypes GG/CC and TT/CT toward an elevated susceptibility (Table 4).

DISCUSSION

Breast cancer continues to be the most prevalent form of cancer among women in the world and its occurrence is often associated with the development index of a particular country .in addition the discovery of biomarkers to facilitate early detection is a critical to reduce to reducing fatalities worldwide¹². Also known as the guardian of the genome the TP53 gene produces the TP53 protein that regulates essential functions such as DNA repair cell cycle arrest and programmed cell death or apoptosis TP53 mutations occur in the highest numbers among BC patients about 30-50% of BC harbor mutations in the TP53¹³. It has been reported that certain mutations within the TP53 protein may also have oncogenic benefits thus making therapy difficult and highlighting the requirement forTP53 based therapeutic approaches¹². The current study highlights the relationship between some specific TP53 polymorphisms and an increased risk of BC for instance the polymorphism RS17878362, involving a-16 base pair duplication in intron 3 shows a market change in the frequency of the genotypes in control (CC equal 72% to CT 58%)with the presence of the minor allele T being associated with a higher risk of disease development this results aligns with other meta-analysis in the identification of the minor allele of RS17878362 as a risk factor for developing BC moreover another polymorphism Rs 1042522 which is responsible for the Arg 72 pro substitution^{14,15}. The most common genotype in control is GG Arg 62%, but in BC patents the GC genotype and the C allele have significantly higher frequencies 62% it is possible that the latter can affect the ability of TP53 gene product to undergo apoptosis and environmental stimuli¹⁶.

The genotypes for two TP53 mutations, RS1042522 and RS17878362, were analyzed among BC patients and healthy controls based on the findings, the genotypes for the RS1042522 mutations are significantly out of equilibrium in the BC patients ($P = 0.014$) but not in the controls $P = 0.07$ the RS1042522 mutation is located at the codon 72 locus, which results in the arginine to proline amino acid change affecting the protein function of apoptosis sell cycle control¹⁶. There is a significant departure from Hardy Weinberg equilibrium for the Rs 17878362 allele in the BC and control groups ($p = 0.003, p = 0.004$ respectively). Another important point to be noted is that genetic polymorphism of the TP53 gene, which is located on the 17 P13.1 chromosome may lead to

inactivation of the protein and increased vulnerability to various cancers¹⁷. Particularly, this is the case for the 16 base pair repeat in the RS17878362, which alters mRNA processing decreases TP53¹⁸. The findings indicate that the non-random localization of alleles in the genes encoding tumor suppressors can become a significant marker of predisposition to cancer and genomic instability and high-risk groups.

There appears to be a statistically strong correlation between TP53 mutations and increased risk of BC more specifically, when analyzing the Rs1042522 mutations, patients processing the C allele or GC genotype have significantly increased chances of suffering from BC (P 0.001). The findings confirm the role of the Arg 72 pro mutation as an essential regulator in the process of cancer development through its influence on the degradation of TP53 protein. Bunked in turn, with regard to the RS17878362 mutation, the genotype with the highest risk is the uh TT one with the odds ratio being 3.11^{19,20}. Mutations in the pathways of tumor suppressors act as markers for assessing the risk of developing cancer. There is a common pattern among the genotypes (GC + CC and CT + TT) that shows an inclination towards the greater BC predisposition in this experiment.

This specific gene mutation shows a close link with the clinical result. Modern scientific literature between 2023 and 2025 support this claim by showing the role of TP53 mutations and promoting carcinogenesis¹⁶. Thus, it becomes imperative to recognize these high-risk genotypes for the development of customized approaches and cancer therapy.

CONCLUSION

The genotype rs17878362 and rs1042522 show a strong relationship with BC among the current Iraq samples specifically occurring that the T allele in the former or the C allele in the later wall raise the change of developing BC. The two genetic mutations probably affect the activity of the TP35 gene leading to increase genomic instability additionally significant difference from Hardy-Weinberg equilibrium toward the non-random distribution of this genetic mutation in connection with the occurrence of breast cancer finely dedicating such high-risk genetic profiles represents a useful tool in cancer genetic for early detection and targeted treatment.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Soura Alaa Hussein, Ahmed Mudher Al-Khaykanee
Drafting or Revising Critically:	Soura Alaa Hussein, Tiba A.H. Al-Rubaye
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. FTU-1401/Approval/ JSDJNEHU Dated 25.11.2023

REFERENCES

- Freihat O, Sipos D, Kovacs A. Global burden and projections of breast cancer incidence and mortality to 2050: a comprehensive analysis of GLOBOCAN data. *Frontiers Public Health* 2025;13:1622954.
- Roheel A, Khan A, Anwar F, Akbar Z, Akhtar MF, Khan I, et al Global epidemiology of breast cancer based on risk factors: a systematic review. *Frontiers Oncol* 2023; 13: 1240098.
- Li L, Doppalapudi A, Escamilla J, Karithara A, Pham C, Phillip A, et al. Precision medicine and beyond: Evolving roles of targeted therapy, immunotherapy, and artificial intelligence in oncology. *INNOSC Theranostics Pharmacol Sci* 2025;43.
- Khan AQ, Touseeq M, Rehman S, Tahir M, Ashfaq M, Jaffar E, et al. Advances in breast cancer diagnosis: a comprehensive review of imaging, biosensors, and emerging wearable technologies. *Frontiers Oncol* 2025;15:1587517.
- Marouf SS. Molecular subtypes of breast cancer: distribution and prognostic implications. *Zanco J Med Sci* 2025;470-80.
- Gurau AM, Mihalache D, Satala CB, Rață AM, Rebegea LF. HER2-low breast cancer: biological framework and determinants of HER2 Instability. *Medicina* 2026;62(2):304.
- Brücher BL. The erosion of healthcare and scientific integrity: A growing concern. *J Healthcare Leadership* 2025; 23-43.
- Wali AF, El-Tanani M, Talath S, Rabbani SA, Rangraze IR, Satyam SM, et al. Antibody - drug conjugates and beyond: next-generation targeted therapies for breast cancer. *Cancers* 2025;17(24): 3943.
- Baliakas P, Soussi T. The TP53 tumor suppressor gene: From molecular biology to clinical investigations. *J Internal Med* 2025;298(2):78-96.
- Flores-López LA, Enríquez-Flores S, García-Torres I, López-Velázquez G, Viedma-Rodríguez R, Ávalos-Rodríguez A, et al. Pancreatic β cell apoptosis in type 2 diabetes is related to post translational modifications of p53. *Molecular Med Reports* 2024;30(5): 1-16.
- Du T, Jiang T, Li X, Rahaman MM, Grzegorzec M, Li C. Prediction of TP53 mutations across female reproductive system pan-cancers using deep multimodal PET/CT radiogenomics. *Frontiers Med* 2025;12:1608652.

12. McDaniel JM, Morrissey RL, Chau GP, Su X, Ruff ZV, et al. Multi-omics analysis identifies intrinsic Trp53 driven metastatic breast cancer subtypes. *bioRxiv* 2026;2026-02.
13. Marvalim C, Datta A, Lee SC. Role of p53 in breast cancer progression: An insight into p53 targeted therapy. *Theranostics* 2023;13(4):1421.
14. Morten BC, Chiu S, Oldmeadow C, Lubinski J, Scott RJ, Avery-Kiejda KA. The intron 3 16 bp duplication polymorphism of p53 (rs17878362) is not associated with increased risk of developing triple-negative breast cancer. *Breast Cancer Res Treatment* 2019; 173(3): 727-33.
15. Sagne C, Marcel V, Amadou A, Hainaut P, Olivier M, Hall J. A meta-analysis of cancer risk associated with the TP53 intron 3 duplication polymorphism (rs17878362): geographic and tumor-specific effects. *Cell Death Dis* 2013; 4(2): e492.
16. Zhang XQ, Bai XH, Zhang HZ, He XF. Association between the p53 polymorphisms and cervical cancer risk: an updated meta-analysis. *Frontiers Oncol* 2025; 15: 1461737.
17. Dawood HH, Mohammed RK. The association between single nucleotide polymorphisms rs1042522 and rs1642785 in the TP53 gene and acute myeloid leukemia in a sample of the Baghdad/Iraq population. *Baghdad Sci J* 2024; 21(3): 10.
18. Macedo JMB, Silva AL, Pinto AC, Landeira LFL, Portari EA, Santos-Rebouças CB, et al. TP53 and p21 (CDKN1A) polymorphisms and the risk of systemic lupus erythematosus. *Adva Rheumatol* 2023; 63(1): 43.
19. Alhadheq AM, Parine NR, Shaik JP, Alhadheq R, Alkahtani S, Aljarba NH, et al. Polymorphisms of TP53 gene and its association with colorectal cancer: A case-control investigation. *J King Saud Univ Sci* 2023;35(10):102936.
20. Barnoud T, Parris JL, Murphy ME. Common genetic variants in the TP53 pathway and their impact on cancer. *J Molecular Cell Biol* 2019; 11(7): 578-85.

Prevalence of Entamoeba Gingivalis and Trichomonas Tenax among Nursing Staff and Students: Study their Relationship with Some Health Parameters

Entamoeba
Gingivalis and
Trichomonas
Tenax among
Nursing Staff and
Students

Wijdan Dhaidan Shnain Al-Abbas¹, Kawther Alqaseer¹, Ohood Aqeed Radhi²
and Nadia Habeeb Sarhan¹

ABSTRACT

Objective: To investigate the prevalence of Entamoeba Gingivalis and Trichomonas Tenax among nursing staff and students: study their relationship with some health parameters

Study Design: Descriptive study

Place and Duration of Study: This study was conducted at the Nursing College, Kufa University of Najaf City, Iraq, from 1st January 2026 to 31st March 2026.

Methods: A total of 100 participants consisting of staff and students of both males and females from 19 to 50 years were enrolled.

Results: There were 58.9% non-infected and 50%, 42.9% were shown with Trichomonas Tenax and Entamoeba Gingivalis respectively. In the 19-34-year age group, 44.4%, 41.7% were infected with Entamoeba Gingivalis and both male and female recorded 33.3% with Trichomonas Tenax. Also, in the 35-50 years' group, 55.6%, 58.3% were noticed with Entamoeba Gingivalis and 66.7%, with T. tenax for both male and female. In contrast, all participants in the non-infected group, both males and female (100%), exhibited normal amylase levels, with no cases of elevated amylase. Furthermore, for males and females with parasitic infections, no significant associations were found ($p > 0.05$), with sinusitis, pharyngitis, nasal allergy, and gingivitis in both sexes in relation with malondialdehyde.

Conclusion: No significant difference in terms of oral parasite infections within the educated population. The health parameters did not show statistically significant results related to these infections, which may be due to factors such as the severity of the infection, sample size, and the population's level of awareness. However, the presence of disturbances in the parameters (either high or low) compared to normal values suggests the need for further research. This is particularly important for all population groups, especially those with periodontitis and poor oral hygiene, as studying the relationship between these parameters and the two parasites could serve as an important indicator.

Key Words: Prevalence, Entamoeba Gingivalis, Trichomonas Tenax, Alpha-amylase, Malondialdehyde.

Citation of article: Al-Abbas WDS, Alqaseer K, Radhi OA, Sarhan NH. Prevalence of Entamoeba Gingivalis and Trichomonas Tenax among Nursing Staff and Students: Study their Relationship with Some Health Parameters. Med Forum 2026;37(9):67-71. doi:10.60110/medforum.370913.

INTRODUCTION

Entamoeba Gingivalis and Trichomonas Tenax are protozoan parasites that inhabit the human oral cavity, and possibly those of domestic animals, as noted in existing research. Since their discovery, numerous studies have shown a strong link between periodontal diseases such as caries, gingivitis, and periodontitis and

both of these protozoa¹. The prevalence of these parasites is often associated with factors like deficient oral hygiene, older age, tartar and calcareous deposits being present, and dental disease. To thrive, these protozoa rely on a variety of food sources, including epithelial cells, food fragments, microorganisms, hemocytes and white blood cells².

Although T. tenax and E. gingivalis are not usually considered harmful protozoa, their presence in the mouth could indicate underlying oral issues like gingivitis or oral trichomoniasis. However, more studies are needed to completely understand these protozoa, their role, also their connection to more risk factors, as they might be a major reason of various problems of orally health³.

Salivary alpha-amylase is basically an enzyme already present in the human saliva that is an essential role for starch digesting. Besides its role in digesting carbohydrates, salivary alpha-amylase has garnered

1. Lecturer / Assistant Dean², Department of Basic Science, Nursing College, University of Kufa, Iraq.

Correspondence: Kawther Alqaseer, Lecturer, Department of Basic Science, Nursing College, University of Kufa, Iraq.
Contact No: 07815150315
Email: kawthera.hasan@uokufa.edu.iq

Received: May, 2026
Reviewed: June-July, 2026
Accepted: August, 2026

interest for its potential involvement in various pathophysiological processes, including gums disease⁴. Amylase is a vital protein released in both saliva and serum as part of the body's immune response to periodontal disease⁵.

Oxidative stress occurs when there's an imbalance between reactive oxygen species (ROS) and antioxidants in the body, leading to cell damage⁶. Malondialdehyde is a key marker used to measure oxidative stress and antioxidant levels⁷.

Oral parasites typically emerge when a person overlooks or is unaware of existing oral health issues⁸. The present study was conducted to investigate oral parasites in an educated population, specifically the staff and students of the nursing college, and to examine certain parameters.

METHODS

This descriptive study was conducted at Nursing College, Kufa University of Najaf City, Iraq from 1st January 2026 to 31st March 2026 vide letter MDA-KU-901 /Approval/JSDJNEHU dated December 28, 2025. A total of 100 participants consisting of staff and students of both males and females from 19 to 50 years were enrolled.

At room temperature (25-28°C), the saliva was diluted using normal saline in a sterilized cup, were stained with Giemsa stain immediately after dilution. Then swabs were immersed in a solution (saliva sample with normal saline), and the swab was rolled onto a slide. A light microscope (40×-400×) was used to investigate these parasites in samples. Saliva were gathered in a quiet room at 8 AM to 12 PM. Participants were instructed to abstain from eating or drinking for at least a one hour before the examination. After this waiting period, they rinsed their mouths with water sundry times and waited 1-2 minutes until the water was clear before the saliva collection began. Then at 4,000 rpm for 10 minutes the samples were centrifuged, and stored in small Eppendorf tubes at -20°C until analysis.

Salivary malondialdehyde levels (nMol/ml) were measured using a method where malondialdehyde reacts with Thio Barbituric Acid, producing a pink-colored complex. This complex was analyzed spectrophotometrically, with absorbance readings taken at 532 nm. For salivary alpha-amylase (U/ml) analysis, the Amylase-Test Wako (Osaka, Japan) was used. The manufacturer's instructions. (FUJIFILM Wako Pure Chemical Corp). Participants aged between 19 and 50 years who do not have any chronic or systemic diseases, such as diabetes or cardiovascular issues, signs of gingivitis, sinusitis, pharyngitis, or allergies were included. Individuals with systemic conditions,

such as diabetes or heart disease, pregnant or breastfeeding women, used antibiotics or anti-inflammatory medications in the past three months, or have undergone periodontal treatment in the last six months were excluded. The data was analyzed through SPSS-26. For qualitative data, Chi-square (non-parametric test) was used. Statistically significant was considered at $P \leq 0.05$.

RESULTS

There were 73% non-infected with parasites, while 21% had *E. gingivalis* infections and 6% had *T. tenax* (Table 1). Table 2 observed the males, 58.9% were non-infected and 50% were infected with *T. tenax*, while females represented 41.1% of the non-infected group and 50% of the infected group ($P > 0.05$). In males, 42.9% were an infected with *E. gingivalis* but 57.1% were infected group ($P > 0.05$). Table 3 showed in the 19-34-year age group, among males 44.4% were recorded with *T. tenax* and 33.3% with *E. gingivalis*. On the other hand, in the 35-50years' group, 55.6% were infected with *T. tenax* and 66.7% with *E. gingivalis*, no statistically significant. Regarding females, in 19-34-year age group, 41.7% of infections were due to *T. tenax* and 33.3% to *E. gingivalis*. In the 35-50years' group, 58.3% were infected with *T. tenax* and while 66.7% with *E. gingivalis* statistically not significant ($P < 0.05$)

Table No. 1: Frequencies of parasite (N=100)

Type of infection	No.	%
Non-Infected	73	73.0
Entamoeba Gingivalis	21	21.0
Trichomonas Tenax	6	6.0

Table No. 2: Relationship between sex and Trichomonas Tenax Infection (N=79) and Entamoeba Gingivalis Infection (N=94)

Variable	Normal Level	High Level	Total	Chi-square (df)	P-value (Sig.)
Trichomonas Tenax					
Male	43 (58.9%)	3 (50%)	46 (58.2%)	0.181 (1)	0.671 (NS)
Female	30 (41.1%)	3 (50%)	33 (41.8%)		
Entamoeba Gingivalis					
Male	43 (58.9%)	9 (42.9%)	52 (55.3%)	0.699 (1)	0.192 (NS)
Female	30 (41.1%)	12 (57.1%)	42 (44.7%)		

Table No. 3: Relationship between age groups (years) and parasitic infection among males (N=12) and females (N=15)

Variable		Parasitic Infection		Total	Chi-square (df)	P-value (Sig.)
		E. gingivalis	T. tenax			
Male age (years)	19 – 34	4 (44.4%)	1 (33.3%)	5 (41.7%)	0.114 (1)	0.735 (NS)
	35 – 50	5 (55.6%)	2 (66.7%)	7 (58.3%)		
Female age (years)	19 – 34	5 (41.7%)	1 (33.3%)	6 (40%)	0.175 (1)	0.675 (NS)
	35 – 50	7 (58.3%)	2 (66.7%)	9 (60%)		

Table No. 4: Relationship between disorders and parasitic infection among males (N=12) and females (N=15)

Variable		Type of Infection		Total	Chi-square (df)	P-value (Sig.)
		E. gingivalis	T. tenax			
Male Disorders	Sinusitis	2 (22.2%)	-	2 (16.7%)	4.000 (3)	0.261 (NS)
	Pharyngitis	3 (33.3%)	-	3 (25%)		
	Nasal allergy	1 (11.1%)	-	1 (8.3%)		
	Gingivitis	3 (33.3%)	3 (100%)	6 (50%)		
Female Disorders	Sinusitis	2 (16.7%)	-	2 (13.3%)	3.281 (3)	0.350 (NS)
	Pharyngitis	2 (25%)	-	3 (20%)		
	Nasal allergy	2 (16.7%)	-	2 (13.3%)		
	Gingivitis	5 (41.7%)	3 (100%)	8 (53.3%)		

Table No. 5: Relationship between Gender and Amylase Level among parasitic infected (N=27) and non-infected (N=73) sample

Variable and Gender		Amylase Level		Total	Chi-square (df)	P-value (Sig.)
		Normal Level	High Level			
Infected	Males	10 (83.33%)	2 (16.67%)	12 (100%)	1.741 (1)	.187 (NS)
	Females	9 (60%)	6 (40%)	15 (100%)		
Uninfected	Males	43 (100%)	-	43 (100%)	N/A	
	Females	30 (100%)	-	30 (100%)		

Table No. 6: Relationship between disorder and malondialdehyde level among parasitic infected males (N=12), parasitic infected females (N=15), non-infected males (N=13), and non-infected females (N=9) sample

Disorder of Gender		Malondialdehyde levels (nMol/ml)		Total	Chi-square (df)	P-value (Sig.)
		Normal Level	High Level			
Males infected	Sinusitis	1 (50%)	1 (50%)	2 (100%)	1.714 (3)	.634 (NS)
	Nasal allergy	-	1 (100%)	1 (100%)		
	Pharyngitis	2 (33.33%)	1 (66.67%)	3 (100%)		
	Gingivitis	2 (33.33%)	4 (66.57%)	6 (100%)		
Females infected	Sinusitis	-	2 (100%)	2 (100%)	1.313 (3)	.726 (NS)
	Pharyngitis	1 (33.33%)	2 (66.67%)	3 (100%)		
	Nasal allergy	1 (50%)	1 (50%)	2 (100%)		
	Gingivitis	3 (37.50%)	5 (62.50%)	8 (100%)		
Males uninfected	Sinusitis	3 (60%)	2 (40%)	5 (100%)	21.591 (3)	<.0001 (HS)
	Pharyngitis	-	-	-		
	Nasal allergy	4 (100%)	-	4 (100%)		

	Gingivitis	2 (50%)	2 (50%)	4 (100%)		
Females uninfected	Sinusitis	1 (50%)	1 (50%)	2 (100%)	10.962 (3)	.012 (S)
	Pharyngitis	1 (50%)	1 (50%)	2 (100%)		
	Nasal allergy	3 (60%)	2 (40%)	5 (100%)		
	Gingivitis	-	-	-		

Table 4 showed among males, sinusitis, 22.2% were recorded with *E. gingivalis* and none with *T. tenax*. For those with pharyngitis, 33.3% had *E. gingivalis* and none had *T. tenax*. One case of nasal allergy (11.1%) was associated with *E. gingivalis* infection only. In contrast, males with gingivitis showed the highest association, with 33.3% infected by *E. gingivalis* and 100% by *T. tenax*, no statistically significant. In females, sinusitis and nasal allergy, 16.7% in each group were observed with *E. gingivalis*, with no cases of *T. tenax* infection. For those with pharyngitis, 25% were showed with *E. gingivalis*, again with no *T. tenax* cases. Females with gingivitis showed 41.7% infected by *E. gingivalis* and 100% by *T. tenax*. Statistically no significant ($P>0.05$) was difference.

Table 5 observed in the infected group, 16.67% of males exhibited high amylase levels compared to 40% of females, while 83.33% of males and 60% of females had normal amylase levels. no significant association. Table 6 showed in infected males 50% for both normal and high levels with sinusitis, 100% high levels with nasal allergy with no normal cases, 33.33% and 66.67% for both normal and high level of pharyngitis and gingivitis respectively. For infected females, this table observed 100 % for high level with sinusitis with no cases for normal level, 50 ,50 % normal and high level respectively with nasal allergy, 33.33% and 66.67% for both normal and high levels of pharyngitis and 37.50%,62.50% normal and high levels of gingivitis respectively. For males and females with parasitic infections, no significant associations were found ($p > 0.05$), as seen with sinusitis, pharyngitis, nasal allergy, and gingivitis in both sexes.

DISCUSSION

The mouth serves as the entry point between the outside world and the body, helping to maintain oral balance, safeguard the mouth, and prevent illness⁹.

This study observed the majority of participants were non-infected, while infections caused by *E. gingivalis* were reported in 21% of participants and *T. tenax* in 6%. The higher proportion of non-infected individuals suggests that the study sample is generally healthy. A study by Malaa et al⁸ found a correlation between lower frequencies of oral parasites and higher education levels among individuals. However, the presence of infections, even in small proportions, among this healthy, educated population underscores the importance of continued research into oral and nasal pathogens.

This study focused on the staff and students of a nursing college, selecting this sample is necessary in order to increase their knowledge and awareness, due to their educational role regarding these diseases in society, the study of Al-Abbas et al¹⁰ showed the role of the educational program in improving and enhancing nurses' knowledge about Trichomoniasis. However, the results of this study do not align with previous surveys conducted on patients attending dental departments in different provinces¹¹. In our results the percentage of these oral parasites were higher than the results of Oladokun et al¹². The higher rates of *E. gingivalis* in patients with periodontal gums compared to healthy not only indicates the presence of the disease but may also be connected to the severity of the condition and its tendency to worsen over time¹³.

The current study revealed no significant association in prevalence of *T. tenax* and *E. Gingivalis* among male and females. These results are similar to previous studies that found no gender differences in the incidence of *T. tenax* in individuals with periodontal disease¹⁴, and disagree with Gichki et al³, in contrast, incidence of *E. gingivalis* align with findings of Stensvold et al² and Oladokun et al¹². The lack of a significant difference of age on the incidence of either *T. tenax* or *E. gingivalis* in the present study is similar to findings from previous studies^{2,12}, and differ from those of Nuaimi¹⁴. While certain infections may appear to be more common in one gender, these differences often become insignificant when accounting for other factors, such as socio-economic status and access to healthcare¹⁵. This lower rate of *T. tenax* in both males and females in our study compared to some other studies may be due to the fact that *T. tenax* is more spread in individuals with poor oral hygiene and periodontitis, factors that were not established in our study.

The current study showed that different disorders were linked to varying rates of *E. gingivalis* infections in both males and females. The sample with gingivitis had a stronger association with *E. gingivalis* and *T. tenax*, with no significant association between sinusitis, nasal allergy, pharyngitis, and gingivitis. The findings of these two parasites in individuals with gingivitis align with other studies^{3,8,12,14}. Other studies confirmed that *T. tenax* is documented to have a greater occurrence in periodontitis patients than gingivitis and other groups^{3,11}.

In term of alpha-amylase levels, our findings showed in both male and female no significant difference. These results contrasting with those of Bharadwaj et al⁵ and

Al-Nuaimi et al¹⁶. The significant increase in amylase levels observed in other studies compared to our finding, may be influenced by various factors. Increased alpha-amylase level has also been linked to greater severe periodontal disease, further signifying its possible as a biomarker for severity of periodontitis¹⁷.

CONCLUSION

There is no significant difference between both infected males and females of various ages and the non-infected group in terms of oral parasite infections within the educated population. The health parameters did not show statistically significant results related to these infections.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Wijdan Dhaidan Shnain Al-Abbas, Kawther Alqaseer
Drafting or Revising Critically:	Ohood Aqeed Radhi, Nadia Habeeb Sarhan
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. MDA-KU-901/Approval/JSDJNEHU Dated 28.12.2025

REFERENCES

- Santos JO, Roldán WH. Entamoeba Gingivalis and Trichomonas Tenax: protozoa parasites living in the mouth. Arch Oral Biol 2023; 147: 105631.
- Stensvold CR, Nielsen M, Baraka V, Lood R, Fuursted K, Nielsen HV. Entamoeba Gingivalis: epidemiology, genetic diversity and association with oral microbiota signatures in North Eastern Tanzania. J Oral Microbiol 2021; 13(1): 1924598.
- Gichki AS, Moon M, Hasni MT, Ghilzi D. Oral protozoa Entamoeba Gingivalis and Trichomonas Tenax among periodontitis and gingivitis patients in periodontology department dental section Quetta. J Ayub Med Coll Abbottabad 2023; 35(4 (Suppl 1): 732-9.
- Caloian CS, Şurlin P, Ciurea A, Pop D, Caloian B, Leucuţa DC, et al. Exploring periodontal conditions, salivary markers, and systemic inflammation in patients with cardiovascular diseases. Biomedicines 2024; 12(6): 1341.
- Bharadwaj P, Kalburgi V, Harsha NSS. Comparative evaluation of alpha amylase levels in serum and saliva of healthy individuals and subjects with chronic periodontitis. Int J Appl Dent Sci 2020; 6(2): 15-8
- Sies H. Oxidative stress: concept and some practical aspects. Antioxidants 2020; 9(9): 852.
- Merino de Paz N, García-González M, Gómez-Bernal F, Quevedo-Abeledo JC, de Vera-González A, López-Mejías R, et al. Relationship between malondialdehyde serum levels and disease features in a full characterized series of 284 patients with systemic lupus erythematosus. Antioxidants 2023; 12(8): 1535.
- Malaa FAS, Abd Ali Abd Aun Jwad B, Khalis Al-Masoudi, H. Assessment of Entamoeba Gingivalis and Trichomonas Tenax in patients with chronic diseases and its correlation with some risk factors. Arch Razi Inst 2022; 77(1): 87-93.
- Lee YH, Chung SW, Auh QS, Hong SJ, Lee YA, Jung J, et al. Progress in oral microbiome related to oral and systemic diseases: an update. Diagnostics 2021; 11(7): 1283.
- Al-Abbas WDS, Mohammad RL, Alqaseer K, Radhi OA. Educational program to assess and promote knowledge of Al-Zahraa hospital nurses about trichomoniasis disease, Al-Najaf city. Wiad Lek 2025; 78(2): 316-27.
- Yaseen A, Mahafzah A, Dababseh D, Taim D, Hamdan AA, Al-Fraihat E, et al. Oral colonization by Entamoeba Gingivalis and Trichomonas Tenax: a PCR-based study in health. Human Microbiota Periodont 2022; 78280574.
- Oladokun AO, Ogboru P, Opeodu OI, Lawal AO, Falade MO. Prevalence of Entamoeba Gingivalis and Trichomonas Tenax among patients with periodontal disease attending Dental Clinic, University College Hospital, Ibadan. Trop Parasitol 2023; 13(2): 107-13.
- Vlasa A, Bud A, Lazar L, Lazar AP, Herbert A, Bud E. Association of Entamoeba Gingivalis with Periodontal disease - systematic review and meta-analysis 2024; 60(5): 736.
- Nuaimi BN. Isolation Trichomonas Tenax and study of the relationship between parasite prevalence and periodontal disease and diabetes in Nineveh Governorate, Iraq. Int J Health Sci 2022; 6(S5): 7752-61.
- Adamu VE, Amaechi AA, Ajaero CMU, Enejo NIF, Nwoke BEB, Ukaga CN. Prevalences and trends of human oral protozoan parasites. Orapuh J 2020; 1(2): e711-15.
- Al-Nuaimi BN, Al-Kattan MM, Al-Taei AF. Study the effect of Entamoeba Gingivalis in some variables of blood and serum in male patients with periodontal disease and healthy in Iraq. Ann Romanian Soci Cell Biol 2021; 25(3): 2915-26.
- Hashim NT, Fathima S, Hisham NM, Shivappa P, Magaogao MV, Islam MS, et al. Exploring salivary alpha-amylase as a biomarker in periodontitis: a comparative analysis of disease stages and clinical correlations. Curr Issues Molecular Biol 2024; 46(11): 12230-243.

Effectiveness of an Educational Program in Improving Knowledge of Pregnancy Danger Signs among Primigravida Women

Educational
Program in
Improving
Knowledge of
Pregnancy
Danger Signs

Zuhoor Jasim Ahmed¹ and Suhaila Fadhil Al-Shaikh²

ABSTRACT

Objective: To assess effectiveness of an educational program in improving knowledge of pregnancy danger signs among primigravida women.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the Primary Health Care Centers, Governorate of Karbala, Iraq from 1st September 2024 to 31st January 2025.

Methods: A quasi-experimental study was conducted at Primary Health Care Centers, Governorate of Karbala, Iraq and 98 first time pregnant mothers were enrolled.

Results: There were big improvements in post-test 69.4% as good knowledge in contrast 100% of the participants have a poor level of knowledge in pre-test stage. The high education, employed and high economic status tend to high knowledge levels ($P < 0.05$) while in post-test knowledge, no significant ($P > 0.05$) association between total knowledge score (post-test stage) and socio-demographic variables.

Conclusion: A well program of teaching can be an equally effective and more equitable intervention to improve the overall level of knowledge toward the warning signs of pregnancy in the first-time pregnant woman. Integrating evidence-based education into routine antenatal care for first-time pregnant women can promote timely care-seeking and reduce preventable maternal complications.

Key Words: Primigravida, Danger signs, Maternal, Knowledge, Obstetrics

Citation of article: Ahmed ZJ, Al-Shaikh SF. Effectiveness of an Educational Program in Improving Knowledge of Pregnancy Danger Signs among Primigravida Women. Med Forum 2026;37(9):72-76. doi:10.60110/medforum.370914.

INTRODUCTION

The state of being pregnant is characterized by increased physical stress and demands for energy. The mother's body undergoes many changes as it adapts to support the growing baby and placenta. In order for health care providers to recognize any medical problems that may arise during pregnancy, they must first learn how to identify what is normal for a woman who is expectantly pregnant. By knowing these changes, providers will have an easier time identifying medical problems when they occur.¹

¹. Master's Scholar, Department of Maternal and Neonate Nursing, College of Nursing, University of Babylon, Iraq

². Professor, Department of Gynaecology, College of Medicine, University of Babylon, Hillah, Babylon, Iraq

Correspondence: Zuhoor Jasim Ahmed, Master's Scholar, Department of Maternal and Neonate Nursing, College of Nursing, University of Babylon, Iraq.

Contact No: +09647735299881

Email: nur344.zhwr.jasem@student.uobabylon.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

Other physiological changes that occur throughout the entire course of pregnancy, from conception through the postpartum period, affect the mother's anatomy and physiology to meet the mother's changing metabolic and physical needs in order to nourish the growing fetus. In addition to affecting how the fetus grows and develops, some of these changes can also contribute to pregnancy-related complications such as preeclampsia, postpartum cardiomyopathy, and perhaps postpartum depression.²

Danger signs during pregnancy indicate that a serious complication has developed either in the mother or the fetus which is at risk of resulting in adverse health effects or even death to the mother or fetus at the time of delivery or within the initial hours after delivery.³

Pregnancy, childbirth, and postpartum have many symptoms - the severe vaginal bleeding, swollen hands or faces, blurry vision, severe abdominal pain, leaking fluid from the vagina, cramps, pelvic pressure, backache, nausea, headaches, burning or pain with peeing, decreased fetal movement, a long time in labor, convulsions, a retained placenta (where the placenta stays inside after delivery), being unconscious, and feeling very weak - that a woman and her healthcare provider should know about so they can quickly diagnose any life-threatening issues.⁴

Maternal mortality remains a serious burden in most of the developing countries such as Iraq and hence, change towards Millennium Development Goal (MDG) five has been specifically overlooked. The mortality ratio of motherhood in developing countries is 15 times higher than in developed countries.⁵ The first step to the proper and timely referral to the basic and necessary obstetric care is the knowledge of danger signs of obstetric complications.⁶

Limited knowledge on the signs of obstetric danger leads to delays in seeking and getting skilled care⁷, and the recognition of danger signs early is highly relevant to prevent delay in decision seeking to get healthcare.⁸ Although the level of health-seeking action was low in the developing countries, over three-fourths of the women were now undertaking suitable health-seeking action following the warning signs in their pregnancy.^{9,10}

This study performed in an Iraqi setting will give locally specific evidence of whether the knowledge of the primigravida women on the signs of pregnancy danger can be increased by the use of an educational program. It is hoped that the findings will enlighten the healthcare providers, planners, and policymakers about the importance of incorporating the educational interventions that are specifically chosen into regular antenatal care services. In the long-term, this would help to increase maternal awareness, prompt care-seeking, complications and maternal and neonatal health outcomes in Iraq. The hypothesis was the knowledge of primigravida women regarding pregnancy danger signs will greatly increase after the intervention through a structured educational program compared to the knowledge of these women before the intervention. Increasing awareness and knowledge of the most important warning signs is likely to make the program result in the timely health-seeking behavior and help to prevent avoidable maternal complications.

METHODS

A one-group pre-test/post-test quasi-experimental study was conducted at six Primary Health Care Centers (Al-Nasr, Al-Tahadi, Al-Malhaq, Al-Wafaa, Al-Iskan, and Al-Muwazafeen) in Karbala, Iraq, from September 1, 2024, to January 31, 2025 vide letter No. 3434/QM/Approval/JSDJNEHU dated August 6, 2024. A total of 98 primigravida women were recruited through purposive sampling. Women receiving antenatal care at the study centers who provided written informed consent were included, while those who declined participation or withdrew after the intervention were excluded.

Data were collected through face-to-face interviews using a two-part questionnaire covering sociodemographic characteristics and 43 knowledge items related to major pregnancy danger signs, including vaginal bleeding, headache, seizures, severe abdominal pain, fever, edema, reduced fetal movement,

and sickness, fatigue, or exhaustion. Correct responses were scored 1 and incorrect responses 0, giving a maximum score of 43. Knowledge was classified as poor (<22; <50%), moderate (22–32; 50%–<75%), or good (≥33; ≥75%). The questionnaire demonstrated high internal consistency (Cronbach's $\alpha=0.935$).¹¹

The study comprised assessment, implementation, and evaluation phases. Baseline sociodemographic and knowledge data were obtained before the intervention. Participants then received a single structured educational session based on Iraqi Ministry of Health guidance, delivered through interactive lectures, group discussions, flipcharts, and demonstrations. An immediate post-test was subsequently administered to assess changes in knowledge.

Data were analyzed using SPSS version 27. Pre- and post-test mean knowledge scores were compared using paired t-tests, while multiple linear regression was used to assess associations between sociodemographic characteristics and total knowledge scores. A P-value ≤0.05 was considered statistically significant.

RESULTS

Table No. 1: Sociodemographic characteristics of pregnant women (n=98)

Variables	No.	%
Age (years)		
16-20	36	36.7
21-25	40	40.8
26-30	17	17.3
31-35	5	5.1
Level of Education		
Unable to read and write	13	13.3
Read and write	8	8.2
Primary school	32	32.7
Intermediate school	5	5.1
Secondary school	19	19.4
University and above	21	21.4
Occupation status		
Housewife	58	59.2
Student	18	18.4
Employed	22	22.4
Economic status		
Insufficient	29	29.6
Sufficient to some extent	56	57.1
Sufficient	13	13.3
Residency		
Rural	46	46.9
Urban	52	53.1
Trimester of current pregnancy		
First trimester	53	54.1
Second trimester	35	35.7
Third trimester	10	10.2
Do you currently receive regular prenatal care visits		
Yes	93	94.9
No	5	5.1

Most participants were aged 21–25 years (40.8%), had primary-level education (32.7%), were housewives (59.2%), reported partially sufficient income (57.1%), lived in urban areas (53.1%), and were in the first trimester (54.1%). Regular prenatal care was reported by 94.9% of participants (Table 1).

Knowledge scores improved significantly after the educational intervention across all assessed domains ($P < 0.05$) (Table 2). Before the intervention, all participants had poor knowledge; after the intervention,

69.4% had good knowledge and 30.6% had moderate knowledge (Table 3).

On multivariable analysis, higher education ($\beta = 0.269$, $P = 0.007$), employment ($\beta = 0.393$, $P < 0.001$), and higher economic status ($\beta = 0.285$, $P = 0.004$) were significantly associated with greater pre-test knowledge. Age, residence, trimester, and regular prenatal care were not significant predictors (Table 4). No sociodemographic variable was significantly associated with post-test knowledge scores ($P > 0.05$) (Table 5).

Table No. 2: Comparison of mean scores between knowledge domains for pre- and post the test

Knowledge domains	Pre-test		Post-test		P-value
	Mean $\pm$ SD	Ass.	Mean $\pm$ SD	Ass.	
Vaginal Bleeding	0.53 $\pm$ 0.30	Moderate	0.90 $\pm$ 0.08	Good	0.008
Headache	0.50 $\pm$ 0.39	Moderate	0.79 $\pm$ 0.21	Good	0.012
Seizures (epilepsy)	0.27 $\pm$ 0.26	Poor	0.83 $\pm$ 0.12	Good	0.006
Severe abdominal pain	0.50 $\pm$ 0.35	Moderate	0.90 $\pm$ 0.10	Good	0.049
Fever	0.26 $\pm$ 0.24	Poor	0.85 $\pm$ 0.12	Good	<0.001
Edema (swelling)	0.07 $\pm$ 0.14	Poor	0.69 $\pm$ 0.15	Moderate	<0.001
Low Fetal Movement	0.31 $\pm$ 0.30	Poor	0.86 $\pm$ 0.21	Good	0.007
Feeling sick, tired and exhausted during pregnancy	0.49 $\pm$ 0.41	Poor	0.86 $\pm$ 0.17	Good	0.020
The overall knowledge	0.37 $\pm$ 0.16	Poor	0.84 $\pm$ 0.07	Good	<0.001

Mean score: Poor <50% (<0.5 mean score), Moderate 50%–<75% (0.5–<0.75 mean score), and Good $\geq$ 75% ($\geq$ 0.75 mean score)

Table No. 3: The distribution of the primigravida according to levels of knowledge

Knowledge Level	Pre-test		Post-test	
	No.	%	No.	%
Poor (<22 score)	98	100.0	-	-
Moderate (22–<33 score)	-	-	30	30.6
Good ($\geq$ 33 score)	-	-	68	69.4

Table No. 4: Multiple linear regression to identify the association between total knowledge score (pre-test stage) and socio-demographic variables

Model	Unstandardized coefficients		Standardized Coefficients	t	P-value	95.0% Confidence Interval for B	
	B	SE	Beta			Lower Bound	Upper Bound
Age (Older age)	0.101	0.072	0.140	1.389	0.168	-0.043	0.245
Level of Education (high education)	0.446	0.163	0.269	2.735	0.007	0.122	0.769
Occupation status (Employed)	1.329	0.317	0.393	4.192	<0.001	0.700	1.958
Economic Status (high)	1.252	0.430	0.285	2.910	0.004	0.398	2.107
Residency (Urban)	-0.781	0.565	-0.140	-3.383	0.170	-1.902	0.340
Trimester of Current Pregnancy (third)	-0.637	0.419	-0.153	-1.521	0.132	-1.469	0.195
Do you currently receive regular prenatal care visits	-1.075	1.289	-0.085	-0.834	0.406	-3.633	1.483

Table No. 5: Multiple linear regression to identify the association between total knowledge score (post-test stage) and socio-demographic variables

Model	Unstandardized coefficients		Standardized Coefficients	t	P-value	95.0% Confidence Interval for B	
	B	SE	Beta			Lower Bound	Upper Bound
Age (Older age)	0.636	0.533	0.121	1.193	0.236	-0.422	1.694
Level of Education (high education)	0.014	0.274	0.005	0.050	0.960	-0.530	0.558
Occupation status (Employed)	0.009	0.558	0.002	0.016	0.987	-1.099	1.117
Economic Status (high)	-0.050	0.727	-0.007	-0.068	0.946	-1.493	1.393
Residency (Urban)	-0.395	0.923	-0.044	-0.428	0.670	-2.226	1.437

Trimester of Current Pregnancy (third)	0.130	0.687	0.019	0.189	0.850	-1.233	1.493
Do you currently receive regular prenatal care visits	0.340	2.094	0.017	0.162	0.871	-3.817	4.497

DISCUSSION

The current study revealed an improvement in the level of knowledge after the educational program about danger signs during pregnancy. This result agreed with the study findings conducted in Saudia Arabia¹² which found that the increase in the knowledge about birth preparedness and pregnancy outcomes in women was highly statistically significant between preprogram and after program. In Indonesia, maternal class education is a key factor in enhancing the capacity of pregnant women to recognize early signs of danger in the carrying of the pregnancy.¹³ The notable improvement in the post-intervention scores with the help of Wilcoxon test result ($p = 0.003$) proves that the educational sessions are effective in improving the maternal knowledge and vigilance.

In Uganda, Lee et al¹⁴ reported that all items of knowledge were better after the educational program. Also, another study in Indonesia found that there was improvement in post-test knowledge about danger signs compared to pre-test knowledge.¹⁵ In Egypt, health teaching sessions do have an effect on enhancing the knowledge of women about danger signals during pregnancy.¹⁶

The current study showed that that the level of knowledge increased after the educational program is possibly due to the formality and concentration of the intervention, which presented clear and evidence-based pieces of information about danger signs of pregnancy clearly and understandably. Interactive teaching approaches and straightforward explanations must have promoted the attention, understanding and memory of the key concepts in the participants. Besides, the presentation of the program in form of the antenatal visits formed a suitable learning environment, since the information was directly associated to the current experiences in the lives of the women, enhancing motivation and interest to attend the sessions. Recall might also be reinforced by the use of immediate content reinforcement by post-testing. Moreover, the primigravida women are generally unprepared in terms of previous information, so they are more open to new information, and hence such gains can be significant after specific education.

In pre-test knowledge, the results indicated that the participants have high education, and employed, and high economic status tend to high knowledge levels at significantly level less than 0.05. The results were consistent with different countries revealed maternal and husband education level, occupation, family size, and family income were some of the predictors of mothers' knowledge of obstetrics danger signs of pregnancy¹⁷⁻²⁰ This substantial correlation between greater pre-test knowledge and the level of education,

employment, and economic status of participants ($P < 0.05$) is potentially explained by the better access to health information, resources, and healthcare services by those groups. More educated women have more chances to know the information about health and seek knowledge about pregnancy and work-related women may have more access to health education because of their employment and more social contacts. The increased economic status will facilitate easier access to antenatal care, educational resources, and online information channels that will collectively improve the perception of the signs of pregnancy danger. These have been associated with a better level of knowledge after the educational intervention which can be seen as the part of socioeconomic determinants in determining the level of maternal health awareness.

After intervention, the current outcomes revealed that the difference between total knowledge score and socio-demographic variables did not show any statistical significance ($P > 0.05$). The findings are consistent with the findings carried out in India²¹, the research was on the knowledge level on childbirth among the primigravida mothers who visited the antenatal outpatient unit of one of the chosen hospitals in Bhopal. They discovered that knowledge and socio-demographic features of the studied sample did not have any statistically significant relationship. From our point of view, the fact that the differences between post-test scores of total knowledge and sociodemographic variables ($P > 0.05$) do not demonstrate significant differences may suggest that the program was equally efficient in all groups of participants, irrespective of their age, education, occupation, economical status, and residency. The educational session was structured and standardized, which also contributed to the similarity of the information offered to all primigravida women, and this situation reduced the differences in the baseline and produced similar knowledge gains.

CONCLUSION

A well-designed program of teaching can be an equally effective and more equitable intervention to improve the overall level of knowledge toward the warning signs of pregnancy in the first-time pregnant woman. Integrating evidence-based education into routine antenatal care for first-time pregnant women can promote timely care-seeking and reduce preventable maternal complications.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zuhoor Jasim Ahmed, Suhaila Fadhil Al-Shaikh
Drafting or Revising	Zuhoor Jasim Ahmed,

Critically:	Suhaila Fadhil Al-Shaikh
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. 3434/QM/Approval/JSDJNEHU
Dated 06.08.2024

REFERENCES

1. Pei M, Gibson CJ, Grady D, Huang AJ. Addressing the impact of high placebo response in hot flash treatment trials. *Am J Obstet Gynecol* 2025;232:e78.
2. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy: review articles. *Cardiovasc J Afr* 2016;27:89-94.
3. Hussein J, Kanguru L, Astin M, Munjanja S. The effectiveness of emergency obstetric referral interventions in developing country settings: a systematic review. *PLoS Med* 2012;9:e1001264.
4. Berihun S, Asefa F, Hawulte MB. Prevalence, inequalities, and factors associated with the completion of continuum of maternal health care among reproductive age women in Ethiopia. *Haramaya University* 2023; 45.
5. Hibstu DT, Siyoum YD. Knowledge of obstetric danger signs and associated factors among pregnant women attending antenatal care at health facilities of Yirgacheffe town, Gedeo zone, Southern Ethiopia. *Arch Public Heal* 2017;75:35.
6. Dessu S, Gedamu G, Tamiso A. Assessment of knowledge on danger sign of pregnancy and associated factors among ANC attendant pregnant women in Arbaminch Town Governmental Institutions, Southern Ethiopia. *Ann Med Health Sci Res* 2018;8:64-9.
7. Gesese SS, Mersha EA, Balcha WF. Knowledge of danger signs of pregnancy and health-seeking action among pregnant women: a health facility-based cross-sectional study. *Ann Med Surg* 2023;85:1722-30.
8. Jammeh A, Sundby J, Vangen S. Barriers to emergency obstetric care services in perinatal deaths in rural Gambia: a qualitative in-depth interview study. *Int Sch Res Not* 2011;2011:981096.
9. Ameen WA, Salma KJ. Maternal risk factors for hypertensive disorders during pregnancy. *Age (Omaha)*. 2021;17:5.
10. Teshoma Regasa M, Markos J, Habte A, Upashe SP. Obstetric danger signs: knowledge, attitude, health-seeking action, and associated factors among postnatal mothers in Nekemte Town, Oromia Region, Western Ethiopia - a community-based cross-sectional study. *Obstet Gynecol Int* 2020;2020:6573153.
11. Gobran MA, Fatah MTA, Ramadan MSH, Amer GA, Rabeh MM, Elshafei RM, et al. Educational Program for Pregnant Women Regarding Obstetrics Dangerous Signs in Rural Areas. *Open J Obstet Gynecol* 2021;11:529-52.
12. Tawfik HM, Ahmed SS, Nady FS, Goda AA. Effect of educational program on promoting birth preparedness awareness, and pregnancy outcomes among primigravida women. *J Heal Care Res* 2025; 2:43-57.
13. Sabriana R, Jusni J, Hartati H, Saleh MFM. Effectiveness of antenatal class education in improving early detection of pregnancy danger signs. *J Ilm Kesehat Sandi Husada* 2025;14:462-70.
14. Lee S, Kasibante S, Emaini A, Wani S, Opii DJ, Levine LD, et al. Effectiveness of a community health worker-led education intervention on knowledge, attitude, and antenatal care attendance among pregnant women in Eastern Uganda. *J Health Popul Nutr* 2025;44:232.
15. Triguno Y, Wardana KEL, Wulandari KA. Health education on the danger signs of pregnancy in primigravida and multigravida. *J Appl Nurs Heal* 2021;3:71-6.
16. Eme S, Rania AM, Hosni N. Effect of health teaching sessions on women's knowledge regarding selected danger signals during pregnancy. *Med J Cairo Univ* 2018;86:4095-106.
17. Bililign N, Mulatu T. Knowledge of obstetric danger signs and associated factors among reproductive age women in Raya Kobo district of Ethiopia: A community based cross-sectional study. *BMC Pregnancy Childbirth* 2017;17:70.
18. kidanu Yibalih N, Dugassa W, Alemu A. Awareness of obstetric danger signs and associated factors among pregnant women who attend antenatal care in public health institutions of Adama city, oromia, Ethiopia. *Int Med Sci Res J* 2020;48.
19. Hussien A. Knowledge about pregnancy danger signs among mothers attending antenatal care in Jugal Hospital, Harari Regional State, Ethiopia, 2019. *Public Heal Indones* 2019;5:73-9.
20. Bintabara D, Mpembeni RNM, Mohamed AA. Knowledge of obstetric danger signs among recently-delivered women in Chamwino district, Tanzania: a cross-sectional study. *BMC Pregnancy Childbirth* 2017;17:276.
21. Priya KM, Podder L, Martis M. An experimental study to assess the effectiveness of video-assisted childbirth education programme on knowledge regarding childbirth among primigravida mothers attending antenatal out patient department of a selected hospital in Bhopal. *J Fam Med Prim Care* 2024;13:2604-8.

Enhancing Empathy and Caring Behavior among Nursing Interns: An Interventional Study with Gender-Based Analysis

Enhancing
Empathy and
Caring Behavior
among Nursing
Interns

Ali Hamza¹, Shima Sadat Aghahosseini² and Sarfraz Masih²

ABSTRACT

Objective: To assess the effect of a structured educational intervention on empathy and caring behavior among nursing interns and to examine gender-based differences in these outcomes

Study Design: Two group quasi-experimental study

Place and Duration of Study: This study was conducted at the University of Lahore, from 1st February 2026 to 31st March 2026.

Methods: Forty six interns were enrolled. Data were gathered using a demographic questionnaire, the Toronto Empathy Questionnaire (TEQ), and the Caring Dimension Inventory-25 (CDI-25).

Results: The median age of participants was 25 years (20–29) and the median GPA was 3.40 (2.90–3.80). Among males, empathy increased from 16.38 $\pm$ 7.939 to 55.25 $\pm$ 2.212 ($p < 0.001$), while among females it increased from 20.04 $\pm$ 6.684 to 55.09 $\pm$ 2.151 ($p = 0.034$). Caring behavior increased from 48.65 $\pm$ 5.605 to 87.87 $\pm$ 5.137 among males ($p = 0.012$) and from 48.04 $\pm$ 5.287 to 88.00 $\pm$ 5.551 among females ($p = 0.014$). Females had significantly higher baseline empathy than males ($p = 0.034$); however, no significant gender differences were observed in post-test empathy ($p = 0.183$) or caring behavior ($p = 0.190$).

Conclusion: The structured educational intervention was associated with significant improvements in empathy and caring behavior among both male and female nursing interns. Although females demonstrated higher baseline empathy, post-intervention scores became comparable between genders, suggesting that structured educational strategies may effectively enhance empathy and caring competencies regardless of gender.

Key Words: Empathy, Caring behavior, Nursing interns, Educational intervention, Gender differences.

Citation of article: Hamza A, Aghahosseini SS, Masih S. Enhancing Empathy and Caring Behavior among Nursing Interns: An Interventional Study with Gender-Based Analysis. *Med Forum* 2026;37(9):77-82. doi:10.60110/medforum.370915.

INTRODUCTION

Empathy and caring behavior are considered central attributes of professional nursing practice. Nursing is not limited to the performance of technical procedures or the management of physical symptoms; it also involves understanding patients' experiences, responding to their emotional needs, respecting their individuality, and providing care with compassion and dignity¹. Empathy enables nurses to recognize and understand the feelings, perspectives, and concerns of

patients, whereas caring behavior reflects the practical expression of compassion, respect, support, attentiveness, and commitment within the nurse-patient relationship. Together, these attributes contribute to the delivery of holistic and patient-centered care².

The importance of empathy in nursing extends beyond interpersonal communication. An empathic nurse is better able to establish therapeutic relationships, identify patients' concerns, communicate effectively, and provide emotional support during illness and recovery. Similarly, caring behavior allows nurses to translate professional values into observable actions³. Behaviors such as active listening, respectful communication, timely response to patient needs, emotional reassurance, and the preservation of privacy and dignity can influence patients' perceptions of the quality of nursing care⁴. Therefore, empathy and caring behavior are essential competencies that should be developed throughout nursing education and reinforced during clinical practice.

The nursing internship represents a particularly important period for the development of these competencies. During this transitional stage, nursing interns move from a predominantly academic learning

¹. MSN Scholar / Professor², Lahore School of Nursing, The University of Lahore, Pakistan.

Correspondence: Shima Sadat Aghahosseini, Professor, Lahore School of Nursing, The University of Lahore, Pakistan.

Contact No: 03203480789

Email: Shimaagahosseini@yahoo.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

environment into clinical settings where they are expected to apply theoretical knowledge and develop professional competence⁴. They encounter patients with complex health conditions, interact with families and multidisciplinary healthcare teams, and experience the practical realities and pressures of healthcare delivery⁵. Although clinical exposure provides valuable opportunities for professional development, factors such as heavy workload, time constraints, emotional exhaustion, and adaptation to new responsibilities may create challenges in maintaining consistent empathic and caring interactions⁶.

Educational institutions have an important responsibility to prepare nursing students and interns not only with clinical knowledge and technical competence but also with the interpersonal and humanistic skills required for professional nursing practice⁷. Traditional nursing education often emphasizes knowledge acquisition and psychomotor skills; however, the deliberate development of empathy and caring behavior may require structured educational approaches⁸. Educational interventions involving interactive discussions, reflective learning, case-based scenarios, role-playing, patient narratives, and guided clinical reflection may help nursing interns better understand patients' perspectives and incorporate empathy and caring into their daily clinical practice⁹.

A structured educational intervention can strengthen empathy and caring by helping nursing interns reflect on clinical experiences, understand patient needs, practice empathic communication, and apply caring behaviors in patient-centered care¹⁰. Gender may also influence empathy and caring through differences in professional socialization, communication, emotional expression, and clinical experiences. However, previous studies have reported inconsistent gender differences; therefore, examining gender-based variations and responses to educational interventions is important¹¹.

Despite the recognized importance of empathy and caring in nursing, there remains a need for interventions specifically designed to strengthen these competencies during the internship period¹². Nursing interns are at a critical stage of professional development, where educational support may influence the formation of long-term attitudes and behaviors toward patient care¹³. Evaluating the effectiveness of a structured educational intervention can provide evidence regarding whether targeted teaching strategies improve empathy and caring behavior beyond routine clinical exposure. Therefore, the present study purpose was to evaluate the effectiveness of a structured educational intervention in improving empathy and caring behavior among nursing interns. In addition, we will examine gender-based differences in empathy and caring behavior. The findings may contribute to the development of evidence-based educational strategies that promote compassionate, patient-centered, and

gender-responsive approaches to nursing education and clinical training.

METHODS

This two group quasi-experimental study was conducted at The University of Lahore and the data were collected from Ittefaq Hospital Trust and Government Teaching Hospital Shahdara from 1st February 2026 to 31st March 2026 letter No. UOL/IREB/26/16/03/10 dated January 14, 2026. A total of 46 BSN nursing interns aged 20-30 years, comprising 23 males and 23 females were included. The intervention focused on patient-centered care, effective communication, active listening, compassion, emotional understanding, and caring behaviors through interactive lectures, case discussions, clinical scenarios, role-play, and reflective activities. Data were collected using a demographic questionnaire, the Toronto Empathy Questionnaire (TEQ), and the Caring Dimension Inventory-25 (CDI-25). The TEQ contained 16 items with a total score ranging from 0 to 64, while the CDI-25 consisted of 25 items with scores ranging from 25 to 125. The validity and reliability of the instruments were established, with the TEQ demonstrating a content validity index (CVI) of 0.85 and Cronbach's alpha of 0.81, while the CDI-25 showed a CVI of 0.79 and Cronbach's alpha of 0.91. Data were analyzed using SPSS-26. The paired t-tests assessed pre- and post-intervention changes and independent-samples t-tests examined gender-based differences. Statistical significance was considered at $p < 0.05$.

RESULTS

There were 23 (50%) males and 23 (50%) females. Majority of participants were from urban areas 30 (65.2%), while 16 (34.8%) were from rural areas (Table 1). The median age was 25 years, with a minimum age of 20 years and a maximum age of 29 years. Regarding academic performance, the median GPA was 3.40, ranging from a minimum of 2.90 to a maximum of 3.80 (Table 2).

Table No. 1: Demographic characteristics of nursing interns (N=46)

Variable		No.	%
Gender	Male	23	50.0
	Female	23	50.0
Place of residence	Urban	30	65.2
	Rural	16	34.8

Among male participants, the mean empathy score increased from 16.38 ± 7.939 at pre-test to 55.25 ± 2.212 at post-test, while among female participants it increased from 20.04 ± 6.684 to 55.09 ± 2.151 . Similarly, the mean caring behavior score among males increased from 48.65 ± 5.605 to 87.87 ± 5.137 , whereas among

females it increased from 48.04 ± 5.287 to 88.00 ± 5.551 . The reported p-values were statistically significant ($p < 0.05$), indicating significant improvement in empathy and caring behavior among both male and female nursing interns following the intervention (Table 3).

At baseline, the mean empathy score was significantly higher among female interns (20.04 ± 6.684) than male interns (16.38 ± 7.939), with a mean difference of -3.66 ($p = 0.034$). However, no statistically significant gender difference was observed in post-test empathy scores, with males scoring 55.25 ± 2.212 and females 55.09 ± 2.151 ($p = 0.183$). Similarly, no significant gender differences were found in pre-test caring behavior scores (48.65 ± 5.605 vs. 48.04 ± 5.287 , $p = 0.168$) or post-test caring behavior scores (87.87 ± 5.137 vs. 88.00 ± 5.551 , $p = 0.190$). These findings indicate that

although females had significantly higher baseline empathy, the post-intervention scores for both empathy and caring behavior were comparable between male and female nursing interns.

Table No. 2: Descriptive statistics of nursing interns (N = 46)

Statistics	Age (years)	GPA/%
Median	25.0000	3.4000
Minimum	20.00	2.90
Maximum	29.00	3.80
Percentiles	25	3.1000
	50	3.4000
	75	3.6000

Table No. 3: Comparison of empathy and caring behavior scores of genders

Outcome Variable	Gender	Pre-test (Mean $\pm$ SD)	Post-test (Mean $\pm$ SD)	p-value
Empathy Score	Male	16.38 ± 7.939	55.25 ± 2.212	< 0.001
	Female	20.04 ± 6.684	55.09 ± 2.151	0.034
Caring Behavior Score	Male	48.65 ± 5.605	87.87 ± 5.137	0.012
	Female	48.04 ± 5.287	88.00 ± 5.551	0.014

Paired t test with $p < 0.05$

Table No. 4: Comparing of empathy and caring behavior scores between male and female nursing interns

Variable	Male (n=23) Mean $\pm$ SD	Female (n=23) Mean $\pm$ SD	Mean Difference	p-value
Empathy – Pre-test	16.38 ± 7.939	20.04 ± 6.684	-3.66	0.034
Empathy – Post-test	55.25 ± 2.212	55.09 ± 2.151	0.16	0.183
Caring Behavior – Pre-test	48.65 ± 5.605	48.04 ± 5.287	0.61	0.168
Caring Behavior – Post-test	87.87 ± 5.137	88.00 ± 5.551	-0.13	0.190

Independent t test with $p < 0.05$

DISCUSSION

The present study was demonstrated substantial improvement in both empathy and caring behavior among male and female nursing interns following the intervention. The demographic findings showed an equal distribution of male and female nursing interns, with 23 (50%) in each group, while 65.2% were from urban and 34.8% from rural areas. The participants had a median age of 25 years (20-29 years) and a median GPA of 3.40 (2.90-3.80), indicating a relatively young and academically comparable group of nursing interns at a similar stage of professional development¹⁴.

A notable finding was the marked improvement in empathy among both male and female nursing interns following the educational intervention. Among male participants, the mean empathy score increased from 16.38 ± 7.939 at pre-test to 55.25 ± 2.212 at post-test, while female participants increased from 20.04 ± 6.684 to 55.09 ± 2.151 . The paired-samples analysis indicated statistically significant improvement among both males and females ($p < 0.001$ and $p = 0.034$, respectively). This substantial increase suggests that empathy can be strengthened through structured educational

experiences rather than being regarded solely as a fixed personal characteristic¹⁵. The intervention's combination of interactive lectures, reflection, role-playing, simulation, patient narratives, and clinical discussion may have provided interns with opportunities to understand patients' perspectives and practice empathic responses in realistic situations¹⁶.

The improvement in caring behavior followed a similar pattern. Male interns demonstrated an increase from 48.65 ± 5.605 before the intervention to 87.87 ± 5.137 after the intervention, while female interns increased from 48.04 ± 5.287 to 88.00 ± 5.551 . There were statistically significant for both males ($p = 0.012$) and females ($p = 0.014$). These findings indicate that the intervention may have helped participants translate empathy-related knowledge into observable caring behaviors. This is particularly important in nursing because empathy alone may not result in improved patient care unless it is expressed through behaviors such as active listening, respectful communication, emotional support, responsiveness, and individualized attention to patients¹⁷.

The gender-based findings provide an additional important dimension to the study. At baseline, female nursing interns demonstrated significantly higher empathy than male interns, with mean scores of 20.04 ± 6.684 and 16.38 ± 7.939 , respectively ($p=0.034$). This finding suggests that gender-related differences may exist in empathic orientation before structured educational exposure. Possible explanations include differences in socialization, communication patterns, emotional expression, and culturally influenced expectations regarding caring and interpersonal relationships. However, these explanations should be interpreted cautiously because gender itself does not necessarily determine an individual's capacity for empathy¹⁸.

Importantly, the gender difference in empathy was no longer statistically significant after the intervention. Post-test empathy scores were highly comparable between male (55.25 ± 2.212) and female (55.09 ± 2.151) nursing interns ($p=0.183$). This finding is particularly meaningful because it suggests that the educational intervention may have reduced the initial gender disparity in empathy. Both groups reached similarly high post-intervention scores, indicating that structured learning activities may provide an effective means of developing empathy irrespective of gender. The finding supports the use of inclusive educational interventions rather than assuming that empathy development should be approached differently solely on the basis of gender¹⁹.

A similar pattern was observed for caring behavior. There was no statistically significant gender difference in pre-test caring behavior scores, with males scoring 48.65 ± 5.605 and females 48.04 ± 5.287 ($p=0.168$). Following the intervention, the scores remained highly comparable, with males scoring 87.87 ± 5.137 and females 88.00 ± 5.551 ($p=0.190$). The absence of significant gender differences suggests that caring behavior may be more strongly influenced by professional education, clinical experience, and nursing values than by gender alone. The nearly identical post-intervention scores further suggest that the educational intervention was similarly applicable and beneficial to both male and female nursing interns.

The reduction in gender differences after the intervention is an important finding for nursing education²⁰. Although, female participants initially demonstrated higher empathy, both male and female interns achieved comparable post-intervention empathy and caring behavior scores. This may indicate that structured opportunities for reflection, communication practice, role-playing, simulation, and exposure to patient experiences can provide a common learning environment in which interns develop similar professional competencies²¹. Such approaches may be particularly valuable in contemporary nursing education, where both male and female nurses are

expected to demonstrate the same standards of compassionate, person-centered care²⁰.

The intervention's multidimensional structure may explain the observed improvements. Rather than relying solely on didactic teaching, the program incorporated interactive lectures, self-directed learning, reflective discussions, clinical scenarios, role-playing, simulation, narrative activities. These approaches allowed participants to move from understanding the theoretical concept of empathy toward practicing empathic communication and reflecting on actual or simulated patient experiences. Experiential learning may have been particularly influential because it enabled interns to consider patients' vulnerability, emotional needs, dependence, and perspectives within realistic clinical situations²². The findings also highlight the importance of incorporating empathy and caring education into the nursing internship period. Academic nursing programs commonly emphasize knowledge and technical competencies; however, professional nursing requires the integration of cognitive, psychomotor, interpersonal, and affective competencies²³. The current findings suggest that targeted educational activities can strengthen the affective and interpersonal dimensions of nursing practice during internship. Integrating such interventions into routine clinical education may therefore help nursing interns enter professional practice with stronger communication skills and a greater orientation toward compassionate patient care.

Nevertheless, the findings should be interpreted in light of the study design and sample characteristics. The relatively small sample size was included for analysis and the use of two clinical settings may limit the generalizability of the findings to all nursing interns. In addition, the quasi-experimental design may be influenced by factors such as clinical exposure, individual motivation, and experiences occurring during the internship period. Self-reported measures may also be affected by social desirability, particularly when assessing desirable professional attributes such as empathy and caring^{24,25}. Future studies with larger samples, multiple institutions, longer follow-up periods, and objective or observational measures of caring behavior could provide stronger evidence regarding the sustainability of intervention effects.

The findings of the present study suggest that empathy and caring are developable professional competencies that can be strengthened through structured, reflective, and experiential educational strategies. Incorporating such interventions into nursing internship programs may promote more compassionate, patient-centered nursing practice.

CONCLUSION

The structured educational intervention was effective in improving empathy and caring behavior among nursing interns. Significant pre- to post-intervention

improvements were observed in both male and female participants. Although female interns had significantly higher empathy at baseline, the gender difference was no longer significant after the intervention, while caring behavior remained comparable between genders.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ali Hamza, Shima Sadat Aghahosseini
Drafting or Revising Critically:	Ali Hamza, Sarfraz Masih
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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

Source of Funding: None

Ethical Approval: No. UOL/IREB/26/16/03/10
Dated 14.01.2026

REFERENCES

- Ahmad A, Wahab H, Seman N. The effect of educational intervention on enhancing the empathy among nursing students. *Enfermería Clínica* 2020; 30:100-3.
- Atta MHR, Hammad HA-H, Elzohairy NW. The role of empathy in the relationship between emotional support and caring behavior towards patients among intern nursing students. *BMC Nurs* 2024;23(1):443.
- Badawy W, Shaban M. Educational interventions to enhance empathy in undergraduate nursing students: an integrative review. *Teaching Learning Nurs* 2025; 45.
- Baugh RF, Hoogland MA, Baugh AD. The long-term effectiveness of empathic interventions in medical education: a systematic review. *Adv Med Educ Prac* 2020:879-90.
- Cho M-K, Kim MY. Effectiveness of simulation-based interventions on empathy enhancement among nursing students: a systematic literature review and meta-analysis. *BMC Nurs* 2024; 23(1):319.
- Ding X, Wang L, Sun J, Li D-y, Zheng B-y, He SW, et al. Effectiveness of empathy clinical education for children's nursing students: A quasi-experimental study. *Nurs Educ Today* 2020; 85:104260.
- Erica B, Chiara A, Silvia C, Carmela R, Eleonora G, Michela B, et al. The impact of an interprofessional simulation-based education intervention in healthy ageing: A quasi-experimental study. *Clin Simulation Nurs* 2022;64:1-9.
- Evans W, Lisiecka D, Farrell D. Exploring the impact educational interventions have on nursing and medical students' attitudes and empathy levels towards people with disability. a systematic review. *J Intellectual Disabilities* 2026;30(2):336-55.
- Fernández-Gutiérrez M, Bas-Sarmiento P, del Pino-Chinchilla H, Poza-Méndez M, Marín-Paz AJ. Effectiveness of a multimodal intervention and the simulation flow to improve empathy and attitudes towards older adults in nursing students: a crossover randomised controlled trial. *Nurs Educ Prac* 2022; 64:103430.
- Ferri P, Rovesti S, Bonetti L, Stifani S, Panzera N, Di Lorenzo R. Evaluation of empathy among undergraduate nursing students: a three-year longitudinal study. *Acta Bio Medica: Atenei Parmensis* 2019;90(Suppl 11):98.
- Habib H, Niinuma SA, Alrefaie K, Khalaf HAA, Hani MJ, Al-Rawi ZYM, et al. Unfolding the empathic insights and tendencies among medical students of two gulf institutions using interpersonal reactivity index. *BMC Med Educ* 2024;24(1):976.
- Huang Z-e, Qiu X, Fu Y-q, Zhang A-d, Huang H, Liu J, et al. Clinical internship environment and caring behaviours among nursing students: A moderated mediation model. *Nurs Ethics* 2024; 31(8):1481-98.
- Inocian EP, Hill MB, Felicilda-Reynaldo RFD, Kelly SH, Paragas ED, Turk MT. Factors in the clinical learning environment that influence caring behaviors of undergraduate nursing students: An integrative review. *Nurs Educ Prac* 2022; 63: 103391.
- Labrague LJ, Obeidat AA. Pedagogical approaches to foster caring behaviors among nursing students: A scoping review. *Nurs Educ Today* 2025; 146: 106547.
- Menezes P, Guraya SY, Guraya SS. A systematic review of educational interventions and their impact on empathy and compassion of undergraduate medical students. *Front Med* 2021;8:758377.
- Levett-Jones T, Cant R, Lapkin S. A systematic review of the effectiveness of empathy education for undergraduate nursing students. *Nurs Educ Today* 2019;75:80-94.
- Murphy A, Griffiths P, Duffield C, Brady NM, Scott AP, Ball J, et al. Estimating the economic cost of nurse sensitive adverse events amongst patients in medical and surgical settings. *J Advan Nurs* 2021; 77(8):3379-88.
- Punshon G, Maclaine K, Trevatt P, Radford M, Shanley O, Leary A. Nursing pay by gender distribution in the UK-does the Glass Escalator still exist? *Int J Nurs Studies* 2019;93:21-9.

19. Reid-Searl K, Levett-Jones T, Lapkin S, Jakimowicz S, Hunter J, Rawlings-Anderson K. Evaluation of the 'empathic care of a vulnerable older person' e-simulation. *Nurs Educ Today* 2020; 88:104375.
20. Robinson R, Meluski K, Hellem T, Hedwig T, Hansen N, Adams J, et al.. Rapid scoping review: empathy in health sciences curriculum. *Healthcare* 2023; 55.
21. Stokken R, Flaten K, Sørheim KL, Høydalsvik T. Understanding empathy in Norwegian social work education. *Social Work Educ* 2025;44(6):1418-36.
22. Wang Y, Zhang Y, Liu M, Zhou L, Zhang J, Tao H, et al. Research on the formation of humanistic care ability in nursing students: A structural equation approach. *Nurs Educ Today* 2020;86: 104315.
23. Woo BFY, Goh YS, Zhou W. Understanding the gender gap in advanced practice nursing: A qualitative study. *J Nurs Management* 2022; 30(8): 4480-90.
24. Yang C, Zhu YL, Xia BY, Li YW, Zhang J. The effect of structured empathy education on empathy competency of undergraduate nursing interns: A quasi-experimental study. *Nurs Educ Today* 2020; 85: 104296.
25. Woo MWJ, Newman SA. The experience of transition from nursing students to newly graduated registered nurses in Singapore. *Int J Nurs Sci* 2020; 7(1):81-90.

Effect of an Instructional Antenatal Care Program on Knowledge Enhancement among Primigravida Women

Antenatal Care
Program on
Knowledge
among
Primigravida

Sumaya Abdulhakeem Hasan¹ and Bushra Jaber Omran AL-Rubayae²

ABSTRACT

Objective: To evaluate the effect of an instructional antenatal care program on knowledge enhancement among primigravida women.

Study Design: A quasi-experimental study

Place and Duration of Study: This study was conducted at the Al-Hindiya Teaching Hospital and the Obstetrics and Gynecology Teaching Hospital in Karbala from 1st June 2025 to 30th November 2025.

Methods: A total of 150 primigravida women were purposively assigned to a study group (n=75), which received a structured instructional program, and a control group (n=75), which received routine care.

Results: Pre-intervention, the majority of participants in both groups had low knowledge (study group: 85.3%, control group: 86.7%), with no significant difference between groups (p=0.551). Post-intervention, 81.3% of the study group achieved high knowledge levels compared to 0% in the control group. The study group's mean knowledge score increased significantly from 25.97±8.08 to 36.42±11.25 (p<0.001, Cohen's d =1.00), whereas the control group remained unchanged. Repeated measures ANOVA showed a significant time × group interaction (F=113.99, p<0.001, η^2 =0.44), confirming the program's effect.

Conclusion: The instructional antenatal care program significantly improved knowledge among primigravida women. Incorporating structured instructional sessions into routine antenatal care is recommended to enhance maternal knowledge and promote safe pregnancy practices.

Key Words: Antenatal care, Primigravida women, Knowledge enhancement, Instructional program

Citation of article: Hasan SA, AL-Rubayae BJO. Effect of an Instructional Antenatal Care Program on Knowledge Enhancement among Primigravida Women. Med Forum 2026;37(9):83-88. doi:10.60110/medforum.370916.

INTRODUCTION

Antenatal care (ANC) is a critical component of maternal health services, aiming to ensure the well-being of both the mother and fetus throughout pregnancy.¹ For primigravida women - those experiencing pregnancy for the first time - adequate knowledge about pregnancy, childbirth, and newborn care is essential for making informed decisions and promoting positive health outcomes.²

However, studies indicate that many primigravida women possess limited knowledge about these aspects, which can lead to increased anxiety, poor health

practices, and suboptimal utilization of healthcare services.^{3,4}

Instructional antenatal care programs have emerged as effective interventions to enhance maternal knowledge and self-efficacy. These programs typically incorporate various instructional strategies, including structured sessions, multimedia resources, and interactive discussions, to impart essential information about pregnancy and childbirth.^{5,6} Research has demonstrated that such programs significantly improve primigravida women's knowledge, attitudes, and practices regarding pregnancy and childbirth.⁷

The effectiveness of instructional antenatal care programs is further supported by studies focusing on specific aspects of maternal health instruction. The examining the impact of antenatal instructional sessions on breastfeeding knowledge, attitude, and self-efficacy found significant improvements in these areas among primigravida women who participated in the program.⁸ Similarly, interventions targeting maternal self-care practices have been shown to empower primigravida women, enhancing their confidence and ability to manage pregnancy-related challenges.⁹

The integration of instructional programs into routine antenatal care has been associated with increased compliance with ANC visits. Women with higher levels of knowledge are more likely to adhere to

¹. MSN Scholar, Department of Maternal and Neonatal Nursing, College of Nursing, University of Babylon, Iraq.

². Professor, Department of Obstetrics & Gynecology, College of Medicine, University of Babylon, Iraq

Correspondence: Sumaya Abdulhakeem Hasan, MSN Scholar, Department of Maternal and Neonatal Nursing, College of Nursing, University of Babylon, Iraq.

Contact No: +9647713887610

Email: sumayaabdulhakeem3@gmail.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

recommended ANC schedules, thereby improving maternal and fetal health outcomes.¹⁰ The importance of incorporating comprehensive instructional interventions into ANC services to address knowledge gaps and promote positive health behaviors among primigravida women.¹¹ Therefore, by providing targeted instruction on various aspects of pregnancy and childbirth, these programs empower women to make informed decisions, adopt healthy practices, and engage actively in their care. As such, integrating instructional interventions into routine ANC services is essential for improving maternal health outcomes and ensuring a positive pregnancy experience.

METHODS

A quasi-experimental study was conducted at Al-Hindiya Teaching Hospital and the Obstetrics and Gynecology Teaching Hospital, Karbala, from 1st June to 30th November 2025 letter No. 3434/QM/Approval/JSDJNEHU; May 21, 2025). A total of 150 primigravida women were recruited by purposive sampling and equally allocated to the study (n=75) and control (n=75) groups. Eligible participants were aged 18-45 years, attended antenatal clinics, understood Arabic, and provided informed consent. Women with high-risk pregnancies or severe chronic illnesses were excluded.

Data were collected using a structured questionnaire covering sociodemographic characteristics, antenatal care knowledge (20 items), and attitudes (15 items). The instrument was pilot-tested in 10 women and showed good reliability for the knowledge scale (Cronbach's $\alpha=0.84$).

The intervention consisted of four structured sessions on pregnancy care, nutrition, danger signs, and childbirth preparation, delivered through lectures, demonstrations, role-playing, and group discussions. The control group received routine antenatal care.

Assessments were performed before and after the intervention.

Data were analyzed using SPSS version 24. Independent and paired t-tests assessed between- and within-group differences, respectively. Repeated-measures ANOVA evaluated time, group, and time $\times$ group effects. Chi-square tests analyzed categorical variables. Effect sizes were reported using Cohen's d and partial η^2 , with $p<0.05$ considered statistically significant.

RESULTS

The mean age was 23.30 ± 3.2 years in the study group and 23.81 ± 3.1 years in the control group. Most participants were housewives, lived in urban areas, and had attended one to four antenatal visits. Health professionals were the main source of pregnancy-related information. Baseline sociodemographic characteristics were comparable between groups (all $p>0.05$) (Table 1). Before the intervention, low knowledge was observed in 85.3% of the study group and 86.7% of the control group, with no participants achieving high knowledge levels (Table 2). Mean baseline knowledge scores were similar between groups (25.97 ± 8.08 vs. 25.75 ± 8.11 ; $p=0.551$) (Table 3).

After the intervention, 81.3% of the study group achieved high knowledge compared with none in the control group, where 78.7% remained at a low level (Table 4). The post-test mean knowledge score was significantly higher in the study group than in the control group (36.42 ± 11.25 vs. 25.79 ± 8.13 ; $p<0.001$) (Table 5). Within the study group, knowledge scores increased significantly from 25.97 ± 8.08 to 36.42 ± 11.25 ($p<0.001$; Cohen's $d=1.00$), while no meaningful change occurred in the control group ($p=0.905$) (Table 6). Repeated-measures ANOVA showed a significant time $\times$ group interaction ($F=113.99$, $p<0.001$, partial $\eta^2=0.44$), confirming greater improvement in the intervention group (Table 7).

Table No. 1: Distribution of sociodemographics

Variable	Category	Study Group (n=75)	Control Group (n=75)	Chi-square	p-value
Age (years)	18–22	28 (37.3%)	30 (40.0%)	0.267	0.875
	23–27	35 (46.7%)	32 (42.7%)		
	28–32	12 (16%)	13 (17.3%)		
Level of Instruction	Read and Write	5 (6.7%)	6 (8%)	1.432	0.699
	Primary	12 (16%)	13 (17.3%)		
	Secondary	30 (40%)	28 (37.3%)		
	University	28 (37.3%)	28 (37.3%)		
Occupation	Housewife	40 (53.3%)	42 (56.0%)	0.267	0.876
	Employed	25 (33.3%)	23 (30.7%)		
	Student	10 (13.3%)	10 (13.3%)		
Residence	Urban	45 (60.0%)	43 (57.3%)	0.133	0.715
	Rural	30 (40.0%)	32 (42.7%)		
Frequency of	None	10 (13.3%)	12 (16.0%)	1.267	0.533

antenatal visits so far	1–2 times	20 (26.7%)	21 (28.0%)		
	3–4 times	25 (33.3%)	24 (32.0%)		
	> 4 times	20 (26.7%)	18 (24.0%)		
Source of information on pregnancy	Family/friends	30 (40.0%)	28 (37.3%)	2.134	0.545
	Social media	25 (33.3%)	27 (36.0%)		
	Health professionals	50 (66.7%)	48 (64.0%)		
	TV/Radio	15 (20.0%)	18 (24.0%)		
	Books or leaflets	10 (13.3%)	12 (16.0%)		

Table No. 2: Primigravida women's knowledge about antenatal care (pre-test) [n=100]

Weighted	Study Group			Control Group		
	No.	%	Mean±SD	No.	%	Mean±SD
Low	64	85.3	25.97±8.08	65	86.7	25.75±8.11
Moderate	11	14.7		10	13.3	
High	0	0.0		0	0.0	

Table No. 3: Primigravida women's knowledge about antenatal care between study and control groups (pre-test)

Groups	Mean±SD	t (df=148)	η^2	Sig (p-value)
Study	25.97±8.08	-0.756	0.005	0.551
Control	25.75±8.11			

Table No. 4: Primigravida women's knowledge about antenatal care (post-test)

Weighted	Study Group			Control Group		
	No.	%	Mean±SD	No.	%	Mean±SD
Low	3	4.0	36.42±11.25	59	78.7	25.79±8.13
Moderate	11	14.7		16	21.2	
High	61	81.3		-	-	

Table No. 5: Primigravida women's knowledge about antenatal care between study and control groups (post-test)

Groups	Mean±SD	t (df=148)	η^2	Sig (p-value)
Study	36.42±11.25	12.892	0.45	0.000
Control	25.79±8.13			

Table No. 6: Paired comparison of knowledge scores pre- and post-test by group

Groups	Pre-test	Post-test	Paired t (df=74)	p-value	Cohen's d (Effect Size)
Study	25.97±8.08	36.42±11.25	13.45	<0.001	1.00 (Large)
Control	25.75±8.11	25.79±8.13	0.12	0.905	0.01 (Negligible)

Table No. 7: Repeated measures ANOVA for knowledge scores

Source	SS	df	MS	F	p-value	Partial η^2
Time (Pre/Post)	3125.60	1	3125.60	145.30	<0.001**	0.50
Group (Study/Control)	21.78	1	21.78	1.01	0.317	0.01
Time × Group	2450.92	1	2450.92	113.99	<0.001**	0.44
Error	3191.15	148	21.55			

DISCUSSION

Before the intervention, the overall knowledge of primigravida women about antenatal care was predominantly low in both the study and control groups

in the present study. In the study group, 85.3% of participants demonstrated low knowledge (25.97±8.08), while only 14.7% fell within the moderate category, and none achieved high scores. A similar trend was observed in the control group, where 86.7% had low knowledge levels (25.75±8.11), 13.3% had moderate

scores, and none scored high. These results highlight the insufficient baseline awareness among primigravida women regarding essential antenatal care practices. This emphasizes a critical gap in maternal health literacy during the first pregnancy, which could negatively influence maternal preparedness, pregnancy outcomes, and neonatal well-being. Comparable findings were reported in Thailand, where a large proportion of primigravida women demonstrated inadequate knowledge of antenatal care prior to structured health instruction sessions.¹² Similarly, a study in Ethiopia, found that pregnant women, particularly those in their first pregnancy, often lacked adequate knowledge of antenatal services and pregnancy danger signs before exposure to health interventions.¹³

This study showed that mean knowledge score of the study group (25.97 ± 8.08) was nearly identical to that of the control group (25.75 ± 8.11). The independent sample t-test revealed no statistically ($p=0.551$) significant difference between the two groups, with a negligible effect size ($\eta^2 = 0.005$). This indicates that both groups began the study at an almost equivalent baseline level of knowledge, thus validating the comparability of the groups prior to the intervention. These findings are consistent with Jordan, which demonstrated no significant differences in baseline antenatal knowledge between study and control groups prior to structured prenatal instruction programs.¹⁴ Similarly, in a study conducted in Australia, pre-test assessments revealed comparable low knowledge scores between intervention and control participants, underscoring the uniformity of maternal knowledge deficits before targeted health instruction.¹⁵

The post-test results demonstrated a substantial rise in knowledge levels among primigravida women in the study group following the instructional program. A majority of participants 81.3% achieved high knowledge scores, compared with none in the pre-test, while only a minimal proportion 4% remained at the low level. In contrast, the control group remained largely unchanged, with 78.7% still in the low knowledge category and none achieving high knowledge. This dramatic improvement in the study group reflects the direct impact of the antenatal care program in enhancing maternal awareness and preparedness for pregnancy. This underscores the importance of structured, targeted instruction in overcoming baseline deficiencies in maternal knowledge. These findings are consistent with a study in Brazil, which reported that structured antenatal teaching programs significantly improved pregnant women's knowledge scores post-intervention compared with control participants who received routine care.¹⁶ Similarly, a study in Pernambuco, Brazil found that focused maternal health sessions led to a significant shift from low to high knowledge categories,

reinforcing the value of systematic instructional interventions.¹⁷

This study showed that comparison between groups further confirmed the effectiveness of the intervention. The mean knowledge score of the study group (36.42 ± 11.25) was significantly higher than that of the control group (25.79 ± 8.13), with a large effect size ($\eta^2 = 0.45$). This indicates that nearly half of the variance in post-test knowledge levels was attributable to the intervention itself, highlighting its strong impact. This finding not only establishes the success of the program but also validates the experimental design by demonstrating a clear divergence between intervention and control outcomes. These results align with a study conducted in Kwara State, Nigeria, where women exposed to antenatal health instruction interventions scored significantly higher on knowledge assessments than those who did not receive structured training.¹⁸ Likewise, in Jordan revealed that participation in antenatal instructional workshops was associated with markedly better knowledge outcomes, compared with control groups relying solely on routine care.¹⁹

The within-group analysis of knowledge scores demonstrates a highly significant improvement among primigravida women in the study group following the instructional antenatal care program. The mean knowledge score increased from 25.97 ± 8.08 at pre-test to 36.42 ± 11.25 at post-test, with a paired t-test value of 13.45 ($p < 0.001$) and a large effect size (Cohen's $d = 1.00$). This indicates that the intervention had a substantial positive impact on maternal knowledge acquisition. In contrast, the control group's mean knowledge score remained virtually unchanged, moving from 25.75 ± 8.11 to 25.79 ± 8.13 , with a negligible effect size ($d=0.01$) and a non-significant ($p > 0.905$), confirming that the observed gains were specifically attributable to the instructional program. These findings underscore the importance of structured antenatal instruction in addressing knowledge deficits commonly observed among primigravida women. Supporting this, in Brazil reported significant improvements in pregnant women's knowledge following focused antenatal instruction sessions, while control participants showed no significant change.²⁰ In Egypt, highlighted that structured maternal health instruction significantly enhanced knowledge scores among first-time mothers compared with routine care.²¹

The repeated measures ANOVA for knowledge scores revealed a significant main effect of time ($F=145.30$, $p < 0.001$, $\eta^2=0.50$), indicating that, overall, participants' knowledge about antenatal care improved from pre-test to post-test. However, the main effect of group (study vs. control) was not significant ($F=1.01$, $p=0.317$, $\eta^2=0.01$), reflecting the homogeneity of knowledge levels between groups at baseline. Crucially, the time $\times$ group interaction was significant ($F=113.99$, $p < 0.001$, $\eta^2=0.44$), demonstrating that the observed improvement

over time was significantly greater in the study group than in the control group. This confirms that the instructional antenatal care program was directly responsible for the substantial increase in knowledge among the intervention participants, rather than natural progression or repeated testing effects. These findings are consistent with international evidence emphasizing the effectiveness of structured antenatal instruction. For example, a study in Brazil found that repeated instructional sessions produced significantly greater knowledge gains in women who received structured programs compared with those exposed only to routine care.²² Similarly, in Nigeria reported that primigravida women participating in targeted antenatal workshops had greater knowledge improvement than controls, confirming the role of focused instruction in maternal health instruction.²³

CONCLUSION

The instructional antenatal care program effectively enhanced primigravida women's knowledge regarding various aspects of pregnancy care, including nutrition, recognition of danger signs, and preparation for childbirth. The intervention demonstrated a clear positive impact compared to routine antenatal care, indicating that structured, interactive instructional programs are beneficial in improving understanding and awareness among first-time pregnant women.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sumaya Abdulhakeem Hasan, Bushra Jaber Omran AL-Rubayae
Drafting or Revising Critically:	Sumaya Abdulhakeem Hasan, Bushra Jaber Omran AL-Rubayae
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. 3434/QM/Approval/JSDJNEHU Dated 21.05.2025

REFERENCES

- Alkhatib AJ, Alkhatib IA, Alkhatib MA, Alkhatib NA. The critical role of prenatal care: promoting optimal health for expectant mothers and their infants in the modern era. *PSM Vet Res* 2023;8(2):13-9.
- Omotayo R, Akintan A, Akadiri O, Bade-Adefioye A, Omotayo S. Assessment of knowledge base of primigravida about pregnancy and antenatal care at the time of booking in a South West Nigerian Tertiary Hospital. *Revista Internacional de Investigación de Medicinaria Ciencias M* 2020; 55.
- Hassan HE, Ahmed WF, Mahmoud AA. Impact of tailored educational program on primigravida anxiety and knowledge regarding minor discomforts in upper Egypt. *Int J Studies Nurs* 2020;5(1):1.
- Alatawi M, Faheem WA, Alabdulaziz H. Knowledge, attitude, and practice of primigravida women on birth preparedness. *Open Nurs J* 2021;15(1).
- Mohammed HH, Hamed AA, Afefy NA, Sherif NA, Ibrahim SM. The effectiveness of nurse-led antenatal education on maternal self-efficacy: an evidence-based approach. *BMC Nurs* 2025; 24(1):895.
- Zaman A, Fadlalmola HA, Ibrahim SE, Ismail FH, Abedelwahed HH, Ali AM, et al. The role of antenatal education on maternal self-efficacy, fear of childbirth, and birth outcomes: A systematic review and meta-analysis. *Eur J Midwifery* 2025;9:10-8332.
- Rashed MS, Nour SA, Mohamed HS, Ragab AR, Salama NS, Nasr EH. Effectiveness of childbirth education on primigravida women's knowledge about childbirth preparation. *Port Said Sci J Nurs* 2023;10(2):95-113.
- Ibrahim Hamdy Aboraiah M, A Fadel E, Emad Eldien Hussien M, Ezzat Osman N. Effect of antenatal educational sessions on primigravida women's breastfeeding knowledge, attitude, and self-efficacy. *Egypt J Health Care* 2021; 12(4):1521-34.
- Rezaei Z, Yazdanpanahi Z, Asadollahi A, Karimi M, Ghahremani L. Evaluating the impact of an educational self-care intervention on the empowerment of primigravida pregnant women covered by family medicine program in the Estahban City - an application of the Pender's health promotion model. *BMC Pregnancy Childbirth* 2025;25(1):308.
- Tanberika FS, Sansuwito TB, Hassan HC. Knowledge in enhancing antenatal care compliance and improving maternal and neonatal health outcomes. *Integrative Biomed Res* 2024;8(12):1-7.
- Odchigue G. Knowledge, attitude, and nutritional intervention on the quality of antenatal care among pregnant women. *J Interdisciplinary Perspect* 2025;3(5):263-73.
- Timsin N, Wangpitipant S. The effects of a health literacy promotion program for prevention of preterm birth among pregnant women who received antenatal care services in the hospital-based. *Women Child Nurs* 2025; 25.

13. Gesese SS, Mersha EA, Balcha WF. Knowledge of danger signs of pregnancy and health-seeking action among pregnant women: a health facility-based cross-sectional study. *Ann Med Surg* 2023;85(5):1722-30.
14. Abuidhail J, Mrayan L, Jaradat D. Evaluating effects of prenatal web-based breastfeeding education for pregnant mothers in their third trimester of pregnancy: Prospective randomized control trial. *Midwifery* 2019;69:143-9.
15. Hayes BA, Muller R, Bradley BS. Perinatal depression: a randomized controlled trial of an antenatal education intervention for primiparas. *Birth* 2001;28(1):28-35.
16. Nunes RD, Puel AG, Gomes N, Traebert J. Evaluating the effectiveness of an educative workshop for pregnant women using pre and post intervention surveys. *Cadernos de saude publica* 2019;35:e00155018.
17. Melo e Lima TR, Maia PF, Valente EP, Vezzini F, Tamburlini G. Effectiveness of an action-oriented educational intervention in ensuring long term improvement of knowledge, attitudes and practices of community health workers in maternal and infant health: a randomized controlled study. *BMC Med Educ* 2018; 18(1):224.
18. Jibril UN, Saleh GN, Badaki O, Anyebe EE, Umar A, Kamal A. Health education intervention on knowledge and accessibility of pregnant women to antenatal care services in Edu, Kwara State, Nigeria. *Int J Womens Health Reprod Sci* 2018;6(2):154-60.
19. Abu-Baker NN, Abusbaitan HA, Al-Ashram SA, Alshraifeen A. The effect of health education on dietary knowledge and practices of pregnant women in Jordan: A quasi-experimental study. *Int J Women's Health* 2021;433-43.
20. Fernandes LM, Lansky S, Oliveira BJ, Friche AA, Bozlak CT, Shaw BA. Changes in perceived knowledge about childbirth among pregnant women participating in the Senses of Birth intervention in Brazil: a cross-sectional study. *BMC Preg Childbirth* 2020;20(1):265.
21. Ahmed Thabet H, Fathy Heiba Eid M, El Saied Mahmoud Dawoud S, S Abu Almakarem A, et al. Effect of awareness program regarding motherhood preparation on first-time mothers' knowledge, practices, and emotional status. *Egypt J Health Care* 2024;15(1):103-22.
22. Silva FB, Gondim EC, Henrique NC, Fonseca LM, Mello DF. Educational intervention involving young mothers: gaining knowledge on childcare. *Acta Paulista de Enfermagem* 2018;31:32-8.
23. Akinwaare MO, Oluwatosin OA. Effect of goal-oriented prenatal education on birth preparedness, complication readiness and institutional delivery among semi-urban pregnant women in Nigeria: A quasi-experimental study. *PloS one* 2023;18(7):e0289414.

The Role of Soluble Tumor Necrosis Factor Receptor II as a Prognostic Marker in Severe COVID-19

Muayad AL-Dulaimi¹, Saleh A.H² and Mohammad Mutlag Salih²

ABSTRACT

Objective: To investigate the clinical disease characteristics and standard laboratory parameters in relation to specific cytokine biomarkers associated with COVID-19 sequelae and to elucidate their correlations with disease severity, respiratory support requirements, and patient outcomes

Study Design: Case control study

Place and Duration of Study: This study was conducted at the Diyala, Iraq, from 1st December 2024 to 30th April 2025.

Methods: Seventy four participants were enrolled and divided in two groups, patient group and control group. Patient group comprised 44 participants and control group consisting of thirty participants.

The Iraqi Ministry of Health devised the COVID-19 severity protocol, and those steps were used to grade the patients.

Results: There was a significant indicators of disease due to their relationship with COVID-19 and a high correlation between the severity of COVID-19 and inflammatory markers, including TNFR2, while blood cells, neutrophils and lymphocytes. There was a substantial rise in tumor necrosis factor receptor II levels ($P < 0.001$) between the control and sick groups.

Conclusion: The levels of tumor necrosis factor receptor II, white blood cells, neutrophils, and lymphocytes, all of which were consistent with the infection, were correlated with the severity of the COVID-19 infection.

Key Words: COVID-19, Tumor necrosis factor receptor II (TNFR2), White blood cells (WBCs), Neutrophil, Lymphocytes.

Citation of article: AL-Dulaimi M, Saleh AH, Salih MM. The Role of Soluble Tumor Necrosis Factor Receptor II as a Prognostic Marker in Severe COVID-19. Med Forum 2026;37(9):89-93. doi:10.60110/medforum.370917.

INTRODUCTION

The COVID-19 pandemic has created a challenging environment for research, society, and the medical community. The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has an unpredictable clinical course that might worsen the illness and have fatal consequences. For patients to get medicine on time, effective molecular biomarkers that can classify them based on risk must be developed¹⁻³.

The COVID-19 pandemic has led to increased morbidity, placing strain on hospital infrastructure and

resources. Developing trustworthy measures of disease severity and death is crucial for distributing healthcare funds and improving treatment results⁴.

The role of many biomarkers in predicting COVID-19 patient prognosis is now under investigation. TNFR2 is one such biomarker of interest, along with lymphocytes, neutrophils, and WBCs. This is particularly significant since in the past, people with other viral infections who had higher concentrations of the chemical experienced poorer results. The pleiotropic cytokine tumor necrosis factor (TNF) is thought to be crucial for coordinating a range of immune responses, the onset of illness, and the survival and division of cells. TNF limits the inflammatory reaction's spread after the invasion is mitigated or stopped by triggering a quick and strong immune response in the host's defense against infections⁵. Pro-inflammatory cytokines including IL-6, IL-8, chemokines, and even the substance itself are stimulated by it⁶. TNF- α promotes inflammation by attracting immune cells to the site of inflammation, such as neutrophils, monocytes, and lymphocytes⁷. Under some circumstances, Tumor necrosis factor is capable of inducing both apoptotic and necrotic cell death pathways⁸. Interestingly, macrophages and monocytes are among the main producers of Sensitivity

¹. Researcher, Department of Biology, Directorate of Education, Diyala, Iraq

². Associate Professor, Department of Biology, College of Education for Pure Sciences, Tikrit University, Salah Al Deen, Iraq

Correspondence: Muayad AL-Dulaimi, Researcher, Department of Biology, Directorate of Education, Diyala, Iraq
Contact No: 07700788972
Email: moaedchemistry@yahoo.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

to TNF is cell-type dependent. While some cells display a strong sensitivity, others, including T cell subsets, natural killer cells, dendritic cells, B cells, and astrocytes, are producers of TNF, but at lower concentrations⁹. Tumor necrosis factor is a stable form of 233 amino acid homotrimers and a type II transmembrane protein¹⁰. Multiple sources substantiate TNF's anti-inflammatory effect in the chronic phase of numerous inflammatory illnesses and infections, in addition to its prominent pro-inflammatory activity¹¹. Leukocytes, another name for WBCs are immune system cells that help defend the human body employs leukocytes as a defense mechanism against exogenous pathogens and infectious agents. Hematopoietic stem cells, residing within the bone marrow and characterized by their multipotency, serve as the origin for all leukocyte lineages. These leukocytes are distributed throughout the organism, including within the circulatory and lymphatic systems¹². White blood cell count is a significant component of total blood count because leukocyte count is frequently used as a sign of illness. In microliters of blood, a typical white cell count is often stated as 4,000–11,000 WBC.

It was discovered that, in comparison to patients with less severe illness, Patients presenting with severe or critical COVID-19 exhibited elevated markers indicative of innate immune system activation. Significantly reduced amounts of lymphocytes and their subtypes was another marker of a compromised adaptive immune response in severe or critical COVID-19 patients¹³.

METHODS

This case control study was conducted at Diyala, Iraq from 1st December 2024 to 30th April 2025 vide letter No. MEC-24044/Approval/JSDJNEHU Dated November 4, 2024. Seventy four participants were enrolled. They were divided in two groups, patient group and control group. In patient group, 44 participants were enrolled. The control group, consisting of thirty participants, displayed a healthy physiological state and was serologically negative blood test findings for antibodies against harmful bacteria, in order to check for COVID-19 IgG/IgM. They also employed the AFIAS-6 New instrument. Nasopharyngeal swabs and genetic testing were used to determine the COVID-19 status of patients. Common complaints were headaches, constipation, runny noses, sore tongues, dyspnea, and decreased appetite. Ten milliliters of venous blood were taken from the patient, and four tubes were used for coagulation, biochemical, hematological, and coagulation testing. The ELISA test sera were split into aliquots and stored. The control group, consisting of thirty participants, displayed a healthy physiological state and was serologically

negative blood test findings for antibodies against harmful bacteria, in order to check for COVID-19 IgG/IgM. They also employed the AFIAS-6 New instrument. The data was entered and analyzed through SPSS-25.

RESULTS

The serum TNFR II concentrations, expressed as mean $\pm$ standard deviation, for both the patient and control groups. The significant difference ($P < 0.05$) indicated by statistical analysis between critical and moderate patient groups, and a highly significant elevation ($P < 0.001$) in the moderate patient group compared to the control group (Table 1, Fig. 1).

Table 2 revealed no statistically significant differences ($P > 0.05$) in TNFR II levels between females and males within either the control or patient groups. However, a highly significant increase ($P < 0.001$) was observed in male patients compared to male controls, and similarly in female patients compared to female controls.

Table 3 presents the serum levels of white blood cells (WBC), neutrophils, and lymphocytes, expressed as mean $\pm$ standard deviation, for both patient and control groups. A highly significant decrease ($P < 0.001$) in lymphocyte levels and a highly significant increase ($P < 0.001$) in WBC and neutrophil levels were observed in the patient group compared to the control group.

Table No. 1: Mean TNFR II concentrations among the study groups

Biomarker	Control (N = 30)	Moderate Patients (N=42)	Critical Patients (N=18)
TNFR II	62.5 $\pm$ 3.2	91.4 $\pm$ 5.0	97.1 $\pm$ 5.7

Controls vs Moderate patients $P < 0.001$

Control vs Critical patients $P < 0.001$

Moderate patients vs critical patients $P < 0.05$

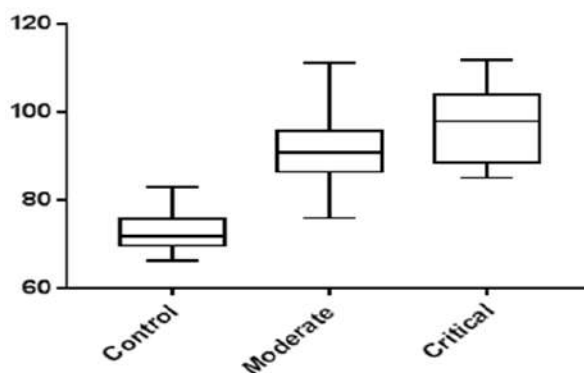


Figure No. 1: TNFR II levels in the control, moderate and critical groups

Table No. 2: Mean TNFR2 levels according to gender and age among controls and patients

Variable	Group	Control (N=30)	Patients (N=44)
Gender	Male	73.0±5.8	88.01±5.90
	Female	73.11±3.9	95.60±9.67
Age (years)	Controls (18–45)	72.07±6.22	85.40±7.52
	Patients (45–75)	73.01±4.88	99.02±8.21

Male control vs male patients $P < 0.001$

Female control vs female patients $P < 0.001$

Table No. 3: Mean leukocyte, neutrophil, and lymphocyte counts were determined for the patient and control groups

Variable	Controls	Patients	P value
White blood cells	6.83±0.23	19.0±0.34	<0.001
Lymphocyte	33.7±4.1	9.6±0.9	<0.001
Neutrophil	60.2±4.72	79.1±6.7	<0.001

DISCUSSION

In the present study, there was no significant ($P > 0.05$) difference in TNFR2 levels between age group in patients and control groups. However, a highly significant elevation ($P < 0.001$) was observed in patients compared to controls. The present results align with the findings of Gohda et al¹⁴ who reported that TNFR levels were significantly elevated in patients with severe disease, but not in those with mild to moderate disease. These differential profiles may elucidate the observed association between elevated TNFR and increased mortality in COVID-19 patients. Specifically, high TNFR2 levels correlated with mortality when relevant clinical parameters were considered. When a serious sickness started, TNFR levels sharply rose. Nonetheless, there was no difference in these biomarker levels between individuals with intermediate II and severe disease. Furthermore, TNFR levels did not change between individuals with mild-to-moderate disease, and the data showed no difference between moderate and critical patients.

Palacios et al¹⁵ shown that elevated ADAM17 expression, not overproduction, is the cause of elevated TNFR levels. Notably, whereas TNFR2 levels were the same in all severity groups, however, no significant difference was observed relative to patients with moderate disease and contrast to their findings, the severity of the illness was correlated with both TNFR levels. These variations might be partly explained by variations in the ELISA kit, variations in the clinical stratification of COVID-19, and variations in ethnicity, among other etiologies.

Prior investigations on COVID-19 have demonstrated that patients presenting with severe symptoms commonly exhibit elevated white blood cell counts upon initial admission¹⁶.

Zhu et al¹⁷ found that in COVID-19, higher total WBC levels were linked to death which, given the observed declines in lymphocyte counts, suggested that elevated neutrophils were the main factor responsible for the rise in WBCs. The inflammatory condition and cytokine storm can account for this activation mechanism¹⁸. Increases in neutrophils have also been linked to lung tissue in addition to the circulation¹⁹. Increased infiltration of immature neutrophils causes an aberrant immunological response in the lungs of individuals with severe illness²⁰.

Neutrophilia, excluding cases complicated by bacterial infection or super-infection, correlates with a hyper-inflammatory state and cytokine storm, both critical components of the COVID-19 pathogenic mechanism. Notably, neutrophils are implicated in the pathogenesis of several viral respiratory illnesses associated with acute respiratory distress syndrome²¹. A subset of individuals exhibits leukocytosis coupled with neutrophilia, which appears to correlate with increased disease severity²². An increase in circulating neutrophil counts correlates with COVID-19 disease progression; consequently, neutrophilia is recognized as a marker of severe respiratory disease and poor clinical outcome²³. Individuals presenting with severe COVID-19 infection exhibited significantly elevated leukocyte and neutrophil counts compared to those with non-severe infections²⁴.

There have been reports of an increase in neutrophils in lung tissue as well as the circulation¹⁹. The pathological immune response observed in the lungs of patients with severe COVID-19 is partially attributable to the increased infiltration of immature and/or dysfunctional neutrophils. Similar to other viral infections, SARS-CoV-2 induces the formation of neutrophil extracellular traps, which may contribute to tissue damage. In COVID-19, dysregulated neutrophil activation can exacerbate the host's inflammatory response²⁵. Neutrophils contribute significantly to the hyper-inflammatory state associated with COVID-19 pathogenesis through enhanced cytokine production and degranulation²⁶. Given the observed correlation between neutrophilia and adverse outcomes, alterations in peripheral blood or tissue neutrophil counts have been proposed as potential exclusive indicators of pulmonary injury in COVID-19 patients^{27,28}. Validation of these data may provide a rationale for targeting neutrophils and their recruitment mediators as a means to attenuate COVID-19 disease severity¹⁸.

It is widely known that various virus types modify the total number of lymphocytes as well as their subsets, suggesting a possible link between changes in the lymphocyte subset and viral pathogenic processes²⁹. A viral attachment may directly induce lymphopenia, or immunological damage from inflammatory mediators may cause it indirectly. Furthermore, lymphopenia may also result from the exudation of circulating

lymphocytes into inflammatory lung tissues. The decrease in lymphocyte subset counts in COVID-19 patients was assessed through a comprehensive analysis of twenty peer-reviewed studies that detailed both lymphocyte subset counts and disease severity¹⁸.

CONCLUSION

Tumor necrosis factor receptor II has therapeutic utility. The correlation was established between the clinical severity of CO-19 and the observed levels of white blood cell counts, neutrophil counts, lymphocyte counts and tumor necrosis factor receptor II.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muayad AL-Dulaimi, Saleh A.H
Drafting or Revising Critically:	Muayad AL-Dulaimi, Mohammad Mutlag Salih
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. MEC- 24044/Approval/JSDJNEHU Dated 04.11.2024

REFERENCES

- Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. *Crit Rev Clin Lab Sci* 2020;57(6): 389-99.
- Keddie S, Ziff O, Chou MK, Taylor RL, Heslegrave A, Garr E, et al. Laboratory biomarkers associated with COVID-19 severity and management. *Clin Immunol* 2020; 221: 108614.
- Tjendra Y, Al-Mana AF, Espejo AP, Akgun Y, Millan NC, Gomez-Fernandez C, et al. Predicting disease severity and outcome in COVID-19 patients: a review of multiple biomarkers. *Arch Pathol Lab Med* 2020; 144(12): 1465-74.
- Baloch S, Baloch MA, Zheng T, Pei X. The coronavirus disease 2019 (COVID-19) pandemic. *Tohoku J Experiment Med* 2020; 250(4): 271-8.
- Mokhtar Z, et al. Synthesis, characterization, molecular modelling, and antimicrobial evaluation of novel drug-polymer composites grafted with polyvinylpyrrolidone. *J Mol Struct* 2026;145842.
- Bonizzi G, Karin M. The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol* 2004; 25(6): 280-88.
- Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. *Int J Molecular Sci* 2019; 20(23): 6008.
- Morgan MJ, Kim YS, Liu G. TNF α and reactive oxygen species in necrotic cell death. *Cell Res* 2008; 18(3): 343-9.
- Mokhtar Z, Ali AJ, Awad SH. Preparation of new Schiff base derivatives from chitosan grafted with polyvinylpyrrolidone and study of their antimicrobial activity. *Trop J Pharm Res* 2025; 24(7):935-48.
- Al-Qazzan M, Al-Balas Q, Alnajjar B, Al-Akeedi M. Discovery of hydroxytriazole as a potential glyoxalase-I inhibitor utilizing computer-aided drug design techniques. *Sci Rep* 2026; 16(1): 9954.
- Zakharova M, Ziegler HK. Paradoxical anti-inflammatory actions of TNF- α : inhibition of IL-12 and IL-23 via TNF receptor 1 in macrophages and dendritic cells. *J Immunol* 2005; 175(8): 5024-33.
- Khan A, Butt MI, Manan A. Estimation of alanine transaminase (ALT), alkaline phosphate (ALP) and aspartate (AST) irrespective of dietary supplementation, body mass index and nature of exercise. *J Pharmaceutical Res Int* 2021; 33(28B): 33-44.
- Arum D. Evaluation of anti-hyperglycemic effect of ethanol leaf extract of annona senegalensis persoon (annonaceae) in alloxan-induced diabetic rats 2018; 45.
- Gohda T, Murakoshi M, Suzuki Y, Hiki M, Naito T, Takahashi K, et al. Circulating tumor necrosis factor receptors are associated with mortality and disease severity in COVID-19 patients. *Plos One* 2022; 17(10): e0275745.
- Palacios Y, Ruiz A, Ramón-Luing LA, Ocaña-Guzman R, Barreto-Rodriguez O, Sánchez-Monciváis A, et al. Severe COVID-19 patients show an increase in soluble TNFR1 and ADAM17, with a relationship to mortality. *Int J Mol Sci* 2021; 22(16): 8423.
- Feng X, Li S, Sun Q, Zhu J, Chen B, Xiong M, et al. Immune-inflammatory parameters in COVID-19 cases: a systematic review and meta-analysis. *Front Med* 2020; 7: 301.
- Zhu B, Feng X, Jiang C, Mi S, Yang L, Zhao Z, et al. Correlation between white blood cell count at admission and mortality in COVID-19 patients: a retrospective study. *BMC Infec Dis* 2021;21(1): 1-5.
- Palladino M. Complete blood count alterations in COVID-19 patients: A narrative review. *Biochemia Medica* 2021; 31(3): 1.
- Buja LM, Wolf DA, Zhao B, Akkanti B, McDonald M, Lelenwa L, et al. The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of

- autopsy findings from other United States cities. *Cardiovasc Pathol* 2020; 48: 107233.
20. Hassan KH, Al-Hussaniy HA, Oraibi AI, Al-Qazzan MB, et al. Thermodynamic and kinetic assessment of cobalt II adsorption using green synthesized NiO/ γ -Al₂O₃ nanoparticles. *Salud Cienc Tecnol Ser Conf* 2025; 4:1167.
21. Camp JV, Jonsson CB, A role for neutrophils in viral respiratory disease. *Front Immunol* 2017; 8: 550.
22. Al-Qazzan M, Al-Balas Q, Alnajjar B, Kasabri V, Al-Hiari Y, Zayed A, et al. Cytotoxic, anti-inflammatory, antioxidant, and anti-glyoxalase-I evaluation of chelating substances: in silico and in vitro study. *PLoS One* 2025;20(10):e0333405.
23. Zhang L, Huang B, Xia H, Fan H, Zhu M, Zhu L, et al. Retrospective analysis of clinical features in 134 coronavirus disease 2019 cases. *Epidemiol Infect* 2020; 148: e199.
24. Soraya GV, Ulhaq S. Crucial laboratory parameters in COVID-19 diagnosis and prognosis: an updated meta-analysis. *Medicina Clinica* 2020; 155(4): 143-51.
25. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. *J Exp Med* 2020; 217(6): e20200652.
26. Al-Zuhairi MI, Shakya AK, Numan NA, Al-Tameemi SJ, Al-Qazzan MB. Evaluating the activity of *Momordica charantia* (bitter melon) fruit and *Curcuma longa* (turmeric) rhizome extract as anti-hyperlipidemic agents in experimental animals. *Drug Deliv* 2025;15(3):1101-8.
27. Wang J, Li Q, Yin Y, Zhang Y, Cao Y, Lin X, et al. Excessive neutrophils and neutrophil extracellular traps in COVID-19. *Front Immunol* 2020; 11: 2063.
28. Alzghoul M, Hiary Y, Kasabri V, Hamdan I. Unique bidentate chelators of functionalized heterocyclic fluoroquinolones with dual anti-inflammatory and selective cytotoxic effects via a C7-C8 ethylene diamine bridge. *Curr Med Chem* 2026; 33.
29. Li T, Qiu Z, Zhang L, Han Y, He W, Liu Z, et al. Significant changes of peripheral T lymphocyte subsets in patients with severe acute respiratory syndrome. *J Infect Dis* 2004; 189(4): 648-51.

Assessment of TCF7L2 Gene rs7903146 Polymorphism as Type 2 Diabetes Genetic Risk Factor within Southern Iraqi Population

Assessment of
TCF7L2 Gene
rs7903146
Polymorphism as
Type 2 Diabetes

Manar Qasim Mohammed¹ and Afrodet A. Saleh¹

ABSTRACT

Objective: To investigate the association between the TCF7L2 rs7903146 polymorphism and type 2 diabetes mellitus susceptibility in a southern Iraqi population

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Al-Fayhaa Teaching Hospital, Basrah, Iraq, from 1st November 2025 to 31st March 2026.

Methods: A total of 184 participants, including 103 patients with T2DM and 81 healthy controls were enrolled. Demographic and clinical data were collected, and genotyping of rs7903146 was performed using the tetra-primer amplification refractory mutation system polymerase chain reaction (Tetra-ARMS PCR). Selected samples were further validated by deoxyribonucleic acid (DNA) sequencing and basic local alignment search tool (BLAST) analysis.

Results: The frequency of C allele was significantly higher in controls (90.1%) than in patients (55.3%), suggesting a protective effect. In contrast, the T allele was more prevalent among patients (44.7%) than controls (9.9%), indicating its role as a risk allele. Significant associations were observed between the T allele and increased susceptibility to type 2 diabetes mellitus, with both heterozygous (CT) and homozygous (TT) genotypes showing elevated risk ($p < 0.01$ and $p < 0.002$, respectively).

Conclusion: A strong association between the TCF7L2 rs7903146 polymorphism and type 2 diabetes mellitus susceptibility in the studied population.

Key Words: TCF7L2, rs7903146, Type 2 diabetes mellitus, Wnt signalling pathway.

Citation of article: Mohammed MQ, Saleh AA. Assessment of TCF7L2 Gene rs7903146 Polymorphism as Type 2 Diabetes Genetic Risk Factor within Southern Iraqi Population. Med Forum 2026;37(9):94-98. doi:10.60110/medforum.370918.

INTRODUCTION

Diabetes mellitus (DM) is known as a major induce for mortality and morbidity worldwide, influencing people across various residence, ethnicity, age, or sex¹. It classifies as modern society illness, as it represents a complicated, multifaceted metabolic condition², where the body failed to deal with sugar efficiently³. As a metabolic syndrome, diabetes has led to many related health issues for those affected and is categorized by the World Health Organization (WHO) as one of the most lethal diseases globally and a quiet threat⁴, these issues include but are not limited to diabetic

neuropathy, diabetic retinopathy, diabetic nephropathy, atherosclerosis and a cardiovascular risk (CVR) which is 2 to 3 times greater compared to those who do not have diabetes⁵. Also, individuals with diabetic Foot face a higher chance of experiencing foot ulcers, infections, and possibly amputations due to inadequate blood flow and nerve damage⁶. Diabetes caused an estimated 3.4 million deaths worldwide in 2024 and led to a staggering total health expenditure of about 1.015 trillion USD globally, with expenditures reaching 1.043 trillion USD in low- and middle-income countries⁷.

Diabetes mellitus has three main categories that can be identified. These are gestational diabetes, type 1 diabetes, and type 2 diabetes. The early phase of type 2 diabetes is characterized by insulin resistance, where cells fail to respond appropriately to insulin. As the condition progresses, there may be an insufficient supply of insulin, this disorder was previously known as "adult-onset diabetes" with clinical signs like frequent urination, excessive thirst, increased hunger, loss of weight, unclear vision, and difficulties with erectile function^{8,9}.

T2DM which makes up over 90% of diabetes incidences is influenced by a variety of interconnected

¹. MSc Scholar / Assistant Professor², Pathological Analysis Department, College of Science, University of Basrah, Iraq

Correspondence: Afrodet A. Saleh, Assistant Professor, Pathological Analysis Department, College of Science, University of Basrah, Iraq.

Contact No: 964 780 315 9713

Email: afrodet.salih@uobasrah.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

risk factors that may be partially adjustable. The primary modifiable factors include dyslipidemia, characterized by elevated levels of cholesterol and triglycerides, along with excess body fat, both of which significantly drive the advancement of the disease⁷, also many Hormones include insulin, glucagon, incretin hormones play a vital role in the onset and regulation of the diabetes because of their impact on key metabolic functions such as appetite, insulin sensitivity, fat accumulation, and the burning of energy⁸. Conversely, non-modifiable factors encompass genetic susceptibility, which includes both personal and familial diabetes history particularly in first-degree relatives as well as inherited genetic variations that increase vulnerability to T2DM^{10,11}. Numerous genes are associated with T2DM, the polymorphisms rs7903146 of the TCF7L2 is among the most vulnerable genes identified related to T2DM so far¹². Genome-wide association studies (GWAS) have pinpointed more than 100 genetic locations associated with T2D, many of which pertain to insulin production, glucose control, and body fat¹³.

METHODS

This case-control study was conducted at Al-Fayhaa Teaching Hospital, Basrah, Iraq from 1st November 2025 to 31st March 2026 vide letter No. MEC-1101-UB/Approval/JSDJNS dated October 21, 2025. A total of 184 participants, including 103 patients with T2DM and 81 healthy controls were enrolled. The case group covered 62 females and 41 males, while the control group encompassed 40 females and 41 males. The diagnosis was validated according to the standards delineated by the American Diabetes Association (ADA), which encompass random blood glucose ≥ 140 mg/dL and/or HbA1c $\geq 6.5\%$ in addition to certain clinical signs and symptoms. The cohort act as control group exhibited comparable ages and lacked any family or personal medical history indicative of diabetes or cardiovascular disease¹⁴. Validated protocols were aligned with anthropometric measurements, including height and weight, to facilitate the calculation of BMI. Subsequent of efficient human genomic DNA extraction from blood samples in respective manufacturer protocol, from the Geneaid™ DNA Isolation Kit (Blood) from Taiwan. The PCR was performed with utilizing a Tetra primers ARMS technique and the theoretical frame works suggest that the copies of target sequence approximately reached a million.

The forward inner primer: (5-TAGAGAGCTAAGCACTTTTATAGATAC-3, reverse inner primer: (C 5-CTC ATA CGG CAA TTA AAT TAT ATA A-3), and two outer primers (forward outer: 5-AAT TTT TTC ACA TGT GAA GAC ATA C-3, reverse outer primer (5-TTT ATA GCG AAG AGA TGA AAT GTA G-3). 12.5 μ l of master mix (A

GoTaq® G2 Green Master Mix, Promega, USA), 1 μ l of each primer, 3 μ l of template DNA, and 5.5 μ l of nuclease free water mixed to reach a total volume of 25 μ l, and the PCR cycles begin with an initial denaturation phase at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, then annealing at temperature of 54°C for 60 second, extension at 72°C for 120 second, and conducted with a final extension at 72°C for 5 minutes. PCR products were exposed to gel electrophoresis on 2% agarose gels, visualized under ultraviolet light, and subsequently documented. Following sequencing, the resulting raw sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to identify similar sequences in the National Center for Biotechnology Information (NCBI) database. The samples, which exhibited different genotypes were categorized as heterozygotes (TC) or (TT) subsequently. The data was analyzed through SPSS-22. The Chi-squares test or the Fisher Exact Probability test was used, as well the T-test which utilized to make comparisons between two distinct numeric variables.

RESULTS

There was a significant variation in the CC genotype for SNP rs7903146 among T2DM patients, with 28 individuals (27.2%) in the patient group, compared to 67 individuals (82.7%) in the control group. furthermore, the results for the TT genotype (Homozygous) revealed a strong elevated in prevalence in T2DM patients compare to the control group. Additionally, a significant differentiation was observed in the CT genotype (heterozygous), with T2DM patients showing 58 cases (56.3%) versus 12 cases (14.8%) in the control group. In Table (1), C allele frequencies were recorded at 114 (55.3%) for patients and 146 (90.1%) for controls, while T allele frequencies were 92 (44.7%) for patients and 16 (9.9%) for controls. The differences observed in genotype and allele frequencies between T2DM patients and the control group were highly significant ($P=0.01$) for the C allele and $P=0.002$ for the T allele (Table 1).

Genotyping of the TCF7L2 rs7903146 (C>T) polymorphism was performed using (T-ARMS-PCR). The amplified products were resolved by 2% agarose gel electrophoresis and visualized under UV illumination. Genotypes were identified as CC (homozygous wild-type), CT (heterozygous), and TT (homozygous mutant) based on the observed banding patterns (Figs. 1-4).

Accurately determining DNA fragment nucleotide sequences was performed by utilizing Sanger sequencing technique, which known for its precision and dependability. Sanger sequencing reactions were carried out on the purified PCR products for both cases and control group, then the created sequence chromatograms are examined in the national center

biotechnology information (ncbi) to find nucleotide replacements related to SNP changes (Table 2). Chromatograms was used to view and comprehend Sanger sequencing chromatograms and evaluating

overlapping or separate signal peaks, which giving a raise to assess peak quality, find nucleotide sequences, and spot heterozygous or homozygous SNP variations. Chromatogram analysis showed in figure 2.

Table No. 1: TCF7L2 rs7903146 variant exhibits strong genetic association with T2DM across multiple inheritance models

Genotype/SNP	Patients (n=103)	Control (n=81)	Chi-square	P-value	OR(C.I)
Codominant					
C/C	28 (27.2%)	67 (82.7%)	55.98	0.01	Reference
T/T	17 (16.5%)	2 (2.5%)	9.647	0.002	4.52(1.74 to 11.72)
C/T	58 (56.3%)	12 (14.8%)	32.124	0.01	5.76(3.17 to 10.47)
Dominant					
TT+CT	75 (84.3%)	14 (15.7%)	0.49	0.48	1.75(8.63-0.35)
Recessive					
CC+CT	86 (52.1%)	79 (47.9%)	45.0	0.01	0.086(0.185-0.04)
Allele					
C	114 (55.3%)	146 (90.1%)	52.77	0.01	0.19(0.12to 0.29)
T	92 (44.7%)	16 (9.9%)	52.916	0.01	5.33(3.39to 8.37)

Table No. 2: BLAST analysis of sequenced sample with homo sapiens reference sequence

Name	Scientific name	Similarity %	Accession No.
J41	Homosapiens	99.39	PQ-074218.1
J40	Homosapiens	99.38	PQ-074218.1
J39	Homosapiens	99.37	PQ-074218.1
J38	Homosapiens	99.38	PQ-074222.1
J37	Homosapiens	99.37	PQ-074222.1
J34	Homosapiens	99.13	NG-012631.1

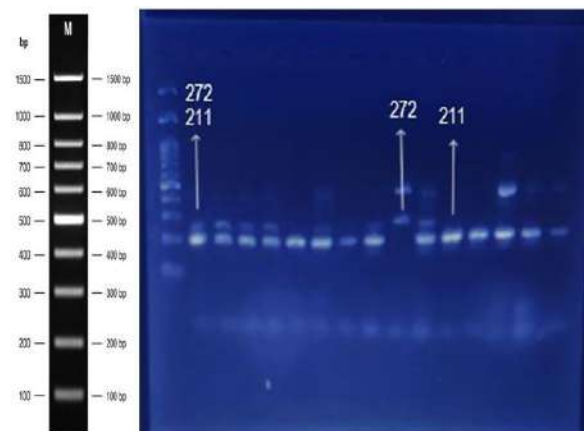


Figure No. 1: Resolution of Tetra-Primer ARMS-PCR Products for TCF7L2 rs7903146 (C>T) Genotyping by 2% Agarose Gel Electrophoresis showing Allele-specific banding patterns (272 bp and 211 bp)

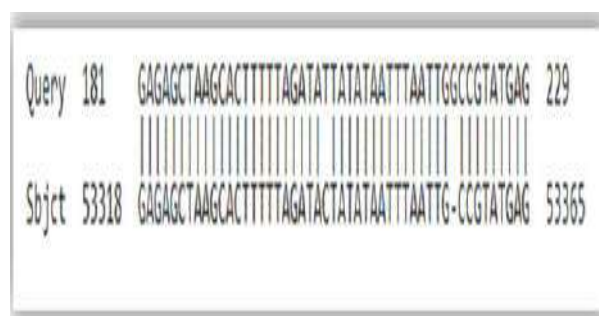


Figure No. 2: Chromatogram by Sanger sequencing (T allele a trs7903146)

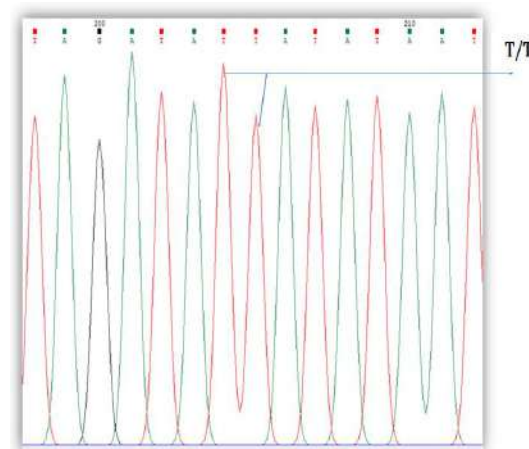


Figure No.3: Alignment of the sample sequence with the reference TCF7L2 gene sequence

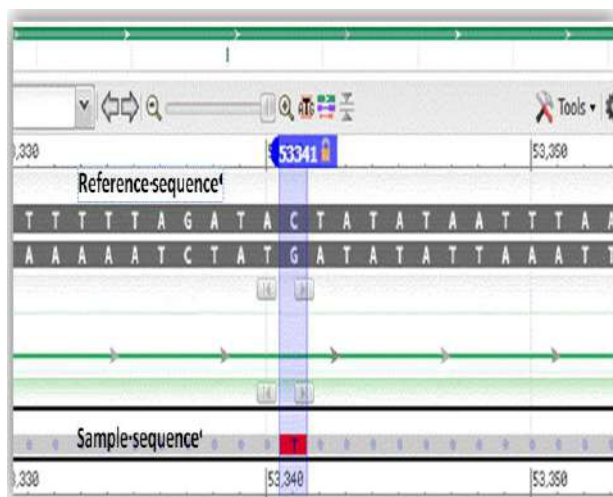


Figure No. 4: Detection of SNP variation by DNA sequence alignment analysis (C>T substitution)

DISCUSSION

The genotype distributions reflect a pronounced skew toward the CC genotype in control subjects (82.7%), whereas patients revealed a predominance of the CT genotype (56.3%), focusing on the possibility of risk allele classification and statistical precision was implemented through chi-square analyses (55.98 for codominant CC versus other categories, $P=0.01$), along with odds ratios with 95% confidence intervals to ascertain effect sizes in the present study.

T2DM, acknowledged as a worldwide metabolic disorder characterized by the impairment of glucose, protein, and lipid metabolism arising from decline tissue responsiveness to insulin, which in consequence leads to impaired insulin secretion. The key clinical manifestation of T2DM including without limitation to elevated blood glucose concentrations and insulin resistance. Despite the Genetic factors considerably effect the etiology of the disease, environmental elements involving an unhealthy diet, excess weight, lack of physical activity, and certain environmental influences, further aid in the onset of diabetes¹².

Table 1 showed a deep analysis of the TCF7L2 rs7903146 differentiation within a case and control groups and revealed a significant allelic imbalance, with more frequent TT genotype among the patients (16.5% compared to 2.5%), leading to OR of 4.52 (95% CI:1.74-11.72, $\chi^2=9.647$, $P=0.002$). While the CT heterozygous genotype is prevalent in-patient groups (56.3% vs. 14.8%), although this is reflected in contrasting recessive patterns (CC-CT versus TT: OR=5.76 CI: 3.17-10.47, $P=0.01$), evidenced the affected by deficiencies in beta-cell in cretin and disturbances in the Wnt/ β -catenin pathway. In dominant models (TT-CT vs. CC), findings were considered non-significant (OR=1.75 [0.35-8.63], $P=0.48$). coupled with the above, allelic analysis explicitly identifies the

T allele as a risk allele (44.7% in cases versus 9.9% in controls; OR=5.33 [3.39–8.37], $\chi^2=52.916$, $P=0.01$).

Correspondingly, Iranian study conducted by Amoli et al¹⁵ demonstrated a robust linked between the rs7903146 T allele and T2DM onset. Their study result given a prove support to the herein conclusions and focused on the crucial role of this SNP as a significant genetic predictor of T2DM. Along the same trajectory, a meta-analysis carried out by Wang and others¹⁶ in China in 2013 evidenced that within the Chinese population, the rs7903146 T allele is conspicuous as one of the most influential genetic contributors to T2DM susceptibility. They noticed that both the CT and TT genotypes strongly linked with T2DM risk, closely aligned with the findings of the current study. Moreover, Mustafa and Younus¹⁷, Iraqi researchers conducted a study in 2021 among the Kurdish people in Erbil, and revealed that, the CT and TT genotypes had significant relation with T2DM onset tendency. They demonstrated that patients with T allele had a significantly elevated risk of developing T2DM, agree with the current outcome, which shows that the T allele founded in 44.7% of patients and purely 9.9% of healthy participants. Similarly, Chaudhary and colleagues¹⁸ in Kuwait in 2024 study the TCF7L2 polymorphisms and demonstrated significant association with increased T2DM susceptibility, especially among participants carrying alleles linked β -cell dysfunctionality, insulin production and activity. Their findings evidenced the existing concept that the T allele is major contributor to both disease susceptibility an dim paired metabolic regulation In same direction, Nouri and Altaee¹⁹ in Iraq during 2025 revealed a strong relation between the TCF7L2 rs7903146 polymorphism and T2DM onset, with the T allele being more frequencies in case cohort. Their outcomes reinforcement the existed findings, particular in regarding to prevalence of CT and TT genotypes in patients and the protective function of the C allele and considerable concordances with previous studies from Iraq, Iran, Kuwait, and China, thus supporting the significant role of the TCF7L2 rs7903146 polymorphism in increasing T2DM susceptibility.

CONCLUSION

Overall, the TCF7L2 rs7903146 differentiation considered as strongest genetic factor play a robust role in glucose homeostasis within patient group, with T allele revealed a significant odds ratio of 5.33 (95% CI:3.39–8.37). -his allele affected beta-cell function by negative regulatory and inducing in cretin resistance. This reveals a critical requirement for the necessity of genetic mapping configurations in Arabian genetic and scapes to establish personalized endocrinological defenses against the T2DM susceptibility.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Manar Qasim Mohammed, Afrodet A. Saleh
Drafting or Revising Critically:	Manar Qasim Mohammed, Afrodet A. Saleh
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. MEC-1101_UB/
Approval/JSDJNS Dated 21.10.2025

REFERENCES

- Yameny AA. Diabetes mellitus overview. *J Biosci Appl Res* 2024; 10(3): 641-5).
- Al-odinan MS, Aljefree NM, Almoraie NM, Bakarman MA, Alhadrami HA, Shatwan IM. Interaction between the TCF7L2 gene and dietary intake on metabolic syndrome risk factors among Saudi Arabian adults. *Frontiers Nutr* 2025; 12.
- Salunke DD, Aher AA, Thitame SN. Endocrine disorder: advance in understanding diabetes and obesity a review. *J Pharmacy Bioallied Sci* 2025; 17(Suppl 1): S32-5.
- Abbas AT, Saleh AA, Abbas HJ. Association between the INSR gene rs1799817 polymorphism and type 2 diabetes mellitus in Iraqi patients. *J Biosci Appl Res* 2025; 11(4): 1325-34.
- Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. In *Health Science Reports*. John Wiley and Sons Inc. 2024; 7(3).
- Yang YH, Wen R, Yang N, Zhang TN, Liu CF. Roles of protein post-translational modifications in glucose and lipid metabolism: mechanisms and perspectives. In: *Molecular Medicine BioMed Central Ltd* 2023; 29(1).
- Sabah Abdulridha Budair A, Ismail Al Hamdi K, Ali Mansour A. Non-Infectious Dermatological Manifestations among Patients with Diabetes Mellitus in Basrah, Iraq. *Arch Razi Inst* 2022; 77(1): 441-9.
- Abdulsaid RT, Jabbar AS, Akar TK. Impact of type 2 diabetes mellitus on pulmonary function tests. *Iraqi J Pharmaceutical Sci* 2023; 32(2): 25-32.
- Roman G, Pantea-Stoian A. Cardiovascular risk/disease in type 2 diabetes mellitus. In: *Type 2 diabetes - From Pathophysiology to Cyber Systems*. Intech Open 2021;45.
- International Diabetes Federation. *IDF Diabetes Atlas*. 5th ed. Brussels, Belgium: International Diabetes Federation 2011; 48.
- Ansari S. Therapeutic effect of "QursZiabetusKhas, Habb-e-ZaharMohra, Habb-e-Banafs ha" in Diabetes Mellitus - a case report. *Cell Med* 2025; 48:55
- Olorunfemi BO, Ogunde AO, Almogren A, Adeniyi AE, Ajagbe SA, et al. Efficient diagnosis of diabetes mellitus using an improved ensemble method. *Sci Reports* 2025; 15(1):
- Młynarska E, Czarnik W, Dzieza N, Jędraszak W, Majchrowicz G, Prusinowski F, et al. Type 2 diabetes mellitus: new pathogenetic mechanisms, treatment and the most important complications. *Int J Molecular Sci* 2025; 26(3).
- White J, Jago R, Thompson JL. Dietary risk factors for the development of insulin resistance in adolescent girls: a 3-year prospective study. *Public Health Nutr* 2014;17(2):361-8.
- Amoli MM, Amiri P, Tavakkoly-Bazzaz J, Charmchi E, Hafeziyeh J, et al. Replication of TCF7L2 rs7903146 association with type 2 diabetes in an Iranian population. *Genetics Molecular Biol* 2010; 33:
- Wang J, Hu F, Feng T, Zhao J, Yin L, Li L, et al. Meta-analysis of associations between TCF7L2 polymorphisms and risk of type 2 diabetes mellitus in the Chinese population. *BMC Med Genetics* 2013; 14(1).
- Mustafa S, Younus D. Association of TCF7L2 rs7903146 Polymorphism with the Risk of Type 2 Diabetes Mellitus (T2DM) AmongKurdish Population in Erbil Province, Iraq. *Indian J Clin Biochem* 2021; 36(3): 312-8.
- Chaudhary N, Alawadhi F, Al-Serri A, Al-Temaimi R. TCF7L2 and FTO Polymorphisms are associated with type 2 diabetes mellitus risk in Kuwait. *Med Principles Prac* 2024; 33(2): 157-63.
- Nouri MA, Altaee MF. Association of Tcf7l2 Gene Expression and Polymorphisms with Type 2 Diabetes Mellitus Incidence in A Sample of Iraqi Population. *Iraqi J Sci* 2025; 66(11): 4937-951.

Study of Communication Skills among Psychiatric Nurses in Psychiatric Care Units

Communication
Skills among
Psychiatric
Nurses

Hawraa Haider AI-Zghaibi¹ and Muna Abdulwahab Khaleel¹

ABSTRACT

Objective: Assessing the level of communication skills among nurses working in psychiatric care units and to determine their association with selected sociodemographic variables

Study Design: Descriptive cross-sectional study

Place and Duration of Study: This study was conducted at the Ibn Al-Rushd and Al-Rashaad Teaching Psychiatric Hospitals in Baghdad Governorate, Iraq, from 15th December 2025 to 30th April 2026.

Methods: A total of 134 nurses were selected and among them 12 nurses participated in the pilot study and 122 nurses were included in the main study sample.

Results: Majority of nurses demonstrated a low level of communication skills 57.4%, while 31.1% demonstrated a moderate level, and only 11.5% demonstrated a high level. Statistically significant ($p < 0.05$) relationships were also found between communication skills and age, educational level, years of experience and participation in training programs. No significant differences ($P > 0.05$) were observed in relation to gender, place of residence and marital status.

Conclusion: Most psychiatric nurses lack adequate communication skills, which can negatively impact patient care and therapeutic relationships. Improving these skills is essential to enhancing the quality of psychiatric nursing practice.

Key Words: Communication Skills, Psychiatric nurses, Psychiatric care units.

Citation of article: AI-Zghaibi HH, Khaleel MA. Study of Communication Skills among Psychiatric Nurses in Psychiatric Care Units. Med Forum 2026;37(9):99-104. doi:10.60110/medforum.370919.

INTRODUCTION

Communication skills are essential for effective nursing practice, especially in psychiatric settings where therapeutic interaction is essential to patient care. In order to evaluate patients' mental health, understand their needs, and build therapeutic relationships based on trust, psychiatric nurses mainly rely on communication. Nurses can provide safe, patient-centered care and enhance overall clinical outcomes by communicating effectively¹. Communication skills refer to an individual's ability to understand others effectively through verbal and nonverbal communication, and to express oneself clearly. These skills enable nurses address complex problems, analyze situations logically and effectively, and improve their problem-solving abilities and professional engagement². Psychiatric

settings present particular challenges that make Effective communication more difficult and crucial. Individuals suffering from mental illnesses may find it difficult to communicate their ideas, comprehend information, or engage in social interactions. To guarantee successful interaction and promote patient recovery, psychiatric nurses must employ specific communication strategies like empathy, active listening, and therapeutic communication³. Communication between nurses and patients is essential for strengthening the therapeutic relationship, improving patients understanding of their treatment, and enhancing their overall healthcare experience. Strong communication skills enable healthcare professionals to better interpret clinical symptoms and their patients' emotional and psychological needs⁴.

In mental health settings, accurate assessment of psychiatric symptoms and better patient outcomes depend on effective communication¹. Communication is regarded as a crucial therapeutic tool in psychiatric nursing that promotes patient recovery and increases treatment engagement. Strong communication skills enable nurses to effectively manage complex patient conditions and offer emotional support⁵. Additionally, it has been demonstrated that enhancing psychiatric nurses' communication skills through education and training greatly improves clinical performance and fortifies therapeutic relationships between nurses and patients⁶. Although communication skills are

¹. MSN Scholar / Professor², Department of Mental & Psychiatric Nursing, College of Nursing, University of Babylon, Hilla City, Iraq.

Correspondence: Hawraa Haider AI-Zghaibi, MSN Scholar, Department of Mental & Psychiatric Nursing, College of Nursing, University of Babylon, Hilla City, Iraq.
Contact No: 07845459996
Email: nur262.hawraa.haider@student.uobabylon.edu.iq

Received: May, 2026
Reviewed: June-July, 2026
Accepted: August, 2026

acknowledged as important in nursing practice, previous research has primarily examined nurses' perceptions of communication rather than directly assessing communication skills. Furthermore, not enough research has been done on how sociodemographic characteristics affect psychiatric nurses' communication abilities¹. This study is significant because it emphasizes how crucial communication skills are for psychiatric nurses to provide safe and efficient patient care.

METHODS

This descriptive cross-sectional study was conducted at Ibn Al-Rushd and Al-Rashaad Teaching Psychiatric Hospitals in Baghdad Governorate, Iraq from 15th December 2025 to 30th April 2026 letter No. 96 date 2nd December, 2025 nurses working in psychiatric hospitals including were enrolled. A total of 134 nurses were selected to participate and among them 12 nurses participated in the pilot study and 122 nurses were enrolled as main study sample. Inclusion criteria included nurses currently working in psychiatric care units with at least one year of experience and willingness to participate. The exclusion criteria included nurses working in departments other than psychiatric units, those with less than one year of experience in psychiatric units, and administrative nurses without direct patient contact.

Data were collected using a structured self-administered questionnaire consisting of two parts: socio-demographic characteristics and a communication skills questionnaire. Communication skills were assessed using a 23 items structured questionnaire assessing nurses' communication abilities with patients. Responses to the were rated on a three-point likert scale :(Frequently =3, Occasionally= 2, Never= 1), with higher scores indicating better communication skills. The questionnaire required approximately 20–25 minutes to complete. Reliability testing demonstrated good internal consistency with a Cronbach's alpha level (0.86). The normality of data distribution was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests.

RESULTS

The majority of the samples 41% were between 25 and 34 years, followed by 35 to 44 years 27.9%, less than 25 years 19.7%, and 45 years and above 11.5%, with a mean age of 31.6±7.2 years. There were 63.9% females and 36.1% males. More than half of the nurses 55.7% resided. Most of the nurses 55.7% were married, while 31.1% were single, 8.2% divorced, 3.3% widowed and 1.7% separated. 8.2% held a master's degree, 4.9% had preparatory education, and 1.6% had a PhD. There were 41.0% had 6-10 years, 36.1% had 1-5 years and 22.9% had >10 years' experience. Nearly half the nurses 47.5% had taken part in mental health training courses and 52.5% had not attended any training. Twenty eight

(22.9%) had completed one course, 18 (14.8%) had completed two courses and 12 (9.8%) had completed three or more courses (Table 1).

Table No. 1: Sociodemographic information of the nurses (n=122)

Variable	No.	%
Age (years)		
< 25	24	19.7
25 – 34	50	41.0
35 – 44	34	27.9
> 45	14	11.5
Gender		
Male	44	36.1
Female	78	63.9
Residence		
Urban	68	55.7
Rural	54	44.3
Marital status		
Single	38	31.1
Married	68	55.7
Divorced	10	8.2
Widowed	4	3.3
Separated	2	1.7
Educational level		
Preparatory	6	4.9
Diploma	28	23.0
Bachelor degree	76	62.3
Master degree	10	8.2
PhD	2	1.6
Years of experience in psychiatric hospitals		
1–5	44	36.1
6–10	50	41.0
> 10	28	22.9
Participation in mental health training courses		
Yes	58	47.5
No	64	52.5
Number of training courses attended		
One	28	22.9
Two	18	14.8
Three or more	12	9.8

The findings show that many nurses scored low on several items, especially those related to assertiveness and sharing information. More than 50 percent of the respondents reported low scores namely "I prefer not to tell or share bad information" (65.6%), The organisation lacked something in this aspect either because they were not confident enough or the other reason. In contrast, the other items associated with the patient's rights, along with attentiveness, and understanding emotions, achieved a moderate score category. Like "I respect my patient's freedom of expression" (21.3% agreed, mean score 1.77), or "I give information in understandable terms" (21.4% agreed, mean score 1.74). The overall mean scores for the items were around 1.47 and 1.77. Many of the items are found to be low (L) while few are at moderate (M) level. This means that the nurses do demonstrate some

ability to respect for the autonomy or prescriptive need of the patient but many do so with low confidence, clarity and consistency in their communication skills (Table 2).

Majority of nurses (57.4%) scored in the low communication skills category (mean score 28.77 ± 5.78), suggesting limited ability to communicate effectively with patients. About one-third of the sample (31.1%) demonstrated moderate communication skills, while a small proportion (11.5%) achieved high communication skills. These results reflect that most nurses face challenges in confidently conveying information, using supportive verbal expression, and maintaining clarity and structure in their interactions with patients (Table 3).

In Table 4 showed the statistical differences in overall communication skills scores among nurses according to their socio-demographic characteristics. The findings indicate that age, educational level, years of experience, and participation in mental health training course were significantly ($p < 0.05$) associated with communication skills. Specifically, older nurses, those with higher educational qualifications, and nurses with more years of experience tended to have higher communication skills, as reflected in increasing mean ranks. Similarly, nurses who had attended mental health training courses demonstrated significantly better communication skills than those who had not. In contrast, variables such as sex, residence and marital status did not show significant ($p > 0.05$) differences in communication skills.

Table No. 2: Distribution of nurses' communication skills in psychiatric care units (n = 122)

Communication skills items	Never		Occasionally		Frequently		Total		M.S	Ass.
	No.	%	No.	%	No.	%	No.	%		
It is difficult for me to be demanding or strict with patients	70	57.4	34	27.9	18	14.7	122	100.0	1.57	L
I am attentive to patients' needs, especially regarding their privacy	58	47.5	40	32.8	24	19.7	122	100.0	1.72	M
I respect my patient's right to express themselves freely	54	44.3	42	34.4	26	21.3	122	100.0	1.77	M
I support patients' right to know their health information	56	45.9	38	31.1	28	23.0	122	100.0	1.77	M
I respect the autonomy and freedom of the patient's decision-making	60	49.2	40	32.8	22	18.0	122	100.0	1.69	M
I make sure that my patients have understood the information provided	62	50.8	38	31.1	22	18.0	122	100.0	1.67	M
I provide information in a clear and calm way	64	52.5	36	29.5	22	18.0	122	100.0	1.65	L
I listen to patients fairly without judging	66	54.1	36	29.5	20	16.4	122	100.0	1.62	L
I use pauses when giving information	70	57.4	34	27.9	18	14.7	122	100.0	1.57	L
I give information in simple terms and with examples	68	55.7	36	29.5	18	14.8	122	100.0	1.59	L
I take time to listen to and understand patients' needs	60	49.2	40	32.8	22	18.0	122	100.0	1.69	M
I find it difficult to ask patients for information	72	59.0	32	26.2	18	14.8	122	100.0	1.56	L
I use open questions to obtain information	66	54.1	36	29.5	20	16.4	122	100.0	1.62	L
I speak with patients in a quiet place	64	52.5	38	31.1	20	16.4	122	100.0	1.64	L
I try to explore and understand my patient's emotional state	60	49.2	40	32.8	22	18.0	122	100.0	1.69	M
I get nervous when I speak with patients	74	60.7	30	24.6	18	14.7	122	100.0	1.54	L
I have conflicts with patients when opinions differ	78	63.9	28	23.0	16	13.1	122	100.0	1.49	L
I respect the independence and freedom of the patient	56	45.9	40	32.8	26	21.3	122	100.0	1.75	M
I prefer not to tell or share bad information	80	65.6	26	21.3	16	13.1	122	100.0	1.47	L
I give information in understandable terms	58	47.5	38	31.1	26	21.4	122	100.0	1.74	M
Sharing information reduces my time with others	76	62.3	30	24.6	16	13.1	122	100.0	1.51	L
I use supportive verbal expressions	60	49.2	38	31.1	24	19.7	122	100.0	1.70	M
I provide information within my professional competence	56	45.9	40	32.8	26	21.3	122	100.0	1.75	M

Level of Response: Low = 1-1.66 Moderate = 1.67-2.33 High = 2.34-3

Table No. 3: Overall distribution of nurses' communication skills in psychiatric care units (n = 122)

Parameter	Score	No.	%	Mean±SD
Communication Skills	Low	70	57.4	28.77±5.78
	Moderate	38	31.1	
	High	14	11.5	

Table No. 4: Overall communication skills scores by socio demographic variables (n = 122)

Variables	Classification	No.	Mean Rank	H / U	p-value
Age (years)	< 25	24	105.2	8.967 ^b	0.030*
	25 – 34	50	117.6		
	35 – 44	34	124.8		
	45 and above	14	131.9		
Gender	Male	44	111.0	3.524 ^m	0.061
	Female	78	121.8		
Residence	Urban	68	122.5	2.951 ^m	0.086
	Rural	54	116.0		
Marital status	Single	38	112.3	5.982 ^b	0.201
	Married	68	123.4		
	Divorced	10	118.6		
	Widowed	4	120.5		
	Separated	2	105.2		
Educational level	Preparatory	6	95.4	9.721 ^b	0.045*
	Diploma	28	109.6		
	Bachelor degree	76	122.4		
	Master degree	10	136.2		
	PhD	2	140.5		
Years of experience	1–5	44	112.7	7.006 ^b	0.030*
	6–10	50	123.8		
	More than 10	28	130.5		
Participation in mental health training	Yes	58	128.0	4.018 ^m	0.045*
	No	64	115.0		

b = Kruskal-Wallis test m = Mann-Whitney U test N = Numbersig, = p < 0.05

DISCUSSION

Regarding sociodemographic characteristics, this study showed that largest proportion of participants were aged 25-34 years (41.0%). The majority were females 63.9%, married 55.7%, and held a bachelor's degree in nursing (62.3%). Most participants had 6-10 years of experience in psychiatric hospitals (41.0%), while 47.5% had attended mental health training courses. These findings are consistent with previous studies that reported similar demographic characteristics among nurses⁷⁻⁹.

The results presented in table 2 indicated that nurses demonstrated the low levels of communication skills. This may be attributed to the complexity of psychiatric settings, insufficient training and workload pressures. These finding are consistent with the study conducted by Kindie et al¹⁰, while they are inconsistent with the findings of Demir & Dinç¹¹, who reported higher communication skills among nurses .The finding indicate that several items reflected a moderate level of communications skills among psychiatric nurses, suggesting a basic level of communication competence. However, the complexity of psychiatric care, workload,

and lack of specialized training may limit the consistent use of effective communication skills. These findings are consistent with studies conducted by Hasan et al¹² and Hadi & Mohammed¹³.

The results also showed low levels of assertiveness, reluctance to share unpleasant information, and nervousness during patient interactions. These findings may be attributed to the sensitive nature of psychiatric care, inadequate communication training, and work-related pressures. Similar findings were reported by Ndubuisi et al¹⁴, Yousuf et al¹⁵ and Colomer-Sánchez et al¹⁶. The results presented in Table 3 indicate that the majority of nurses demonstrated low levels of communication skills, while only a small proportion showed high communication competence. This may be attributed to the psychological nature of psychiatric settings and a lack of specialized communication training. These findings are consistent with the study conducted by El-Houssieny et al¹⁷. However, they are inconsistent with the findings of Ruslan & Abdullah¹⁸ and Zeynizadeh et al¹⁹, who reported good and moderate levels of communication skills among nurses. This study showed that significant differences in communication skills according to age, educational

level, years of experience, and participation in mental health training courses (Table 4). These finding may be attributed to the positive effects of experience, education, and training on communication competence and are consistent with previous studies^{20,21}. No statistically significant differences were found according to gender, place of residence, or marital status, suggesting that professional factors may have a greater influence on communication skills than personal characteristics. This finding is consistent with studies conducted by Alshammari et al²² and Chadayan et al²³.

CONCLUSION

Most psychiatric nurses demonstrated low communication skills, which may negatively affect patient care and therapeutic relationships. Therefore, improving communication skills is essential for enhancing the quality of psychiatric nursing practice.

Recommendation: The implementing continuous training programs in therapeutic communication and integrating communication skills education into nursing curricula. Further studies are recommended to identify effective strategies for improving communication skills among psychiatric nurses.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hawraa Haider AI-Zghaibi, Muna Abdulwahab Khaleel
Drafting or Revising Critically:	Hawraa Haider AI-Zghaibi, Muna Abdulwahab Khaleel
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. 96 Dated 02.12.2025

REFERENCES

- Xiang L, Li C, Gao Q, Li L, Shi Z. Nurses' perspectives on communication in acute psychiatric care: A qualitative descriptive study. *J Psychiatric Mental Health Nurs* 2025; 32(6): 1301-13.
- Algrmadi A. The impact of communication skills training on nurses' performance and patient outcomes. *Afr J Biomed Res* 2024; 55.
- Choi J. The role of communication competence in psychiatric nursing practice. *Arch Psychiatric Nurs* 2024; 47: 45-50.
- Yağar F. Investigation of factors affecting nurses' communication levels. *J Human Social Sci* 2023; 6(1): 86-98.
- Zhao Z. Work pressure, empathy, communication skills, and workplace violence among psychiatric nurses: a structural equation model. *BMC Nurs* 2026; 43.
- Khalil AI, Aldehani RF, Aijahdali SA, Almehemadi W. Simulation-based training for improving psychiatric nurses' communication competencies: a quasi-experimental study. *Res Square* 2026; 1-30.
- Razghandi R, Lines L, Smadi O, Gulyani A. Emotional intelligence and its relationship with demographic characteristics among nurses and nursing students: a cross-sectional study. *Nurs Open* 2025; 12(10): e70332.
- Mariati LH, Jakri Y, Djerubu D. The effect of emotional intelligence on nurse's communication skill. *J Ners and Midwifery* 2022; 9(2): 218-26.
- Turjuman F, Alilyyani B. Emotional intelligence among nurses and its relationship with their performance and work engagement: A cross-sectional study. *J Nurs Management* 2023; 48.
- Kindie L, Mitiku HZ, Muche M, Wondimneh BY, Geletie HA. Nurses' communication to patients and associated factors at East Gojjam Zone, governmental hospitals, Amhara Region, Ethiopia, 2022: An institution-based cross-sectional study. *Nurs Res Rev* 2024; 14: 137-48.
- Demir H, Dinç S. Evaluation of the relationship between communication skills and psychosocial care skills of nurses. *J Nursol* 2024; 27(3): 195-201.
- Hasan NN, Mohammed IH, Mahmood YA. Communication skills among nursing staff at Azadi Teaching Hospital. *Al-Kitab J Pure Sci* 2019; 3(1): 62-70.
- Hadi MA, Mohammed SS. Nursing communication skills in preoperative care: Nurses' feedback. *Int J Health Sci* 2022; 6(S2): 10480-88.
- Ndubuisi IF, Okoronkwo IL, Nwaneri AC, Maduakolam I, Nwodoh CO, Mbadugha C. Perceived level of assertiveness and self-esteem of nurse interns in Enugu, Nigeria. *International J Afr Nurs Sci* 2024; 20: 100804.
- Yousuf AAA, Stewart DC, Kane T, Soltani A, Al-Khal A, Sethi A. Health professionals' views and experiences of breaking bad news in the Eastern Mediterranean Region: A scoping review. *Frontiers Med* 2024; 11: 1440867.
- Colomer-Sánchez A, Ayuso-Murillo D, Lendínez-Mesa A, Herrera-Peco I. Anxiety effect on communication skills in nursing supervisors: An observational study. *Nurs Reports* 2021; 11(2): 207-16.
- El-HoussienyTaha NT, Abed Ali FA, Mohamed Abd-Elhady TR, Abdel-Aleem MM. Effect of patient-centered care training program on nurse-patient communication skills at AS-Salam Hospital

- in Port Said: A quasi-experimental study. *Cuestiones de Fisioterapia* 2024; 53(3): 4426-48.
18. Ruslan R, Abdullah SND. Evaluating communication skills of undergraduate healthcare students in Malaysia. *J Palembang Nurs Studies* 2025; 4(1): 9-15.
19. Zeynizadeh S, Pirzadeh A, Tabrizian S. Communication skills and its related factors in Ardabil Alavi Hospital nurses and staff. *Int J Comm Med Public Health* 2020; 7(2): 416-19.
20. Sayılan AA, Koyun ZC. The relationship between nursing students' therapeutic communication skills and their perceptions concerning care behaviors. *J Educ Res Nurs* 2024; 21(2): 174-82.
21. Seger HR, Oleiwi SS. Effect of communication skills training on nurses' and midwives' practices: A randomized controlled trial. *Bahrain Med Bull* 2025; 47(2).
22. Al-Shammari MA, Al-Showaily JS. Assessment of communication skills competence for new graduate nurses in Babylon Province hospitals: A cross-sectional study. *Eur J Med Health Res* 2024; 2(6): 30.
23. Chadayan C, Sweetey MS, Jancy TSC. Appraising the communication skills of nursing students in clinical settings. *J Emerg Technol Innovative Res* 2025; 12(8).

Effectiveness of Educational Program regarding Children Mental and Personal Developmental Stages of Kindergarten Teachers Knowledge

Mohammed Hassan Aloda¹ and Sahar Adham Ali²

Educational Program regarding Children Mental and Personal Developmental

ABSTRACT

Objective: To evaluate kindergarten teachers' knowledge toward early childhood developmental stages, and to evaluate the effectiveness of a prepared educational program in improving their knowledge

Study Design: Quasi-experimental study

Place and Duration of Study: This study was conducted at the College of Nursing, University of Babylon, Iraq, from 12th June 2025 to 31st July 2025.

Methods: 50 kindergarten teachers in nine kindergartens in holy city of Karbala. The data collected consist of twenty multiple choices items related to developmental stages of kindergarten children's age group.

Results: Majority of kindergarten teachers were between 35 and 44 years old and mean age was 36.82 ± 7.5 years, had a bachelor's degree or higher, and at least five years of experience. Most had attended one or two training courses related to child growth and development. Pre-test results indicated that teachers' knowledge of both developmental stages was fair. The educational program demonstrated statistically significant effectiveness in improving knowledge. Teachers' knowledge of developmental stages was strongly associated with education level, years of experience, and training participation, but not with age, child responsibility, or type of kindergarten.

Conclusion: Kindergarten teachers' basic knowledge related to child of developmental stages was insufficient in their pre-test, but the designed educational program effectively enhanced their knowledge through posttest. Educational level, professional experience, and previous training were critical factors in determining knowledge, highlighting the importance of structured professional development.

Key Words: Effectiveness, Educational Program, Personal Developmental Stages, Kindergarten Teachers Knowledge.

Citation of article: Aloda MH, Ali SA. Effectiveness of Educational Program regarding Children Mental and Personal Developmental Stages of Kindergarten Teachers Knowledge. Med Forum 2026;37(9):105-110. doi:10.60110/medforum.370920.

INTRODUCTION

Early childhood is a critical period for social and emotional development, typically lasting from the ages of one to seven. During this period, children learn to recognize the emotions of others, understand their own feelings, and form bonds with friends and caregivers. A child's personality, sense of self, and ability to build

stable attachments are influenced by these stages. Emotional stability and social skills depend on caregiver attention and enjoyable early experiences¹.

According to Erikson's theory of psychosocial development, there are eight stages that occur throughout life, with the early stages being crucial for social interaction and identity construction. The "Initiative vs. Guilt" stage of early childhood (ages 3-6) highlights the importance of fostering children's exploration and independence through play and social interaction. Educators who are aware of this developmental stage can create environments that encourage self-confidence, independence, and emotional stability. This knowledge is essential to the success of educational initiatives for young children because it aligns teaching methods with psychological demands².

Cognitive development in early childhood is a critical area that influences a child's future social and learning abilities. Piaget's stages of cognitive development, particularly the preoperational stage (from two to seven years old), highlight the rapid development of symbolic thinking, creativity, and memory, and the need for

¹. Academic Nurse, Department of Pediatric Nursing, College of Nursing, University of Babylon, Iraq.

². Professor, Department of Adult Nursing, College of Nursing, University of Babylon, Iraq.

Correspondence: Mohammed Hassan Aloda, Academic Nurse, Department of Pediatric Nursing, College of Nursing, University of Babylon, Iraq.

Contact No: 9647732421757

Email: mohammedalawda@gmail.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

teachers to carefully foster these skills¹. Teachers who are aware of these developmental stages can design classrooms that complement children's cognitive abilities, promoting successful learning and skill development³.

Kindergarten teachers' understanding of child developmental has significantly improved as a result of incorporating developmental stages theories into early childhood education programs. Teachers can better enhance learning outcomes by aligning their teaching methods with their students' mental, psychological, functional, and emotional developmental stages. Professional development programs that focus on these developmental stages have been shown to enhance teachers' understanding of educational topics, leading to more successful teaching strategies⁴.

The kindergarten teachers' knowledge and approaches are significantly improved through educational programs based on developmental stages and environmental contexts. The training programs that incorporate role-playing, practical activities, and stage-appropriate learning techniques have been shown to enhance teachers' ability to identify children's cognitive developmental stages and help them achieve them⁵. Teachers who have received training in these techniques are usually better able to modify their lessons to meet the needs of both individuals and groups⁶.

METHODS

A quasi-experimental study was conducted at College of Nursing, University of Babylon, Iraq from 12th June 2025 to 31st July 2025 vide letter No. 76 dated 24th March 2025 and 50 kindergarten teachers in nine kindergartens in holy city of Karbala. A special questionnaire was prepared, and a structured educational program related to childhood growth and developmental stages was developed based on a comprehensive review.

Demographic and employment characteristics of the kindergartens teachers who participate in the present study consist of (age, educational, years of experience in kindergarten, responsibility on child at home, participated in training courses related to factors affecting child growth and development). The developmental stages questionnaire was consist from 20 items with multiple choices answers and the coding in dichotomous (Correct = 2, and Incorrect = 1) and the response options range from 20 to 40, with higher scores indicating good knowledge. The questionnaires assessed by who have over ten years of experience in the specialty field. Content validity was statistical calculator, the results was 0.97 which is acceptable, ethical issues and considerations to ensure participants' privacy and autonomy. Participant (teachers) was voluntary engaged; they take place informed that their data would be confidentially used.

Self-report method selected to complete the prepared questionnaire. The data collection started on 12th June 2025 by carried out pre-test the results indicated that all the participants showed unsatisfied level of knowledge related to prepare developmental stages. The presentation of educational sessions started from 22th to 30th June 2025 through small groups, after completing the session the first post-test carried out, the second post- test obtained. Data collection completes in 23th July 2025 which take about 40 days. The data was analyzed through SPSS-25.

RESULTS

The largest proportion of kindergarten teachers (46.0%) were aged 35-44 years, followed by those aged 25-34 years (30.0%). The mean age was 36.82 ± 8.76 years, with an age range of 18-54 years. Regarding educational qualification, more than half of the participants (52%) held a bachelor's degree or higher, while 30.0% held a diploma and 18.0% had completed secondary school (Table 1).

Table No. 1: Distribution of the participant's sociodemographic and employment characteristics (n=50)

Variable	No.	%
Age group		
Young adults (18-24 years)	4	8.0
Adult (25-34 years)	15	30.0
Middle age adult (35-44 years)	23	46.0
Early middle aged (45-54 years)	8	16.0
Level of qualification		
Secondary School	9	18.0
Diploma degree	15	30.0
Bachelor's degree or above	26	52.0

The largest proportion of teachers (58.0%) had five years or less of experience, followed by those with 6-10 years of experience (20%). Teachers with 11-15 years of experience represented 12% of the sample, while 6% had 16-20 years of experience and 4% had 21 years or more (Fig. 1). Majority of participants (72%) reported having children at home for whom they were responsible for care, whereas 28% did not report such responsibility (Fig. 2). Figure 3 showed that 40% having attended one or two training courses. Nearly equal proportions of governmental and private kindergarten are teachers (Fig. 4).

Table 2 presents the changes in kindergarten teachers' knowledge scores across the pretest, posttest 1, and posttest 2. The overall mean knowledge score increased from 1.41 ± 0.159 in the pretest to 1.83 ± 0.174 in posttest 1 and remained high at 1.80 ± 0.180 in posttest 2. The Friedman test demonstrated a statistically significant difference across the three assessments ($\chi^2=91.9$, $df=2$, $p<0.001$), with a Kendall's W of 0.919, indicating a very strong effect/degree of concordance across the repeated assessments.

Table 3 showed the distribution of kindergarten teachers according to their overall level of knowledge across the three assessments. In the pretest, 30% of teachers had poor knowledge and 70% had fair knowledge. The educational program, all participants demonstrated either fair or good knowledge in posttest 1, with 80.0% classified as having good knowledge. In posttest 2, the proportion with good knowledge remained high at 74%, while 26% had fair knowledge and none had poor knowledge.

Table 4 presents the relationship between kindergarten teachers' knowledge of early childhood developmental stages and their demographic and employment characteristics. Knowledge was significantly associated with educational qualification ($p=0.037$), years of experience ($p<0.001$), and participation in training courses related to growth and development ($p=0.012$). In contrast, no statistically significant associations were found with age ($p=0.294$), responsibility for children at home ($p=0.836$), or type of kindergarten ($p=0.595$).

Table No. 2: Determine the effectiveness of prepared educational program toward early childhood developmental stages on teachers knowledge (posttest)

Pre-test			Post-test			Post-test 2			Friedman Test			
Mean	SD	Assessment	Mean	SD	Assessment	Mean	SD	Assessment	CS	W	df	Sig.
1.48	.505	F	1.78	.418	G	1.76	.431	G	91.9	.919	2	.000
1.42	.499	F	1.82	.388	G	1.82	.388	G				
1.36	.485	F	1.80	.404	G	1.74	.443	G				
1.36	.485	F	1.90	.303	G	1.90	.303	G				
1.36	.485	F	1.86	.351	G	1.76	.431	G				
1.44	.501	F	1.86	.351	G	1.84	.370	G				
1.34	.479	F	1.78	.418	G	1.78	.418	G				
1.28	.454	P	1.74	.443	G	1.76	.431	G				
1.32	.471	P	1.84	.370	G	1.78	.418	G				
1.44	.501	F	1.82	.388	G	1.82	.388	G				
1.42	.499	F	1.82	.388	G	1.82	.388	G				
1.44	.501	F	1.82	.388	G	1.80	.404	G				
1.40	.495	F	1.80	.404	G	1.82	.388	G				
1.44	.501	F	1.84	.370	G	1.82	.388	G				
1.42	.499	F	1.86	.351	G	1.82	.388	G				
1.42	.499	F	1.82	.388	G	1.78	.418	G				
1.36	.485	F	1.80	.404	G	1.70	.463	G				
1.44	.501	F	1.90	.303	G	1.90	.303	G				
1.56	.501	F	1.92	.274	G	1.86	.351	G				
1.46	.503	F	1.82	.388	G	1.80	.404	G				

CS = Chi-square, W = Kendall's W

Table No. 3: Level for kindergarten teacher's knowledge toward early childhood developmental stages through three tests

Level	Pre-test			Post-test			Post-test 2		
	No.	%	Mean±SD	No.	%	Mean±SD	No.	%	Mean±SD
Poor	15	30.0	28.16±3.18	-	-	36.60±3.47	-	-	36.08±3.60
Fair	35	70.0		10	20.0		13	26.0	
Good	-	-		40	80.0		37	74.0	

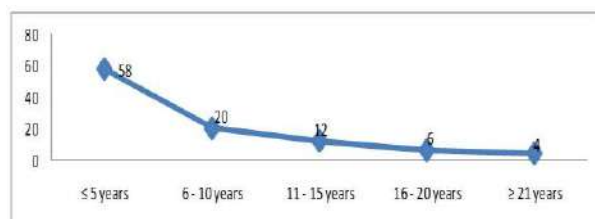


Figure No. 1: Distribution of kindergarten teachers according to their years of experience

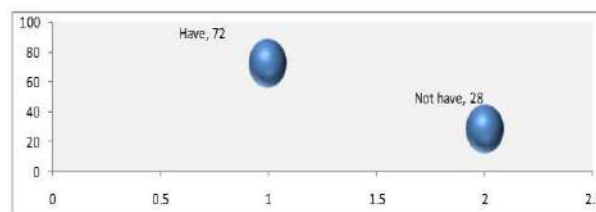


Figure No. 2: distribution of kindergarten teachers according to their responsibility for caring for children at home



Figure No. 3: Distribution of participants according to their participation in training courses related to factors affecting child growth and development

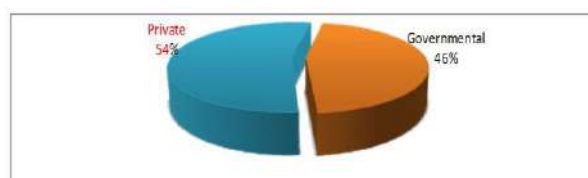


Figure No. 4: Presents the distribution of participants according to the type of kindergarten in which they worked

Table No. 4: The relationship between kindergarten teacher's knowledge about early of childhood developmental stages with their demographic and employment variables

Data	Subgroup	Knowledge		
		Mean Rank	Analysis	P. value
Age (years)	18 – 24	13.88	.152	.294 ^a
	25 – 34	25.93		
	35 – 44	27.22		
	45 – 54	25.56		
Level of qualification	Secondary School	16.22	6.587	.037 ^c
	Diploma degree	23.30		
	Bachelor's degree or above	29.98		
Years of experience (years)	≤ 5	19.69	.562	.000 ^a
	6 – 10	33.30		
	11 – 15	30.67		
	16 – 20	35.83		
	≥ 21	39.75		
Have children at home that you are taking care	Yes	25.76	-.208-	.836 ^b
	No	24.82		
Participated in training courses related to growth and development	Not participate	20.29	.351	.012 ^a
	1 - 2	23.40		
	3 or more	32.69		
Type of kindergarten	Governmental	26.67	-.531	.595 ^b
	Private	24.50		

a=P. value was calculated by Spearman's correlation coefficient b=P. value was calculated by Mann-Whitney U

c=P. value was calculated by Kruskal-Wallis H

DISCUSSION

This study showed that majority falling between 35 and 44 years, and a mean age of 36.82 years, with a standard deviation of 7.5 years. Most had a bachelor's degree or above and most had less than five years. The kindergarten teachers have responsibilities to maintain care for their children at home and most participants were related to training courses that were presented in the study sample attended one or two training courses related to factors affecting child growth and development. The level of kindergarten teachers' knowledge (pretest) toward early childhood developmental stages was fair, and most have fair knowledge. These findings are consistent with a studies by Keung and Fung⁷ and Ball et al⁸ which sought to investigate kindergarten teachers' pedagogical knowledge of content in play-based learning progression and found that teachers often lack a comprehensive understanding of developmental stages, especially in relation to play-based learning.

The majority of kindergarten teachers have adequate knowledge of early life developmental stages. These findings are consistent with research that seeks to assess teachers' competence in developmentally appropriate practices (DAP). For example, a study conducted in Jordan sought to determine kindergarten teachers' knowledge of developmentally appropriate practices and found that their understanding was "average" in some areas (such as curriculum development and integration), but "high" in others. This implies partial consistency: compared to the current study, some knowledge may be strong, while other areas are merely "acceptable" or "average"^{9,10}. In another study, using a recently created scale called the China Kindergarten Teachers' Learning and Development Scale (CKTLDS), another related study sought to determine the learning and development levels of kindergarten teachers in China. It found that the learning and development of Chinese kindergarten teachers was below average^{11,12}. In the present study, there was significant statistical effectiveness of prepared educational program toward

early childhood developmental stages on kindergarten teacher's knowledge. Keung and Fung⁷ reported that their confidence and ability to plan age-appropriate activities and their overall development were all improved through professional development. To assess the effectiveness of an in-service teacher training program in promoting early childhood education in both rural and urban areas, Quaid-e-Azam Academy for Educational Development conducted a study in Punjab, Pakistan, titled "Evaluation of an In-Service Early Childhood Teacher Training Program." The teachers' views on the value and use of early childhood education technologies were improved. Although the details of developmental stage knowledge vary, the current findings that the training improved teachers' competency are consistent with this study¹³.

This study showed that there was significant statistical differences between effect kindergarten teacher's knowledge about early childhood developmental stages with their level of education. There were significant statistical positive correlation between effect kindergarten teacher's knowledge about early childhood developmental stages with their years of experience, and number of participated in training courses related to growth and development. While, there were non-significant statistical relationship between kindergarten teachers knowledge about early of childhood developmental stages with their age, responsibility for children, and type of kindergarten. The results of Koc and Koc¹⁴ and Yusoff¹⁵, which sought to evaluate the impact of teaching experience on the implementation of spatial orientation programs in early education, do not fully align with the current study's findings that the majority of teachers have less than five years of experience, a deeper understanding and consistent integration of developmental knowledge. Furthermore, teachers' knowledge of developmental stages was positively correlated with their years of experience, education level, and participation in training courses, although age and family commitments had a small effect. Overall, the study emphasizes the importance of professional preparation and ongoing training in improving early childhood teachers' knowledge base, which directly impacts their ability to support children's holistic growth and development.

CONCLUSION

The valuable information about kindergarten teachers' understanding of early childhood developmental stages and most of them had at least a bachelor's degree, was in their mid-thirties, and had limited teaching experience. The importance of structured, evidence-based training interventions were confirmed and implementation of the developed educational program which achieved notable success in increasing teachers' knowledge.

RECOMMENDATION

Establishing training and continuous educational programs regarding the developmental stages of kindergarten children is necessary to assess their physical, intellectual, and emotional development. These programs contribute to maintaining normal cognitive and physical activity performance. Furthermore, such educational sessions enable teachers, who are in direct contact with children, to recognize abnormal growth features and participate in the early detection of health abnormalities.

Author's Contribution:

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

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

Source of Funding: None

Ethical Approval: No.76 Dated 24,03.2025

REFERENCES

1. Berk LE. Development through the lifespan. Sage Publications 2020; 48.
2. McLeod S. Erik Erikson's stages of psychosocial development. Simply Psychol 2018; 3(2): 1-3.
3. McDevitt TM, Ormrod JE. Child development and education. Prentice Hall 2010; 65-76.
4. Al-Harbi SS. Theories to guide children development and their impact on early childhood education. J Ecohumanism 2024; 3(3): 1517-29.
5. Martin AJ. Motivation and engagement across the academic lifespan: A developmental construct validity study of elementary school, high school, and university/college students. Educ Psychol Measurement 2019; 69: 794-824
6. Pyle A, DeLuca C, Danniels E. A scoping review of research on play-based pedagogies in kindergarten education. Rev Educ 2017; 5(3): 311-51.
7. Keung CPC, Fung CKH. Exploring kindergarten teachers' pedagogical content knowledge in the development of play-based learning. J Educ Teaching 2020; 46(2): 244-7.
8. Ball DL, Thames MH, Phelps G. Content knowledge for teaching: what makes it special?" J Teacher Educ 2008; 59 (5): 389-407.
9. Jumiaan I, Alelaimat A, Ihmeideh F. Kindergarten teachers' knowledge level of developmentally

- appropriate practice in Jordan. *Education* 2020; 48(8): 1000-11.
10. Al-Khawaldeh M, Alelaimat A, Al-Zyoudeh N, Al-Masa'deh M, Mu'tasem M. The effectiveness of three types of kindergarten curricula in promoting national consciousness among the Jordanian kindergarten children. *Int J Humanities Social Sci* 2018; 8(11): 65-74.
 11. Duan Q, Jiang Y, Wang Y, Zheng C, Liu J, Liu X. Development and validation of Chinese Kindergarten teachers' learning and development scale. *Sustainability* 2023; 15(3): 1992.
 12. Egert F, Dederer V, Fukkink RG. The impact of in-service professional development on the quality of teacher- child interactions in early education and care: a meta-analysis. *Educ Res Rev* 2020; 29: 100309.
 13. Malik S, Khalid L. Evaluation of the training program for in-service teachers of early childhood education conducted by QAED in Punjab. *J Early Childhood Care Educ* 2023; 7(2).
 14. Koc Y, Koç K. Kindergarten teachers' experiences in a spatial orientation skills professional development program. *SAGE Open* 2023; 13(2).
 15. Yusoff MSB. ABC of content validation and content validity index calculation. *Educ Med J* 2019; 11(2): 49-54.

Evaluation of Nurses' Knowledge regarding Correct Electrocardiographic Procedures in Medical Wards of Thi-Qar Governorate Hospitals

Hiba Jasim Hammood¹, Harith Fathi Khudhayer Al-Aubaidy² and Nasir Muwfaq Younis²

ABSTRACT

Objective: This study aimed to evaluate the level of knowledge of nurses working in the medical wards of Thi-Qar Governorate hospitals regarding correct electrocardiographic procedures and to identify the relationship between their knowledge and demographic variables.

Study Design: A descriptive cross-sectional study

Place and Duration of Study: This study was conducted at the multiple government hospitals in the Thi-Qar, southern Iraq from February 2022 and August 2023.

Methods: The study conducted with 60 nurses in Thi-Qar Governorate hospitals. Data were collected using a structured questionnaire that assessed knowledge of cardiac anatomy, physiology, ECG interpretation, and correct lead placement. Descriptive statistics and chi-square tests were used for the data analysis.

Results: Most nurses demonstrated insufficient knowledge of ECG procedures. Specifically, 46.7% of the participants had very low knowledge levels, and 53.3% had low knowledge levels. No participant achieved moderate or high levels of knowledge about the disease. Statistically significant associations were found between nurses' knowledge and both educational attainment ($p = 0.000$) and years of experience in the medical wards ($p = 0.002$).

Conclusion: This study demonstrates that the degree of nursing practice for performing ECGs in Thi-Qar hospitals is appropriate. Not to mention the fact that such a gap in knowledge is potentially dangerous: it is putting patients at risk.

Key Words: Electrocardiography, Nursing Knowledge, ECG Procedures.

Citation of article: Hammood HJ, Al-Aubaidy HFK, Younis NM. Evaluation of Nurses' Knowledge regarding Correct Electrocardiographic Procedures in Medical Wards of Thi-Qar Governorate Hospitals. Med Forum 2026;37(9):111-115. doi:10.60110/medforum.370921.

INTRODUCTION

Electrocardiography is one of those necessary evils in modern medicine you just cannot live without it especially in the ER or on medical floors, where rapid recognition of a heart problem can lead to life-saving interventions. From a practical standpoint, nurses are flexible and capable of performing ECGs, interpreting basic results, and ensuring proper electrode and lead placement for the procedure to be valid. Mistakes made during this phase could lead to misdiagnosis, delayed treatment, and ultimately, unsatisfactory patient care¹.

In particular, in a hospital environment, where cardiovascular disease is common, an accurate ECG must be assured. However, many international studies have reported low levels of nurses' knowledge and skills in ECG performance, particularly in resource-limited healthcare settings². Nurses are part of the emergency services team and their ability to use and interpret ECGs is highly recognized as they perform the majority of the hospital's medical duties and often have contact with patients. Analysis, interpretation and mastery of the clinical symptoms and diagnostic techniques in relation to CVD should be a key aspect of the education priorities for nursing³. The nurse should be knowledgeable of ECG equipment and feel confident in documenting significant and potentially life-threatening arrhythmias and in their response to them⁴. These gaps might be attributed to pre-service nurse training and in-service staff development programs. In addition, insight into how demographic factors (age, level of education, number of years in practice, and attendance in training courses) correlate with ECG proficiency might support focused actions⁵. This study aimed to investigate the actual level of

¹. Medical Laboratory Technology Department, Al-Suwaria Technical Institute, Middle Technical University. Iraq.

². College of Nursing, University of Mosul, Iraq

Correspondence: Nasir Muwfaq Younis, College of Nursing, University of Mosul, Iraq.

Contact No: 009647703000601

Email: nasir.muwfaq@uomosul.edu.iq

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

knowledge towards conducting ECGs properly among nurses working in medical wards of Thi-Qar regarding ECG performance. We further examined whether demographics, such as experience or education, played a role. Lastly, we wanted to know what exactly the nurses struggle with so that we can improve their training and fix the overall system of the hospital.

METHODS

This study utilized a cross-sectional descriptive design, which is a snapshot once off. This cross-sectional study aimed to assess the knowledge of nurses working in the medical wards of Thi-Qar regarding proper ECG techniques at one moment. This type of question is why this approach works so well and is common in nursing education and practice research. The findings of this study were conducted between February 2022 and August 2023 in multiple government hospitals in the base of Thi-Qar, southern Iraq. These hospitals are both referral centers that see all patients and rely on these nurses to perform frontline roles, such as ECGs. Focused on registered nurses operating in these areas of medical wards, hospital wards, and places where ECGs are regularly performed. This is because they are embedded in patients with cardiac symptoms. Hence knowing how to perform an ECG correctly is not just beneficial, but a necessity for these nurses.

The inclusion criteria included that the registered nurse working in a medical ward, at least one year of clinical experience and willingness to participate and provide informed consent. While, the inclusion criteria included that the administrative nurses or those not involved in clinical ECG procedures and nurses on leave during the data collection period. The methods of sampling and sample size the researcher was used a non-probability convenience sampling. A convenience sampling might restrict generalizability, but is suitable for exploratory and feasibility studies where random sampling is not practical. The study involved 60 participants. Similar studies on nursing knowledge of ECG procedures as well as practical realities surrounding the availability of nurses in the participating hospitals guided the number of participants.

To determine the extent of nurses' knowledge about performing ECGs, we compiled a quick, self-directed questionnaire. We developed it based on previous research findings, ECG education literature, and expert opinions. The questionnaire consisted of two parts. Part one: Demographic Data included age, gender, education level, total years of experience working in the nursing field, years of working in a medical department, and previous training in ECG measurement. Part two: Knowledge Assessment: 20 multiple-choice items covering: Cardiac anatomy and physiology, ECG basics and interpretation and electrode and lead placement. Correct answers received 1 point each, while incorrect or unanswered questions received Zero. The content

validity of the questionnaire was established through reviews by cardiologists and senior nursing educators. This was first used to pilot the questionnaire with 10 nurses, whose responses were not included in the study. We wanted to ensure that the questions were clear, of reasonable length, and that the answers made sense. The questionnaire had a Cronbach's alpha of 0.78, indicating an acceptable level of reliability for research purposes.

The data collection procedure for four weeks, we printed the questionnaire and handed it to passing nurses on their breaks so as not to hinder their work. Prior to commencing, we provided the participants with a summary of the study, ensured confidentiality, and obtained written informed consent. Once they completed their questionnaires, the nurses placed them in envelopes to ensure anonymity. To ensure data accuracy and reduce bias: (Researchers remained available on-site to clarify any questions, no identifying information was collected on the survey forms and participation was entirely voluntary, with no incentives offered. The data were analyzed using IBM SPSS Statistics Version 25. The following statistical techniques were applied: Descriptive statistics (frequency, percentage, mean, and standard deviation).

RESULTS

Most nurses demonstrated insufficient knowledge of ECG procedures. Specifically, 46.7% of the participants had very low knowledge levels, and 53.3% had low knowledge levels. No participant achieved moderate or high levels of knowledge about the disease. Statistically significant associations were found between nurses' knowledge and both educational attainment ($p = 0.000$) and years of experience in the medical wards ($p = 0.002$).

Table No.1: Distribution of Demographic Data for Medical Department Nurses

Variables	Categories	Freq.	%
Age Groups	20 – 24	6	10
	25 – 29	15	25
	30 – 34	16	26.7
	35 – 39	7	11.6
	≥ 40	16	26.7
Gender	Male	35	58.3
	Female	25	41.7
Achievement Education	College of Nursing	10	16.7
	Nursing Institute	30	50
	secondary Nursing School	20	33.3
Years of Experience in Nursing Field	≤ 5	13	21.7
	6 – 10	11	18.3
	11 – 15	12	20
	16 – 20	8	13.3
	21 – 25	10	16.7
	26 – 30	6	10

Table No.2: Assessment of Nurses' Understanding of Appropriate ECG Procedures in Medical Wards

Questions	M.S.	S.D.	R.S.%	Eva.
Heart Anatomy				
1- The heart receives freshly oxygenated blood.	0.25	0.44	25	2
2-The amount of blood pumped by the heart per minute in the natural situation	0.30	0.47	30	2
3- The brain contains the primary cardiac control area.	0.23	0.43	23	1
4-The electrical initiation of a heartbeat begins in the chamber wall	0.53	0.51	53	3
5-How many layers composed of cardiac muscle?	0.70	0.47	70	3
6-The primary extrinsic regulator of heart activity is the ANS.	0.17	0.38	17	1
Heart Physiology				
7- As a nursing student, you are aware that the purpose of an ECG is to	0.40	0.50	40	2
8- Which area of the heart is most likely to act as a pacemaker if the SA node is damaged?	0.27	0.45	27	2
9- Which option lists the cardiac conduction system's parts in the proper order?	0.30	0.47	30	2
10- If the AV bundle's cells were damaged, the cardiac impulse would not be able to reach the	0.33	0.47	30	2
11- Junctional fibers in the cardiac conduction system link	0.30	0.47	30	2
12- The following will undoubtedly be impacted if communication between the SA node and the AV node is blocked:	0.20	0.41	20	1
Location Limb Leads:				
13.a. Right arm	0.83	0.38	83	4
13.b .Left arm	0.70	0.47	70	3
13.c. Right leg	0.77	0.43	77	4
13.d. Left leg	0.77	0.43	77	4
14. Just to the right of the sterna border is the V1-fourth intercostals area.	0.73	0.45	73	3
14. b. V2, the fourth intercostal gap immediately to the left of the border of the sterna	0.63	0.49	63	3
14.c. halfway between leads V2 and V4	0.60	0.50	60	3
14.d. V4 middle clavicle line, below the fifth interspace	0.43	0.50	43	2
14.e. V5: anterior axillary line at V4's level	0.40	0.50	40	2
14.f. Leads V4 and V5 are at the same level as the midaxillary line (V6).	0.50	0.51	50	3
The 10 rules for a normal ECG.				
15- After going over his ECG notes, the student nurse would correctly comprehend that the electrodes should be connected.	0.20	0.41	20	1
16- His ECG notes are being reviewed by the student nurse.	0.27	0.46	17	1
17- Ventricular rate and rhythm are determined by measuring a single QRS complex.	0.50	0.57	50	3
18-From an ECG reading , PR interval represents;	0.23	0.43	23	1
19- The AV node's typical rate of spontaneous depolarization is	0.23	0.43	23	1
20- What is happening in the heart according to the electrocardiogram (ECG) QRS wave?	0.63	0.49	63	3

Table No.3: Overall Evaluation of Nurses' Level of Knowledge

Level of Knowledge	Frequency	Percent
Low Low (0 - 24)	28	46.7
Low High (25 – 49)	26	53.3
High Low (50 – 74)	6	0
High High (75 – 100)	0	0
$\bar{x} \pm S.D$	0.44 $\pm$ 0.461	

Table No.4: Relationship Between Nurses' Demographic Factors and Knowledge.

Demographic	Knowledge	X ²	D. F	p	Sig.
Age		1.348	4	0.853	NS
Gender		0.069	1	0.793	NS
Achievement Education		19.260	2	0.000	HS
Years of Experiences in the Nursing		2.012	5	0.848	NS
Years of Expert in the Medical De.		11.456	3	0.002	HS
Training Courses in Nursing		5.600	3	0.133	NS

DISCUSSION

The greatest number of healthcare staff is occupied by nurses, and they deal with patients all the time; therefore, their opinions and perspectives on the quality of care received are highly relevant^{6,7}. Nurses have numerous complementary skills available to them in the Medical Department, and evidence of nursing interventions that are found to be useful in ECG. Therefore, this study was conducted to assess the knowledge of nurses toward the performance of electrocardiograms in the medical field. The table 1: is in agreement with the same line with⁸. In the current study, of the nurses were in the age group (30-34) & (40) 16 (27.7%) and (≥40) respectively, while 35(58.3%) of the nurses were male⁹. Also obtained identical results. With regard to gender, the majority of the studied nurses 35(58.3%) were male. Our findings reveal in table 2 and table 3 an alarming finding that nurses working in medical wards of Thi-Qar Governorate have insufficient knowledge to perform ECG. This is important because electrocardiograms are important for diagnosing and treating cardiac problems. In contrast, the results were alarming: no one scored moderate or high knowledge. The balance of 53.3 per cent were in the low range, and almost half of these (46.7 per cent) were classified as having very low levels. This is a serious skills gap. This is not local either. Similar conclusions have also been catalogued in other parts of the world. For example, Chan et al, in a study on Singaporean nurses,² found that the ECG leads were misplaced and the heart conduction pathways misread by nurses¹⁰⁻¹³. Hospitalists in the clique, mainly frontline nursing. Timely interpretation of ECG relates directly to the accuracy and speed of patient management. One of the more interesting things we learned was that education and experience both count.¹⁴ Those nurses who had more education and experience working in medical wards performed better on the ECG knowledge test. This is in agreement with additional researchers who have discovered targeted education and exposure during an authentic clinical context enhances psychomotor skills. In table 4; there was no significant relationship between good E.C.G knowledge and training. In fact, training had no effect whatsoever. Nurses trained in the skills did not perform better than

those who lacked another training. And this is precisely what¹⁵ demonstrated: that we should not expect the rare or mediocre training to deliver very meaningful advances in improving any-one outcome. The national situation, especially with regard to medical facilities in Iraq, is still characterized by poor staffing and issues over funding and access to further training in Iraq. This could explain for maybe why nurses in our study did not have ECG knowledge even though they were working clinically. In contrast, studies from high-income countries generally show better levels of ECG literacy because of regular competency assessment and access to simulation laboratories¹⁶.

CONCLUSION

The result of this study disclosed the fact that there is a little bit worried about some major defected knowledge about the instruction for taking ECG from all medical wards hospitals in Thi-Qar Governorate. The nurses exhibited low knowledge scores with none achieving moderate to high competency. These results highlight the imperative to implement systemic change surrounding ECG knowledge and skills as nursing competences through educational curricula, continuing professional education initiatives.

Recommendations

The study recommends the implement structured ECG training programs should be integrated into both pre-service education and in-service professional development, conduct regular competency assessments to ensure nurses maintain and apply ECG knowledge accurately, incorporate simulation and hands-on workshops as part of ECG education can help bridge the gap between theory and practice.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hiba Jasim Hammood , Harith Fathi Khudhayer Al-Aubaidy
Drafting or Revising Critically:	Hiba Jasim Hammood, 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.

Source of Funding: None

Ethical Approval: No. 27/332 Dated 13.01.2022

REFERENCES

1. Kim H, Park H, Kim EK. Risk factors for postoperative delirium in patients with colorectal cancer. *J Clin Nursing* 2022;31(1-2):174-83.
2. Chen Y, Kunst E, Nasrawi D, Massey D, Johnston AN, Keller K, Fengzhi Lin F. Nurses' competency in electrocardiogram interpretation in acute care settings: A systematic review. *J Advanced Nursing* 2022;78(5):1245-66.
3. de Lima MJ, Jamil N, Vazhuthakkadu S, Mosleh S, Al-Nsair N. Impact of self-directed e-learning on nurses' competency in arrhythmia interpretation in cardiology. *PLoS One* 2025;20(9):e0331043.
4. Chaiard J, Weaver TE. Update on Research and Practices in Major Sleep Disorders: Part II—Insomnia, Willis-Ekbom Disease (Restless Leg Syndrome), and Narcolepsy. *J Nursing Scholarship* 2019;51(6):624-33.
5. Ayed AY, Younis NM, Ahmed MM. Teachers' knowledge about communicable disease control at primary schools in Mosul City: A cross-sectional study. *Int J Academic Med* 2024;10(2):75-9.
6. Timmis A, Vardas P, Townsend N, Torbica A, Katus H, De Smedt D, et al. European Society of Cardiology: cardiovascular disease statistics 2021: executive summary. *Eur Heart J-Quality of Care and Clin Outcomes* 2022;8(4):377-82.
7. Attar ET. ECG interpretation abilities in clinical practice: Examining the role of expertise, age, and gender. *Medicine* 2025;104(32):e42401.
8. Nabil Malk R, Mostafa Rezk M, Said Mohammed S, Fouad Abd-Allah K. Effect of an education program on nurses performance regarding electrocardiography. *Egypt J Health Care* 2018;9(1):38-49.
9. Tanha HE, Hosseini SM, Sharifzadeh G, Borhanzahi K. Comparison of the effect on social-networking-based teaching and workshop-based teaching on emergency nurse ECG interpretation skills. *J Nursing Education (JNE)* 2024;18(6):57-66.
10. Bdair IA. Electrocardiogram interpretation competency among undergraduate nursing students: A quasi-experimental study. In *Nursing Forum* 2022;57(6):1273-1280.
11. Fathi AM, Awad MS, Abdullah MK, Younis NM. Electrocardiography Knowledge among Cardiac Care Unit Nurses at Al-Salam Teaching Hospital. *Bahrain Medical Bulletin* 2026;48(2).
12. Innes KE, Montgomery C, Selfe TK, Wen S, Khalsa DS, Flick M. Incorporating a usual care comparator into a study of meditation and music listening for older adults with subjective cognitive decline: a randomized feasibility trial. *J Alzheimer's Disease Reports* 2021;5(1):187-206.
13. Saad Soliman E, Mostafa Ragheb M, Abd El-Salam Sheta H, Hamed Mohamed S. Effect of an educational program on nurses' performance regarding reducing pressure ulcer and safety of immobilized patients. *J Nursing Sci Benha Univ* 2022;3(2):856-72.
14. Ahmed MM, Hussein AA, Younis NM. Assessment of nursing student's beliefs about healthy dieting. *J Educ Health Promotion* 2024;13:408.
15. Ayed AY, Younis NM, Ahmed MM. Comparison of infection severity of vaccinated and unvaccinated health workers with Corona Virus: A cohort study. *Educ Health Promotion* 2023;(1):336.
16. Ahmed RA, Ali SA. Efficiency of competency training program on intensive care nurses performance regarding the electrocardiogram interpretation. *Egypt J Nursing Health Sciences* 2023;4(3):8-39.

Comparison of Extension of Analgesic Effect of Bupivacaine Alone and Bupivacaine with Dexmedetomidine in Caudal Anesthesia amongst Children

Ayesha Mirza¹ and Amjad Nadeem²

Analgesic Effect
of Bupivacaine
Alone and with
Dexmedetomidine
in Caudal
Anesthesia
among Children

ABSTRACT

Objective: To compare the duration and quality of postoperative analgesia between caudal bupivacaine alone and bupivacaine combined with dexmedetomidine in children.

Study Design: Prospective randomized comparative study

Place and Duration of Study: This study was conducted at the Department of Anesthesia KRL Hospital Islamabad from 20th April 2026 till 20th July 2026.

Methods: This study included 140 children undergoing elective infraumbilical surgery. Patients were divided equally into two groups.

Results: Mean duration of analgesia was significantly longer with bupivacaine plus dexmedetomidine than with bupivacaine alone (12.86 ± 2.74 vs. 6.42 ± 1.58 hours, $p < 0.001$). Time to first rescue analgesia was also prolonged (12.51 ± 2.68 vs. 6.18 ± 1.51 hours, $p < 0.001$), while rescue analgesia within 24 hours was required less frequently (48.6% vs. 87.1% , $p < 0.001$). Mean rescue doses were lower in the dexmedetomidine group (0.69 ± 0.66 vs. 1.74 ± 0.72 , $p < 0.001$), with significantly lower FLACC scores up to 12 hours. Emergence agitation was reduced (8.6% vs. 21.4% , $p = 0.033$), although early postoperative sedation was greater. Mean intraoperative heart rate and mean arterial pressure were modestly lower with dexmedetomidine.

Conclusion: Adding dexmedetomidine to caudal bupivacaine significantly prolonged postoperative analgesia, reduced pain scores and rescue analgesic requirements, and decreased emergence agitation in children without a significant increase in overall adverse effects.

Key Words: Analgesic Effect, Bupivacaine, Dexmedetomidine, Anesthesia, Children

Citation of article: Mirza A, Nadeem A. Comparison of Extension of Analgesic Effect of Bupivacaine Alone and Bupivacaine with Dexmedetomidine in Caudal Anesthesia amongst Children. Med Forum 2026;37(9):116-120. doi:10.60110/medforum.370922.

INTRODUCTION

Good perianesthetic pain management is a key component of paediatric anaesthesia because inadequate pain management can have a detrimental effect on physiological stress, agitation, postoperative discomfort and recovery times¹. Caudal epidural anesthesia is the most frequently used regional anesthetic in children for infraumbilical surgery, surgery of the lower abdomen, urological surgery, surgery of the perineum and lower limbs². It is an easy procedure to do and useful for intraoperative and early

postoperative pain management, and decreases the need for systemic opioids³. The drawback of single shot caudal block however is that the duration of the block of analgesia is relatively short if a local anesthetic is injected alone⁴. Caudal anesthesia with bupivacaine is often used for caudal anesthesia in children due to its proven regional anesthetic efficacy and relatively long duration of action⁵. However, the pain relieving properties of a caudal injection can become less effective in the postoperative period and may require supplementation with other pain medications⁶. Several adjuvants have thus been studied to extend caudal analgesia without significantly increasing the incidence of adverse effects, such as opioids, clonidine, ketamine, dexamethasone, magnesium, and dexmedetomidine⁷. Of these, dexmedetomidine has garnered special attention due to its α_2 -adrenergic agonist activity and analgesic and sedative properties⁸. Dexmedetomidine has the potential to increase the effects of caudal local anesthetic in the spinal and supraspinal levels, via α_2 -adrenergic mechanisms⁹. Randomized trials have shown that the addition of dexmedetomidine to caudal bupivacaine has a significant effect on the extension of postoperative

¹. Resident / Consultant², Department of Anesthesia KRL Hospital Islamabad.

Correspondence: Dr. Ayesha Mirza, Resident, Department of Anesthesia KRL Hospital Islamabad.

Contact No: 03035568020

Email: aisha.mirza26@gmail.com

Received: July, 2026

Reviewed: August, 2026

Accepted: August, 2026

analgesia when compared to caudal bupivacaine alone¹⁰. Another study that combined effects of caudal anesthesia with dexmedetomidine on children also showed that dexmedetomidine had a significant effect in prolonging time to first rescue analgesic administration¹¹. In more recent comparisons, dexmedetomidine has been shown to be one of the most potent caudal adjuvants to prolong postoperative analgesia in children¹². Although dexmedetomidine has analgesic activity, it can affect sedation and cardiovascular parameters, such as heart rate and blood pressure¹³. A few studies have noted a higher rate of bradycardia, and others have found prolonged analgesia but no excessive number of adverse reactions¹⁴. Thus, the efficacy and safety of dexmedetomidine use as a caudal adjuvant in children should be evaluated. Application of analgesia for a longer duration after pediatric surgery will decrease rescue analgesic needs, enhance postoperative comfort and facilitate a smoother recovery phase¹⁵.

METHODS

This prospective randomized comparative study was conducted at the Department of Anesthesia KRL Hospital Islamabad from 20th April 2026 till 20th July 2026 and included 140 children undergoing elective infraumbilical surgery under general anesthesia with caudal analgesia. Patients were randomly divided into two equal groups of 70 each. Group A received caudal 0.25% bupivacaine 1 mL/kg alone, while Group B received caudal 0.25% bupivacaine 1 mL/kg combined with dexmedetomidine 1 µg/kg. Children aged 2–10 years, belonging to American Society of Anesthesiologists (ASA) physical status I or II and scheduled for elective lower abdominal, urological, perineal, or inguinal surgery were included. Children with known allergy to study drugs, coagulopathy, infection at the caudal injection site, congenital spinal abnormalities, neurological disorders, significant cardiac or respiratory disease, developmental delay interfering with pain assessment, or failed caudal block were excluded.

Data Collection: After approval from the Institutional Ethical Review Committee and obtaining informed consent from parents or guardians, patients were enrolled using a non-probability consecutive sampling technique and randomly allocated to either group using computer-generated random numbers with concealed allocation. Standard monitoring included heart rate, non-invasive blood pressure, oxygen saturation, respiratory rate, and electrocardiography. Following induction of general anesthesia, caudal block was performed under aseptic conditions in the lateral position. The study solution was administered according to group allocation after confirming correct needle placement and negative aspiration for blood or cerebrospinal fluid. Heart rate, blood pressure, oxygen

saturation, and adverse events were recorded intraoperatively and during postoperative recovery. Postoperative pain was assessed using the Face, Legs, Activity, Cry and Consolability (FLACC) score at predetermined intervals. The primary outcome was duration of analgesia, defined as the time from administration of the caudal block to the first requirement for rescue analgesia. Rescue analgesic medication was administered when the FLACC score was ≥ 4 .

Statistical Analysis: Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean $\pm$ standard deviation, while categorical variables were presented as frequencies and percentages. Age, weight, duration of surgery, duration of analgesia, pain scores, hemodynamic parameters, and rescue analgesic requirements were compared between groups using the independent t-test or Mann–Whitney U test, as appropriate. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The two groups were comparable at baseline. Mean age was 5.82 ± 2.14 years in the bupivacaine group and 5.64 ± 2.08 years in the bupivacaine+dexmedetomidine group, while mean weight was 20.46 ± 6.72 versus 19.98 ± 6.54 kg.

Table No. I: Baseline Demographic and Operative Characteristics

Characteristic	Bupivacaine alone (n=70)	Bupivacaine + Dexmedetomidine (n=70)
Age, years, Mean $\pm$ SD	5.82 $\pm$ 2.14	5.64 $\pm$ 2.08
Weight, kg, Mean $\pm$ SD	20.46 $\pm$ 6.72	19.98 $\pm$ 6.54
Male, n (%)	47 (67.1)	45 (64.3)
Female, n (%)	23 (32.9)	25 (35.7)
ASA I, n (%)	55 (78.6)	57 (81.4)
ASA II, n (%)	15 (21.4)	13 (18.6)
Duration of surgery, min, Mean $\pm$ SD	54.72 $\pm$ 14.63	56.18 $\pm$ 15.21
Duration of anesthesia, min, Mean $\pm$ SD	71.84 $\pm$ 16.28	73.26 $\pm$ 16.94
Inguinal surgery, n (%)	27 (38.6)	26 (37.1)
Urological surgery, n (%)	25 (35.7)	26 (37.1)
Perineal/other infraumbilical surgery, n (%)	18 (25.7)	18 (25.7)

Males comprised 67.1% and 64.3%, and ASA-I status was present in 78.6% and 81.4%, respectively. Mean surgical duration was 54.72±14.63 versus 56.18±15.21 minutes and anesthesia duration 71.84±16.28 versus 73.26±16.94 minutes, with no significant baseline differences.

Dexmedetomidine significantly prolonged analgesia (12.86±2.74 vs. 6.42±1.58 hours, $p<0.001$) and delayed first rescue analgesia (12.51±2.68 vs. 6.18±1.51 hours, $p<0.001$). Rescue analgesia within 24 hours was required in 48.6% versus 87.1%, with fewer rescue doses (0.69±0.66 vs. 1.74±0.72, $p<0.001$). Table 2.

Table No.2: Postoperative Analgesic Outcomes

Outcome	Bupivacaine alone (n=70)	Bupivacaine + Dexmedetomidine (n=70)	p-value
Duration of analgesia, hours, Mean±SD	6.42±1.58	12.86±2.74	<0.001
Time to first rescue analgesia, hours, Mean±SD	6.18±1.51	12.51±2.68	<0.001
Rescue analgesia required within 24 h, n (%)	61 (87.1)	34 (48.6)	<0.001
Number of rescue doses in 24 h, Mean±SD	1.74±0.72	0.69±0.66	<0.001
FLACC score at 2 h, Mean±SD	1.18±0.91	0.74±0.72	0.002
FLACC score at 4 h, Mean±SD	2.06±1.12	1.02±0.88	<0.001
FLACC score at 6 h, Mean±SD	3.41±1.36	1.46±1.02	<0.001
FLACC score at 12 h, Mean±SD	3.08±1.27	2.14±1.13	<0.001
FLACC score at 24 h, Mean±SD	2.24±1.08	1.96±1.02	0.116

Table No.3: Hemodynamic Parameters and Postoperative Sedation

Parameter	Bupivacaine alone (n=70)	Bupivacaine + Dexmedetomidine (n=70)	p-value
Baseline heart rate, beats/min, Mean±SD	103.42±14.81	102.76±14.37	0.789
Mean intraoperative heart rate, beats/min, Mean±SD	99.86±13.72	91.38±12.64	<0.001
Baseline MAP, mmHg, Mean±SD	68.72±7.84	69.14±8.06	0.755
Mean intraoperative MAP, mmHg, Mean±SD	66.81±7.26	64.12±7.18	0.029
Mean SpO ₂ , %, Mean±SD	98.41±0.86	98.36±0.91	0.739
Sedation score at 1 h, Mean±SD	1.38±0.49	1.91±0.61	<0.001
Sedation score at 2 h, Mean±SD	1.21±0.41	1.62±0.55	<0.001
Emergence agitation, n (%)	15 (21.4)	6 (8.6)	0.033

Table No.4: Adverse Effects and Recovery Outcomes

Outcome	Bupivacaine alone (n=70)	Bupivacaine + Dexmedetomidine (n=70)	p-value
Bradycardia, n (%)	2 (2.9)	7 (10.0)	0.086
Hypotension, n (%)	2 (2.9)	5 (7.1)	0.245
Nausea/vomiting, n (%)	9 (12.9)	5 (7.1)	0.256
Urinary retention, n (%)	3 (4.3)	4 (5.7)	0.698
Respiratory depression, n (%)	0 (0.0)	0 (0.0)	1.000
Excessive sedation, n (%)	1 (1.4)	4 (5.7)	0.366
Any adverse effect, n (%)	16 (22.9)	19 (27.1)	0.559
Time to full recovery, min, Mean±SD	34.62±9.84	39.28±10.72	0.008
Parent satisfaction, n (%)	51 (72.9)	64 (91.4)	0.004

Baseline heart rate and MAP were similar between groups, but mean intraoperative heart rate was lower with dexmedetomidine (91.38±12.64 vs. 99.86±13.72 beats/min, $p<0.001$), as was MAP (64.12±7.18 vs. 66.81±7.26 mmHg, $p=0.029$). Mean SpO₂ remained comparable (98.36±0.91% vs. 98.41±0.86%). Table 3. Overall adverse effects were comparable between dexmedetomidine and bupivacaine-alone groups (27.1% vs. 22.9%, $p=0.559$). Bradycardia occurred in 10.0% versus 2.9%, hypotension in 7.1% versus 2.9%,

nausea/vomiting in 7.1% versus 12.9%, urinary retention in 5.7% versus 4.3%, and excessive sedation in 5.7% versus 1.4%; no respiratory depression occurred. Table 4.

DISCUSSION

The present study showed that the infusion of dexmedetomidine along with caudal bupivacaine significantly increased the duration of the analgesic

effect in children undergoing infraumbilical surgery. The mean duration of analgesia was 6.42 ± 1.58 hours for bupivacaine alone and 12.86 ± 2.74 hours for bupivacaine with dexmedetomidine ($p < 0.001$). Likewise, the time to SR analgesia was prolonged from 6.18 ± 1.51 to 12.51 ± 2.68 hrs. Several previous studies have also found a significant increase in time to first rescue analgesia with caudal bupivacaine and dexamethasone, of 14.4 ± 7.5 hours versus 6.6 ± 2.5 hours with caudal bupivacaine alone^{16,17}. The results of this study indicate not only that dexmedetomidine helps to postpone the onset of postoperative pain, but also that it decreases the total amount of analgesics used. Dexmedetomidine's analgesic benefit was corroborated by postoperative pain scores. FLACC scores were significantly lower in the combination group at 2 hours (0.74 ± 0.72 vs. 1.18 ± 0.91), 4 hours (1.02 ± 0.88 vs. 2.06 ± 1.12), 6 hours (1.46 ± 1.02 vs. 3.41 ± 1.36), and 12 hours (2.14 ± 1.13 vs. 3.08 ± 1.27). However, the difference decreased by 24 hours and reached non-statistical significance (1.96 ± 1.02 vs. 2.24 ± 1.08 , $p = 0.116$). In another previous study, eight patients in the bupivacaine-only group became agitated but no children agitated in the group receiving caudal dexmedetomidine and bupivacaine¹⁷. Therefore, this moderate postoperative sedation may also help to facilitate a more tranquil emergence and recovery from sedation following surgery, in addition to the analgesia. Dexmedetomidine had a small hemodynamic effect. Mean intraoperative heart rate was significantly lower in the combination group (91.38 ± 12.64 vs. 99.86 ± 13.72 beats/min, $p < 0.001$), while mean arterial pressure was also slightly reduced (64.12 ± 7.18 vs. 66.81 ± 7.26 mmHg, $p = 0.029$). These changes were not reflected in any significant differences in mean oxygen saturation between groups ($98.36 \pm 0.91\%$ vs. $98.41 \pm 0.86\%$), nor in any respiratory depression. Previous studies also have reported statistically significant changes in the hemodynamics following the addition of dexmedetomidine to caudal bupivacaine, but these changes were not deemed clinically significant¹⁸. Caudal dexmedetomidine has also been found to be effective for prolonged periods without a significant rise in overall adverse effects as compared to previous studies¹⁹. Bradycardia and hypotension were more common with dexmedetomidine (10.0% and 7.1% respectively), but neither was statistically significant compared with the controls (2.9% and 2.9% respectively). Frequently, nausea/vomiting, urinary retention, excessive sedation and the percentage of adverse effects did not differ significantly between both groups (27.1% vs 22.9%, $p = 0.559$; 3.9% vs 3.3%, $p = 0.873$; 12.9% vs 11.8%, $p = 0.837$; respectively). Other prior studies have also demonstrated a similar rate of hemodynamic changes and other adverse events when comparing caudal dexmedetomidine regimens to other regimens with a much longer duration of action

for analgesia. Parent satisfaction was significantly higher for the combination regimen (91.4% vs. 72.9%; $p = 0.004$) which may be due to improved pain management and minimizing agitation and the need for rescue analgesics. This study has some limitations. The results might not be generalizable due to the single center design and small sample size. Clinical scoring systems were used to evaluate pain and sedation, and may be subject to observer variability. The study evaluated only one dose of dexmedetomidine, so the optimal dose-response relationship could not be determined.

CONCLUSION

The addition of dexmedetomidine to caudal bupivacaine significantly prolonged postoperative analgesia, delayed the need for rescue analgesia, reduced pain scores and rescue analgesic requirements, and decreased emergence agitation compared with bupivacaine alone. Although dexmedetomidine was associated with greater early sedation, modest reductions in heart rate and blood pressure, and a slightly longer recovery time, the overall adverse-effect profile remained comparable between groups.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Ayesha Mirza, Amjad Nadeem
Drafting or Revising Critically:	Ayesha Mirza, Amjad Nadeem
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.ERC-KRL-HIPUB-ERC/114 dated 16.09.2025 and CPSP Approval Reference No.CPSP/REU/ANS-2021-045-2787 Dated 17.04.2026

REFERENCES

1. Amaambo NNT. A comparative study of analgesic effect of caudal bupivacaine with dexmedetomidine versus bupivacaine alone for infraumbilical surgeries in children in Windhoek Central Hospital. University of Namibia 2025. <https://repository.unam.edu.na/items/e8d20d13-7f18-457a-a4ce-4c228c954d2a>
2. Anil Kumar LN, Raghav A, Chahar S. Analgesic Benefits of Caudal Dexmedetomidine with Bupivacaine in Children Undergoing Infra-Umbilical Procedures. Int J Acad Med Pharm 2026;8(1):27-33.

3. Xu W, Wei H, Zhang T. Methods of prolonging the effect of caudal block in children. *Frontiers in pediatrics* 2024;12:1406263.
4. Kadam S. A comparative study of caudal anesthesia with bupivacaine v/s bupivacaine with dexmedetomidine in lower abdominal surgeries in pediatric age group. *Pain Med* 2021;6(3):25-29.
5. El Asser HM, Kamal H. Dexmedetomidine versus fentanyl for ultrasound guided caudal block in pediatrics. A randomized controlled study. *Ain-Shams J Anesthesiol* 2024;16(1).
6. Malik S, Abbas SM, Ali H, Gazder T, Kumar V, Farooq F. Comparative Analysis of Bupivacaine Versus Bupivacaine with Dexmedetomidine in Caudal Block Anesthesia: Comparison of Bupivacaine with Dexmedetomidine in Caudal Anesthesia. *J Health Rehabilitation Res* 2024;4(3): 1-5.
7. Farid DM, Hegab AS, Elhossieny KM, Elsayed AI. Analgesic Effect of Bupivacaine-Dexmedetomidine versus Bupivacaine-Midazolam in Caudal Anesthesia for Pediatric Hypospadias Surgeries. *Zagazig University Med J* 2024;30(9.1): 5048-5058.
8. Salama HG, AlAnsary AM, Kamal El-Din DM, Eldawy MR. Safety and Efficacy of Combined Dexmedetomidine and Bupivacaine versus Bupivacaine Alone in Caudal Block in Pediatric Patients Undergoing Lower Abdominal Surgery. *QJM: An Int J Med* 2024;117 (Supplement_1):hcae070-006.
9. Siddique MAB, Ali NP, Sarder MAH, Rubaiat MM, Haque MN, Ahmed MNU. Comparative Study between Bupivacaine with Dexmedetomidine and Bupivacaine with Fentanyl for Caudal Epidural Analgesia Perioperatively in Paediatric Infraumbilical Surgery. *J Armed Forces Med Coll Bangladesh* 2025;21(2):29-35.
10. Singh K, Bajaj J, Bathla S, Mehta K. Comparison of caudal bupivacaine and dexmedetomidine with caudal bupivacaine and intravenous dexmedetomidine for postoperative analgesia in children: A randomized controlled trial. *J Anaesthesiol Clin Pharmacol* 2024;41(3):455.
11. Shah UJ, Karuppiiah N, Karapetyan H, Martin J, Sehmbi H, Shah U. Analgesic efficacy of adjuvant medications in the pediatric caudal block for infraumbilical surgery: a network meta-analysis of randomized controlled trials. *Cureus* 2022;14(8).
12. Singh A, Gupta KK, Kaur A, Kaur H. Analgesic Efficacy and Safety of Dexmedetomidine as an Adjuvant to Caudal Levobupivacaine for Infraumbilical Surgeries in Children. *J Anesthesia / Anestezi Dergisi (JARSS)* 2021;29(4):263.
13. Khalil KSA, Rashwan SAA, Hussein HAEW. Dexamethasone versus dexmedetomidine as an adjuvant to bupivacaine for ilioinguinal and iliohypogastric nerve block in pediatrics, comparative randomized double-blind study. *Egypt J Med Res* 2023;4(2):27-42.
14. Fares KM, Mohamed SAB, Abd El-Rahman AM, AbdeLemam RM, Osman AMM. Analgesic effect of intrathecal fentanyl vs dexmedetomidine as adjuvants to bupivacaine following abdominal surgery for cancer in children, a randomized trial. *Pain Med* 2020;21(11):2634-2641.
15. Mostafa MF, Hamed E, Amin AH, Herdan R. Dexmedetomidine versus clonidine adjuvants to levobupivacaine for ultrasound-guided transversus abdominis plane block in paediatric laparoscopic orchiopexy: Randomized, double-blind study. *Eur J Pain* 2021;25(2):497-507.
16. Ram GK, Dubey PK, Akhileshwar P, Ranjan A. Dexmedetomidine and ketamine as an adjuvant to levobupivacaine for pediatric caudal analgesia: a randomized, controlled study. *Anesthesia, essays Researches* 2020;14(2):253.
17. Sultan N, Javed H, Khan A, Mir A, Khan A, Shahid N. A Comparison of Caudal Bupivacaine VS Bupivacaine Tramadol Mixture for Postoperative Analgesia In Children Having Lower Abdomen and Lower Limb Surgeries. *Pak J Intensive Care Med* 2025;5(01):242-242.
18. Agarwal S, Agrawal S, Kamal G, Gupta A, Gupta A, Kalra B. A comparative study of clonidine and dexmedetomidine as an adjuvant to levobupivacaine for caudal analgesia in children undergoing below umbilical surgeries: A randomized double-blind controlled trial. *Ağrı* 2026;38(1):21.
19. Pallapati AP, Jose R, Devadharshini B, Gowri M, Joselyn AS. A double-blinded randomized control trial to compare the duration of analgesia using morphine, clonidine, or dexmedetomidine as adjuvants to ropivacaine in caudal anesthesia in children undergoing infraumbilical surgeries. *J Anaesthesiol Clin Pharmacol* 2023;40(3):388.

Nutritional Profile and Quality of Sleep among Adult Patients on Maintenance Hemodialysis

Nutritional
Profile and
Quality of Sleep
among Adult
Patients

Hina Rahim¹, Zahid Nabi², Humayun Rashid³ and Hifsa Akram¹

ABSTRACT

Objective: To evaluate the nutritional profile and sleep quality, and to determine the correlation between malnutrition and poor sleep quality among adult patients undergoing maintenance hemodialysis.

Study Design: Cross-sectional descriptive study.

Place and Duration of Study: This study was conducted at the Department of Nephrology, KRL Hospital, Islamabad, Pakistan, from October to December 2025.

Methods: A total of 100 patients aged 18 years or above undergoing maintenance hemodialysis for at least 3 months and receiving a minimum of 3 dialysis sessions/week were included in this study. Nutritional status and sleep quality were evaluated using the Malnutrition-Inflammation Score and the Pittsburgh Sleep Quality Index, respectively. The Pearson correlation coefficient (r) was applied to evaluate the relationship between malnutrition and sleep quality.

Results: Mean age in this study was 58.22±10.65 years with a higher percentage of male patients (62%). The mean Malnutrition-Inflammation score was 15.79±5.36. Mild-to-moderate malnutrition was recorded in 63% while severe malnutrition was reported in 25% of patients. The assessment of sleep quality using Pittsburgh Sleep Quality Index score showed a mean of 9.27±4.88, with 72% experiencing poor sleep quality. A significant positive correlation was found between the two scores (r=0.263, p<0.01).

Conclusion: Malnutrition was significantly correlated with poor sleep quality, emphasizing the need for integrated nutritional and sleep management strategies in patients undergoing maintenance hemodialysis.

Key Words: Chronic Kidney Disease, End-Stage Kidney Disease, Hemodialysis, Malnutrition, Sleep Quality.

Citation of article: Rahim H, Nabi Z, Rashid H, Akram H. Nutritional Profile and Quality of Sleep among Adult Patients on Maintenance Hemodialysis. Med Forum 2026;37(9):121-125. doi:10.60110/medforum.370923.

INTRODUCTION

Chronic Kidney Disease (CKD) is characterized by a progressive loss of renal function, which may lead to a terminal phase, called End-Stage Kidney Disease (ESKD). Maintenance hemodialysis (MHD) becomes an essential and life-sustaining requirement at this terminal stage of ESKD for adequate filtration to manage uremic toxicity and fluid balance. However, malnutrition and poor sleep quality (SQ) remain prevalent and debilitating in patients on MHD, severely compromising the quality of life (QoL) and increasing healthcare burdens.^{1,2}

The global prevalence of ESKD requiring dialysis is estimated to affect millions of individuals and impose significant economic and social burdens on healthcare system.

The situation is not different in Pakistan, where burden of CKD continues to rise, with an overall prevalence of 21.2% among all age groups (ranging between 29.9% to 12.5% in different population types).³

Malnutrition is common among patients on MHD with prevalence ranging from 40% to 70%, reported in different studies using various diagnostic criteria. The most common form of malnutrition is protein-energy wasting (PEW), which also contributes to serious complications in these patients.^{4,5} The pathogenesis of PEW is multifactorial and involves inadequate dietary intake, chronic inflammation, hormonal dysregulation, and dialysis-related nutrient losses (6–8g amino acids/session).^{6,7}

The most validated assessment tool for this purpose is Malnutrition-Inflammation Score (MIS) which combines dietary intake, weight change, body mass index (BMI), albumin, and total iron-binding capacity. In this scale, a higher MIS score will reflect a poor nutritional status and greater inflammatory burden, predicting adverse clinical outcomes in patients.⁸ In addition to malnutrition, insomnia, restless legs syndrome, and sleep apnea are commonly reported disorders in patients undergoing MHD with a prevalence reported between 50 to 80%, causing a further deterioration of physical and mental health.⁹

Nutrient deficiencies, particularly magnesium, calcium, and B-vitamins also disrupt melatonin synthesis and

¹. Resident / Head of Department² / Consultant³,
Department of Nephrology KRL Hospital Islamabad

Correspondence: Hina Rahim, Resident, Department of
Nephrology KRL Hospital Islamabad.

Contact No: 03217465894

Email: hina_abdulrahim@yahoo.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

affect circadian rhythm regulation. Frequent impaired cognitive function, increased risk of CV events, depression, and reduced treatment adherence are also observed in patients undergoing MHD and linked with poor SQ. Sleep quality is assessed using the Pittsburgh Sleep Quality Index (PSQI), which provides multidimensional insight by evaluating latency, duration, and daytime dysfunction over the past month. A poor SQ is indicated when the PSQI score is >5 .¹⁰

A holistic management plan therefore requires understanding this interplay between nutritional profile and quality of sleep. The distinct cultural, dietary, and socioeconomic environment of Pakistani population underscores the need for local research on this topic; however, limited data are available in Pakistan on malnutrition and sleep disturbances among MHD patients.

This study was therefore planned to evaluate the nutritional profile and sleep quality of patients undergoing MHD and to determine the correlation between these two parameters. These findings will provide evidence to our local medical professionals to reduce complications, improve QoL, and enhance overall outcomes in patients receiving this life saving therapy.

METHODS

This cross-sectional descriptive study was conducted at the Department of Nephrology, KRL Hospital, Islamabad, Pakistan, from October to December 2025 over a period of three months after getting approval from ethical committee of the hospital.

Sample size was calculated using Open Epi sample size formula with a 95% confidence interval and 10% margin of error. With an expected 60.4% prevalence of poor SQ among malnourished MHD patients, the minimum sample size required was calculated as 92 patients. However, to account for potential incomplete responses and to ensure adequate statistical power, we recruited a total of 100 patients.¹¹ A total of 100 patients of either gender, aged 18 years or above who had been on MHD for at least 3 months and were receiving a minimum of 3 dialysis sessions per week were included in the study. Exclusion criteria consisted of recent hospitalization within the past month, active infection, malignancy, psychiatric illness, cognitive impairment, or those using sedatives or sleep-inducing medications.

Data collection: Patients fulfilling the inclusion criteria were included using non-probability consecutive sampling. Demographic and clinical details of these patients (age, gender, duration of MHD and comorbidities) were recorded. Anthropometric measurements were carried out including height, weight, BMI, and mid-arm circumference (MAC) as per standardized protocols. Laboratory parameters such as serum albumin were obtained from patient's medical

history present in hospital's records. Nutritional status of these patients was evaluated using the MIS. MIS consisted of 10 items including weight change, dietary intake, gastrointestinal (GI) symptoms, functional capacity, comorbidity severity, fat and muscle stores, BMI, albumin, and total iron-binding capacity. Each parameter was given score from 0 to 3, and MIS had a total score between 0 (normal) and 30 (severe malnutrition). Nutritional status was then categorized as normal nutrition (0–8), mild to moderate malnutrition (9–18), and severe malnutrition (>18) based on the total score obtained.¹²

SQ was assessed using the PSQI, which provided a global score ranging from 0 to 21 and patients were categorized as having good sleep if the PSQI was ≤ 5 and poor sleep if the PSQI was >5 .¹³

Data were analyzed using SPSS version 26. Baseline characteristics were summarized by employing descriptive statistics where continuous variables were presented as mean $\pm$ standard deviation (SD), while non-normally distributed variables (assessed using the Shapiro–Wilk test) were expressed as median and interquartile range (IQR). Categorical variables including MIS category and SQ category were shared as frequencies and percentages. Normality of the continuous data was assessed using the Shapiro–Wilk test.

The Pearson correlation coefficient (r) was applied to evaluate the relationship between the study variables including MIS and SQ. A p-value <0.05 was considered statistically significant for all analyses.

RESULTS

Mean age of patients in this study was 58.22 ± 10.65 years which ranged from 35 to 79 years with a higher number of male patients (62%) than the females (38%). The details of demographics, clinical and laboratory findings are shown in Table-I.

Table No. I: Demographics, clinical and laboratory findings at baseline n= 100

Demographics		
Age (Mean $\pm$ SD) years		58.22 $\pm$ 10.65
Gender	Male n (%)	62 (62)
	Female n (%)	38 (38)
Clinical assessments and laboratory findings		
BMI* (Mean $\pm$ SD) Kg/m ²		23.95 $\pm$ 3.32
MAC** (cm)		26.37 $\pm$ 2.82
Hypertension n (%)		66 (66)
Diabetes n (%)		58 (58)
Duration of MHD*** (Mean $\pm$ SD) years		2.35 $\pm$ 1.1
Serum albumin (Mean $\pm$ SD) g/dL		3.41 $\pm$ 0.09

Body mass index*, Mid-arm circumference**, Maintenance hemodialysis***.

The assessment of nutritional profile of these patients showed a mean MIS score as 15.79 ± 5.36 . Based on MIS classification, 63% of patients demonstrated mild to moderate malnutrition, followed by severe malnutrition (25%) and normal nutritional status 12% of patients. The SQ by assessed by PSQI score was 9.27 ± 4.87 , where 72% of patients had poor sleep quality (PSQI > 5) while 28% demonstrated good sleep. The details are shown in Table-2.

The analysis regarding correlation shows several significant associations among nutritional and sleep-related variables. BMI was found to be strongly correlated with MAC ($r = .921$, $p < 0.01$), indicating consistent anthropometric patterns. Higher malnutrition (MIS Score) was also significantly associated with lower BMI ($r = -.324$, $p < .01$) and lower MAC ($r = -.335$, $p < .01$), confirming that reduced body measurements reflect poorer nutritional status. The analysis regarding the primary of study revealed that PSQI significantly correlates with MIS ($r = .263$, $p < .01$), suggesting that worse nutritional status is linked with poorer sleep quality. No significant correlations

were; however, found between age, duration of dialysis, or albumin with sleep quality as shown in Table-3.

Table No.2: Distribution of nutritional profile and sleep quality categories n = 100

Details of nutritional profile		
MIS* score (Mean± SD)		15.79±5.36
MIS Category	Normal nutrition n (%)	12 (12)
	Mild to moderate malnutrition n (%)	63 (63)
	Severe malnutrition	25 (25)
Sleep quality profile		
Mean PSQI** score (Mean± SD)		9.27 ± 4.87
SQ*** category	Good SQ n (%)	28 (28)
	Poor sleep quality n (%)	72 (72)

Malnutrition-Inflammation Score*, Pittsburgh sleep quality index**, Sleep quality***.

Table No.3: Correlation between nutritional and sleep-related variables n=100

Variables	Age	BMI (kg/m ²)	MAC (cm)	Duration of Dialysis	Serum Albumin (g/dL)	MIS Score	PSQI
Age	1	0.15	0.121	-0.027	-0.098	0.015	-0.082
BMI* (kg/m ²)	0.15	1	.921 (p<0.01)	0.099	-0.003	-.324 (p<0.01)	-.138
MAC** (cm)	0.121	.921 (p<0.01)	1	0.12	-0.022	-.335 (p<0.01)	-.146
Duration of Dialysis	-0.027	0.099	0.12	1	0.047	0.001	0.055
Serum Albumin (g/dL)	-0.098	-0.003	-0.022	0.047	1	.229 (p<0.05)	-0.018
MIS*** Score	0.015	-.324 (p<0.01)	-.335 (p<0.01)	.001	.229 (p<0.05)	1	.263 (p<0.01)
PSQI****	-0.082	-0.138	-0.146	0.055	-0.018	.263 (p<0.01)	1

Body mass index*, Mid-arm circumference**, Malnutrition-inflammation score***, Pittsburgh sleep quality index****

DISCUSSION

The mean age of patients in our study was 58.22 ± 10.65 years with males comprising 62% of total study population. The findings revealed a high burden of malnutrition and sleep disturbances in the study patients. The nutritional assessment showed a mean MIS score of 15.79 ± 5.36 where some degree of malnutrition was exhibited by 88% of patients. The details showed that 63% of patients had mild-to-moderate malnutrition while severe malnutrition was present in 25% of patients. Assessment of SQ showed a mean PSQI score of 9.27 ± 4.88 , with 72% of patients experiencing poor SQ. Rizvi and Baig studied Pakistani patients on MHD and found that 63% were malnourished and only 3% were at normal nutritional

status. It is important that MAC was low despite the normal mean BMI and albumin levels, highlighting significant concern among nutritionally compromised dialysis population.¹⁴ Consistent with the above-mentioned high burden of malnutrition, sleep disturbances are also widespread among dialysis populations. The poor QS in patients of MHD was investigated by Mehrabi et al. and Mortazavi et al. with a reported high prevalence of 90.86% and 84%, respectively.^{15,16}

In a study by Soleimani et al. with 423 Iranian hemodialysis patients, the link between diet (using Dietary and Lifestyle Inflammation Scale) and sleep was investigated. A significant correlation was found between higher DLIS score and poor SQ. Patients in the highest DLIS quartile had over three times greater odds

of poor SQ which revealed a strong association between malnutrition-inflammation and impaired SQ.¹⁷ Not only inflammation but specific dietary components also found to influence SQ. Zhang et al. mentioned that adequate nutrition, particularly enriched with higher dietary fiber, is closely linked to better SQ in hemodialysis patients. Increased consumption of total, insoluble, and vegetable-derived soluble fiber was beneficial and associated with a lower prevalence of poor sleep with a clear dose-response trend ($p < 0.05$). The findings further highlighted the importance of addressing malnutrition by optimizing dietary components to enhance SQ and overall well-being of patients.¹⁸ Complementary to these findings, other investigators have evaluated the patterns of macronutrient intake and found their associations with SQ. Azarnoush et al. further investigated the association between different nutritional intakes and SQ. The results of 114 patients under **maintenance dialysis** showed that the foods with higher carbohydrates (OR 3.0; 95% CI 1.32–6.81), fats (OR 3.0; 95% CI 1.32–6.81), and fiber (OR 2.53; 95% CI 1.12–5.67) contents were significantly associated with poor SQ, while protein intake showed no such relationship. These results not only indicated the association of overall dietary intake but also emphasized on the impact of major ingredients of diet on SQ in dialysis patients.¹⁹ The above discussed clinical consequences of malnutrition extend beyond the SQ and substantially impair broader spectrum of QoL. In a study by Visiedo et al. with 120 hemodialysis patients, a high prevalence of malnutrition (62%) was reported. Malnutrition was the most significant predictor of impaired QoL, strongly correlating with both physical and mental health summary scores.²⁰ This correlation between nutritional status and SQ is not supported by some investigators and other predictors including age, BMI, diabetes history, and the calcium-phosphorus product were found to be independent predictors of poor sleep ($p < 0.05$) were mentioned by Mehrabi et al. Similarly, Mortazavi et al. found older age (61.65 vs. 51.12 years) and lower education (31.1% vs. 4%) to significantly affect the SQ ($p < 0.001$).^{15,16} The limitations needed to be shared for this study is its cross-sectional design that limits its causal inference between malnutrition and SQ. Moreover, its single-center nature and relatively small sample size may affect the generalizability of these findings to broader range of patients undergoing MHD.

CONCLUSION

A significant positive correlation is present between malnutrition and poor SQ among patients undergoing MHD. This bidirectional relationship between nutritional status and sleep disturbances necessitates a comprehensive management approach to target both aspects simultaneously. Healthcare providers should

thereby routinely assess nutritional profiles of these patients as early identification and intervention through tailored nutritional counseling, dietary supplementation, and sleep hygiene education may help to improve patient outcomes, reduce complications, and enhance overall QoL in this vulnerable segment of patients.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hina Rahim, Zahid Nabi
Drafting or Revising Critically:	Humayun Rashid, Hifsa Akram
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. KRL-HI-PUB-ERC/Apr25/81
Dated 28.04.2025

REFERENCES

1. Vaidya SR, Aeddula NR. Chronic Kidney Disease. [Updated 2024 Jul 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK535404/>.
2. Maringhini S, Zoccali C. Chronic Kidney Disease Progression—A Challenge. *Biomedicine* 2024;12(10):2203. doi:org/10.3390/biomedicine12102203.
3. Imtiaz S, Alam A. Epidemiology and Demography of Chronic Kidney Disease in Pakistan-A Review of Pakistani Literature. *Pak J Kidney Dis* 2023;7(1):2-7. doi: 10.53778/pjkd71209.
4. Cohen-Hagai K, Nacasch N, Sternschuss A, Ohana M, Wolach B, Benchetrit S, et al. Malnutrition and inflammation in hemodialysis patients: Comparative evaluation of neutrophil reactive oxygen formation. *Nutrition* 2020;78:110793. doi: 10.1016/j.nut.2020.110793.
5. Mali N, Ge J, Su F, Li CY, Fan WX. Review of risk factors of malnutrition in maintenance hemodialysis patients. *Arch Nephrol Urol* 2022; 5:24-33. doi:10.26502/anu.2644-2833049.
6. Richardson T, Gardner M, Salani M. Cardiovascular Disease and Dialysis: A Review of the Underlying Mechanisms, Methods of Risk Stratification, and Impact of Dialysis Modality Selection on Cardiovascular Outcomes. *Kidney Dial* 2025;5(1):5. doi:10.3390/kidneydial5010005.
7. Sabatino A, Regolisti G, Karupaiah T, Sahathevan S, Sadu Singh BK, Khor BH, et al. Protein-energy wasting and nutritional supplementation in patients with end-stage renal disease on hemodialysis. *Clin*

- Nutr 2017;36(3):663-671. doi: 10.1016/j.clnu.2016.06.007.
8. Bint Harun KUH, Kawser M, Nabi MH, Mitra DK. Factors associated with the malnutrition inflammation score (MIS) among hemodialysis patients in Dhaka city: a cross-sectional study in tertiary care hospitals. *Porto Biomed J* 2024;9(1):243. doi: 10.1097/j.pbj.00000000000000243.
 9. Ma X, Yan D, Zhou C, Shi Y, Wang Y, Li J, et al. The correlation between protein energy wasting and the incidence of main adverse cardiovascular events in adult maintenance hemodialysis patients: a single-center retrospective cohort study. *Ren Fail* 2025;47(1):2441399. doi: 10.1080/0886022X.2024.2441399.
 10. Davydov G G, Nashat H, Ghali S, Afifi S, Suryadevara V, Habab Y, et al. Common Sleep Disorders in Patients With Chronic Kidney Disease: A Systematic Review on What They Are and How We Should Treat Them. *Cureus* 2023;15(8): e44009. doi: 10.7759/cureus.44009.
 11. Morvaridi M, Behbahani HB, Alipour M, Javid AZ, Keramatzadeh S, Shokri S, et al. The association of Malnutrition-Inflammation Score with sleep quality and mental health in hemodialysis patients: a multicenter cross-sectional study. *BMC Nephrol* 2025;26:305. doi: 10.1186/s12882-025-04089-0.
 12. Lim LM, Kuo HT, Chao YL, Shen FC, Chen YK, Chiu YW, et al. Malnutrition-Inflammation Score of Patients with Chronic Kidney Disease from Early Stage to Initiation of Dialysis. *Nutrients* 2024;16(23):4014. doi: 10.3390/nu16234014.
 13. Carpi M. The Pittsburgh Sleep Quality Index: a brief review. *Occup Med (Lond)* 2025;75(1):14-15. doi: 10.1093/occmed/kqae121.
 14. Rizvi N, Baig M. Nutritional status of patients on maintenance hemodialysis as assessed by mini nutritional assessment survey-short form and anthropometric measurements. *Pak J Kidney Dis* 2020;4(2):249-53.
 15. Mehrabi S, Sarikhani S, Roozbeh J. Sleep Quality in Patients Undergoing Long-term Hemodialysis Using the Pittsburgh Sleep Quality Index. *Nephro-Urol Mon* 2017;9(2):e13137. doi:10.5812/numonthly.44278.
 16. Mortazavi M, Rouhani MH, Seirafian S, Motamedifard R, Hosseini M. Dietary and Sleep Quality Association in Hemodialysis Patients. *Adv Biomed Res* 2023;12:189. doi: 10.4103/abr.abr_244_22.
 17. Soleimani Damaneh M, Bavi Behbahani H, Alipour M, Zare Javid A, Keramatzadeh S, Shokri S, et al. Associations of dietary and lifestyle inflammation scores with sleep quality and mental health in hemodialysis patients: a multicenter cross-sectional study. *Nutr Metab (Lond)* 2025;22(1):69. doi: 10.1186/s12986-025-00958-5.
 18. Zhang S, Liu SX, Wu QJ, Wang ZH, Liu H, Dong C, et al. Association of dietary fiber with subjective sleep quality in hemodialysis patients: a cross-sectional study in China. *Ann Med* 2023;55(1):558-71. doi: 10.1080/07853890.2023.2176541.
 19. Azarnoush H, Mortazavi M, Rouhani MH, Seirafian S, Atapour A, Hosseini M, et al. Dietary nutrients' intake and sleep quality in peritoneal dialysis patients. *Sleep Sci* 2021;14(Spe 2):174-8. doi: 10.5935/1984-0063.20200102.
 20. Visiedo L, Rey L, Rivas F, López F, Tortajada B, Giménez R, et al. The impact of nutritional status on health-related quality of life in hemodialysis patients. *Sci Rep* 2022;12(1):3029. doi: 10.1038/s41598-022-07055-0.

Serum Ferritin an Indicator of Iron Deficiency Anemia in Children with Normal Hemoglobin

Serum Ferritin
an Indicator of
Iron Deficiency
Anemia in
Children

Hafsa Hashmi¹, Sughra Wahid² and Maliha Akhtar Qureshi³

ABSTRACT

Objective: To determine the diagnostic accuracy of serum ferritin in diagnosis of iron deficiency in children with normal haemoglobin taking composite non-invasive laboratory reference as gold standard.

Study Design: A cross-sectional validation study

Place and Duration of Study: This study was conducted at the Department of Paediatrics KRL Hospital Islamabad Pakistan, from 15th January to 15th July 2026.

Methods: A total of 165 children aged 6 months to 5 years with normal haemoglobin and clinical suspicion of iron deficiency were included. Serum ferritin was compared with a composite non-invasive laboratory reference comprising adjusted serum ferritin, soluble transferrin receptor, transferrin saturation and reticulocyte haemoglobin content. Data were analysed using Statistical Package for the Social Sciences version 26. Diagnostic performance was assessed by calculating sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and receiver operating characteristic curve.

Results: The mean age was 2.86 ± 1.29 years, mean weight was 16.52 ± 3.93 kilograms and mean serum ferritin level was 35.03 ± 22.99 micrograms per litre. There were 91 (55.2%) males and 74 (44.8%) females. Serum ferritin identified iron deficiency in 90 (54.5%) children, while the composite laboratory reference detected 74 (44.8%) cases. Serum ferritin showed sensitivity of 87.84%, specificity of 72.53%, positive predictive value of 72.22%, negative predictive value of 88.00% and overall diagnostic accuracy of 79.39%.

Conclusion: Serum ferritin was a useful and sensitive test for detecting iron deficiency in children with normal haemoglobin.

Key Words: Child, Diagnosis, Ferritin, Haemoglobin, Iron deficiency, Sensitivity and specificity

Citation of article: Hashmi H, Wahid S, Qureshi MA. Serum Ferritin an Indicator of Iron Deficiency Anemia in Children with Normal Hemoglobin. Med Forum 2026;37(9):126-131. doi:10.60110/medforum.370924.

INTRODUCTION

Iron Deficiency Anemia has been regarded as one of the most prevalent nutrient deficiencies in the world. This has been noticed in the case of children in both developing as well as developed countries.¹ Among the numerous markers used in the diagnosis as well as the determination of the severity of the situation of IDA, the role of serum ferritin has been found to be extremely important as it has the ability to identify the iron status in the body.¹ For a long time now, the diagnosis of Anemia has been greatly reliant upon the estimation of Hemoglobin levels.²

Current studies reveal that in the case of children, iron stores can become depleted even when Hemoglobin levels are found to be normal. This clearly goes on to identify the indispensable role of serum ferritin as a more specific marker.²

Children are at high risk of iron deficiency because of their high iron requirements during their rapid rates of growth. Iron has been shown to play a pivotal role in participating in important functions in the human body like oxygen transport, DNA synthesis, and enzymatic reactions.³ Thus, the lack of iron can generate reduced intellectual function, poor physical development, and immune dysfunction in the pediatric population.⁴ The clinical progression of iron deficiency has been shown to develop through sequential stages that include iron depletion leading to the involvement of the iron-recursive pathway. This finally culminates in the development of iron-deficiency anemia.⁵ Among the important markers used in the sequence of iron deficiency development has been the determination of serum ferritin. This has proven very important as it has given the clinician the opportunity to prevent the outcome of iron deficiency progressing towards the development of iron-deficiency anemia.⁵

¹. Resident / Head of Department² / Consultant³, Department of Pediatrics, KRL Hospital Islamabad.

Correspondence: Hafsa Hashmi, Resident, Department of Pediatrics, KRL Hospital Islamabad.

Contact No: 0300 9141719

Email: malaiyka@gmail.com

Received: July, 2026

Reviewed: August, 2026

Accepted: August, 2026

Ferritin in the serum is a intracellular protein that stores iron and releases it in a regulated fashion. This protein has a very important role to play in the iron regulatory functions of the body. When iron levels are sufficient in the body, the serum ferritin concentration ranges between 15-200 ng/ml in children.⁶ When the concentration is lower than that, the iron stores are depleted despite the fact that the patient may not have iron-deficiency anemia.⁷ However, serum ferritin levels must be interpreted cautiously, as they can be influenced by various factors, including inflammation, infection, and chronic disease. During acute or chronic inflammatory states, ferritin behaves as an acute-phase reactant, leading to elevated levels irrespective of iron stores.⁸ Therefore, concurrent assessment of other parameters, such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), is recommended to differentiate between true iron deficiency and inflammation-induced changes.⁸

Thus, the proposed aim of the current researcher proposal has been set to examine the accuracy of the serum ferritin test in the detection of iron deficiency compared to the reference standard

METHODS

This cross-sectional validation study was carried out in the Department of Paediatrics, KRL Hospital, Islamabad, from 15th January 2026 to 15th July 2026. Ethical approval was obtained from the Institutional Ethical Review Committee of the hospital before starting the study. All study procedures were performed according to the institutional ethical guidelines. The sample size was calculated by using a diagnostic accuracy (sensitivity/specificity) calculator with expected sensitivity of 81.6%,⁹ specificity of 37.5%,⁹ prevalence of 44.8%,⁹ 95% confidence level and $\pm 10\%$ absolute precision for both sensitivity and specificity. The calculated sample size was 165 children.

Children aged 6 months to 5 years of either gender, presenting with normal haemoglobin and having clinical suspicion of iron deficiency based on history of fatigue, poor growth or developmental delay were included in the study. Normal haemoglobin was considered as haemoglobin level >10.5 g/dl for children aged 6 months to 2 years and >11.5 g/dl for children aged more than 2 years up to 5 years. Children having history of chronic inflammatory diseases including juvenile rheumatoid arthritis or chronic infections, blood transfusion during the previous 4 weeks, overt anaemia with pallor and fatigue, liver disease, renal dysfunction, severe malnutrition or underweight according to WHO growth standards, coeliac disease, inflammatory bowel disease, or use of iron supplementation within the last 3 months were excluded. Baseline demographic information including age, gender, weight, area of residence and family socioeconomic background was recorded. A detailed

physical examination was performed to identify signs of overt anaemia, nutritional status, and other clinical findings relevant to the study.

A total of 10 ml of venous blood was collected by trained phlebotomists under aseptic conditions. Five ml of blood was placed in an EDTA tube for complete blood count analysis, while another 5 ml was collected in a yellow-top vacutainer for biochemical investigations. All specimens were immediately transported to the hospital laboratory for analysis. Laboratory investigations included serum ferritin adjusted for inflammation using C-reactive protein, soluble transferrin receptor (sTfR), transferrin saturation (TSAT), and reticulocyte haemoglobin content (Ret-He). The results of serum ferritin and composite non-invasive laboratory reference were documented on the study proforma for every participant. Iron deficiency by serum ferritin was considered when serum ferritin level was <30 $\mu\text{g/L}$. Iron deficiency by composite non-invasive laboratory reference was considered when at least 2 iron parameters were abnormal, including adjusted serum ferritin <12 $\mu\text{g/L}$ or <30 $\mu\text{g/L}$ in the presence of inflammation, soluble transferrin receptor-log ferritin index >5.6 , transferrin saturation $<20\%$ or reticulocyte haemoglobin <27 pg.

The collected data were entered and analysed using IBM SPSS version 26. Quantitative variables including age and weight were presented as mean $\pm$ standard deviation. Qualitative variables including gender, area of residence, socioeconomic background, serum ferritin results and composite non-invasive laboratory reference were expressed as frequencies and percentages.

RESULTS

The mean age of children was 2.86 ± 1.29 years and mean weight was 16.52 ± 3.93 kg, with serum ferritin level recorded as 35.03 ± 22.99 $\mu\text{g/L}$.

Table No. 1: Patient Demographics.

Demographics	Mean $\pm$ SD
Age (years)	2.86 ± 1.29
Weight (kg)	16.52 ± 3.93
Serum Ferritin Level ($\mu\text{g/L}$)	35.03 ± 22.99
Gender	
Male n (%)	91 (55.2%)
Female n (%)	74 (44.8%)
Residential Status	
Rural n (%)	90 (54.5%)
Urban n (%)	75 (45.5%)
Family Socioeconomic Status	
Low n (%)	58 (35.2%)
Middle n (%)	90 (54.5%)
High n (%)	17 (10.3%)

Among the participants, male was 91(55.2%) and female were 74(44.8%). Regarding residential status, majority were belonged to rural area 90(54.5%) while 75(45.5%) were from urban area. Concerning family socioeconomic status, low status was found in 58(35.2%), middle status in 90(54.5%) and high status in 17(10.3%) of children (Table-I).

On comparing iron deficiency diagnosis, serum ferritin detected positive result in 90(54.5%) and negative in 75(45.5%) of cases, whereas composite laboratory reference showed positive in 74(44.8%) and negative in 91(55.2%) cases, total sample being 165(100%) for both parameter (Table-2).

Table No.2: Overall results of Serum Ferritin and Composite Laboratory Reference in diagnosis of Iron Deficiency.

Iron Deficiency	Serum Ferritin	Composite Laboratory Reference
Positive	90(54.5%)	74(44.8%)
Negative	75(45.5%)	91(55.2%)
Total	165 (100%)	165 (100%)

When serum ferritin was compared against composite laboratory reference, true positive were found in 65 cases, false positive in 25 cases, false negative in 9 cases and true negative in 66 cases, with total positive on composite reference being 74 and total negative being 91 (Table-3).

Table No.3: Comparison of Serum Ferritin versus Composite Laboratory Reference in diagnosis of Iron Deficiency.

Serum Ferritin	Composite Laboratory Reference	Total
	Positive	Negative
Positive	65 (TP)	25 (FP)
Negative	9 (FN)	66 (TN)
Total	74	91

Key: TP = True positive, FP = False positive, FN = False negative, TN = True negative

The diagnostic performance of serum ferritin in diagnosing iron deficiency showed sensitivity of

87.84%, specificity of 72.53%, diagnostic accuracy of 79.39%, positive predictive value of 72.22% and negative predictive value of 88% (Table-4).

Table No.4: Sensitivity, Specificity, Diagnostic Accuracy, PPV and NPV of Serum Ferritin in diagnosis of Iron Deficiency.

Diagnostic Parameter	Result
Sensitivity	87.84%
Specificity	72.53%
Diagnostic Accuracy	79.39%
PPV	72.22%
NPV	88%

Diagnostic accuracy was best in high socioeconomic group (94.12%) and least in middle socioeconomic group (74.44%). Negative predictive value was notably high across most subgroup, particularly in high socioeconomic group (100%) and urban residents (95.74%), whereas positive predictive value were comparatively low in urban (50%), weight >15kg (50%) and middle socioeconomic (48.78%) subgroup (Table-5).

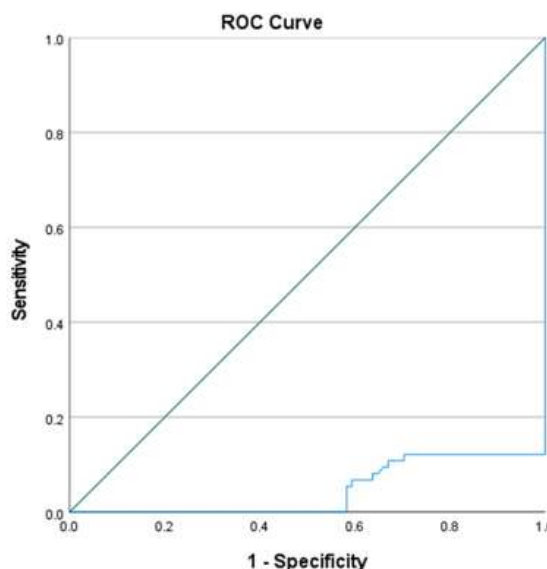


Figure No.1: ROC Curve

Table No.5: Stratified analysis of Sensitivity, Specificity, Diagnostic Accuracy, PPV and NPV of Serum Ferritin in diagnosis of Iron Deficiency with demographics.

Variables	Groups	Diagnostic Parameter	Result
Age (years)	≤3	Sen	88.33%
		Spec	68%
		DA	82.35%
		PPV	86.89%
		NPV	70.83%
	>3	Sen	85.71%
		Spec	74.24%
		DA	76.25%

Gender	Male	PPV	41.38%
		NPV	96.08%
		Sen	92.50%
		Spec	72.55%
		DA	81.32%
	Female	PPV	72.55%
		NPV	92.50%
		Sen	82.35%
		Spec	72.50%
		DA	77.03%
Weight (kg)	≤15	PPV	71.79%
		NPV	82.86%
		Sen	93.75%
		Spec	54.55%
		DA	86.44%
	>15	PPV	90%
		NPV	66.67%
		Sen	76.92%
		Spec	75%
		DA	75.47%
Residential Status	Rural	PPV	50%
		NPV	90.91%
		Sen	87.93%
		Spec	65.63%
		DA	80%
	Urban	PPV	82.26%
		NPV	75%
		Sen	87.50%
		Spec	76.27%
		DA	78.67%
Family Socioeconomic Status	Low	PPV	50%
		NPV	95.74%
		Sen	84.09%
		Spec	78.57%
		DA	82.76%
	Middle	PPV	92.50%
		NPV	61.11%
		Sen	90.91%
		Spec	69.12%
		DA	74.44%
	High	PPV	48.78%
		NPV	95.92%
		Sen	100%
		Spec	88.89%
		DA	94.12%
		PPV	88.89%
		NPV	100%
		Sen	100%

The optimal cutoff value of serum ferritin for diagnosis of iron deficiency was determined as $\leq 21.90 \mu\text{g/L}$, at which sensitivity was 87.80%, specificity was 100% and area under curve was 0.954 (Fig-1)

DISCUSSION

The present study was conducted to evaluate role of serum ferritin as indicator of iron deficiency in children

having normal hemoglobin level. Total of 165 children were enrolled, among them serum ferritin detected iron deficiency in 90(54.5%) of cases as compared to composite laboratory reference which detected 74(44.8%) cases, suggesting that serum ferritin alone may overestimate the burden of iron deficiency when used without another supportive parameter. Serum ferritin showed sensitivity of 87.84% and specificity of

72.53% in detecting iron deficiency, with diagnostic accuracy of 79.39%. The high sensitivity of this parameter implies that serum ferritin test is quite dependable in diagnosing iron deficiency, and this could be due to the fact that the serum ferritin levels represent the level of iron content within the body, hence falling when there is an iron shortage, even before any effect is experienced on the hemoglobin levels. Relatively low specificity on the other hand implies that it is not always dependable in ruling out iron deficiency because serum ferritin levels are sometimes influenced by conditions unrelated to iron such as infections.

The present study found serum ferritin positive for iron deficiency in 90(54.5%) of children while composite laboratory reference detected only 74(44.8%) cases, with sensitivity of 87.84% and specificity of 72.53%. This is comparable to findings of Khan and Shah¹⁰ who also reported serum ferritin as most sensitive marker among iron indices, with sensitivity of 85%, though their specificity was markedly lower at 15%, possibly because their sample comprised already anemic children where overlap of inflammatory and depletion state is more common, reducing specificity of test further than seen in present study.

Findings of present study, where 39 children had no overt anaemia yet abnormal ferritin reflecting subclinical iron deficiency, are in line with study by Ibrahim and Habeeb¹¹ who studied 150 children with normal haemoglobin but low MCV and MCH and found mean ferritin of 86.5 ± 7 ng/mL, supporting that ferritin can detect deficiency even before haemoglobin become affected, similar principle being demonstrated in present cohort where iron deficiency was identified despite normal Hb status.

Zaman et al.¹² reported sensitivity of anaemia for predicting depleted iron store as only 33.8%, much lower than sensitivity of serum ferritin (87.84%) observed in present study, indicating that ferritin is far superior marker compared to haemoglobin alone for detecting iron deficiency, this difference likely arise because anaemia is late manifestation of iron depletion whereas ferritin decline early in course of negative iron balance.^{13,14}

Khan et al.¹⁵ reported higher ferritin in rural compared to urban children and positive correlation with hygienic condition, somewhat differing from present study where diagnostic accuracy was comparatively better in urban children, this discrepancy may be due to difference in nutritional and healthcare access pattern across different population, with urban setting in present cohort possibly having better access to balanced diet reducing confounding inflammatory influence on ferritin.

The variation observed across socioeconomic group in present study, with best diagnostic accuracy in high socioeconomic class, is consistent with epidemiological

pattern described by Animasahun et al.¹⁶ highlighting that nutritional deprivation and lower socioeconomic status increase burden and diagnostic complexity of iron deficiency, particularly in non-industrialised setting, reinforcing that socioeconomic factor remain important determinant of both prevalence and diagnostic performance of ferritin based testing.

CONCLUSION

The current study found out that serum ferritin acts as an accurate and sensitive marker in assessing iron deficiency in children with normal hemoglobin status. It can accurately diagnose subclinical iron deficiency prior to hemoglobin being affected.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Hafsa Hashmi, Sughra Wahid
Drafting or Revising Critically:	Hafsa Hashmi, Maliha Akhtar Qureshi
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. CPSP/REU/PED-2022-045-7351
Dated 14.01.2026

REFERENCES

- Kumar SB, Arnipalli SR, Mehta P, Carrau S, Ziouzenkova O. Iron deficiency anemia: efficacy and limitations of nutritional and comprehensive mitigation strategies. *Nutrients* 2022;14(14):2976.
- Aksu T, Ünal Ş. Iron deficiency anemia in infancy, childhood, and adolescence. *Turk Arch Pediatr* 2023;58(4):358-362.
- Kumar A, Sharma E, Marley A, Samaan MA, Brookes MJ. Iron deficiency anaemia: pathophysiology, assessment, practical management. *BMJ Open Gastroenterol* 2022; 9(1):e000759.
- Chouraqui JP. Dietary approaches to iron deficiency prevention in childhood-a critical public health issue. *Nutrients* 2022;14(8):1604.
- Yang J, Li Q, Feng Y, Zeng Y. Iron deficiency and iron deficiency anemia: potential risk factors in bone loss. *Int J Mol Sci* 2023;24(8):6891.
- Kotla NK, Dutta P, Parimi S, Das NK. The role of ferritin in health and disease: recent advances and understandings. *Metabolites*. 2022;12(7):609.
- Chaber R, Helwich E, Lauterbach R, Mastalerz-Migas A, Matysiak M, Peregud-Pogorzelski J, et al. Diagnosis and treatment of iron deficiency and

- iron deficiency anemia in children and adolescents: recommendations of the Polish Pediatric Society, the Polish Society of Pediatric Oncology and Hematology, the Polish Society of Neonatology, and the Polish Society of Family Medicine. *Nutrients* 2024;16(21):3623.
8. Garcia-Casal MN, Pasricha SR, Martinez RX, Lopez-Perez L, Peña-Rosas JP. Serum or plasma ferritin concentration as an index of iron deficiency and overload. *Cochrane Database Syst Rev* 2021;5(5):CD011817.
 9. Jonker FAM, Boele van Hensbroek M, Leenstra T, Vet RJWM, Brabin BJ, Maseko N, et al. Conventional and novel peripheral blood iron markers compared against bone marrow in Malawian children. *J Clin Pathol* 2014;67(8): 717-723.
 10. Khan AS, Shah SA. Iron deficient children and significance of serum ferritin. *J Pak Med Assoc* 2005;55(10):420-421.
 11. Ibrahim TH, Habeeb HO. Serum ferritin level in children with normal haemoglobin and low MCV and MCH in Al Sajad General Hospital in Al Najaf. *J Med Res Rev* 2024;2(4):138-146. doi: 10.5455/JMRR.20240822080712.
 12. Zaman IF, Junaid A, Adil MM. Value of anaemia as a predictor of iron deficiency in children. *Rawal Med J* 2009;34:61-64.
 13. Bignonbhai PR, Khanna PPC. Assessment of serum ferritin level in detection of iron deficiency anaemia in paediatric patients: prospective analysis. *Int J Paediatr Geriatr* 2021;4(2):07-09. doi: 10.33545/26643685.2021.v4.i2a.148.
 14. Sheikh WH, Iqbal H, Younas M, Hamdani NR, Ali O, Ain UQ. Determination of the association between serum ferritin and C-reactive protein in children presented with iron deficiency anaemia in Combined Military Hospital Pathology Department Multan. *Pak Armed Forces Med J* 2022;72(Suppl-2): S99-S102. doi: 10.51253/pafmj.v72iSUPPL-2.3036.
 15. Khan MS, Shahnaz, Sultana R, Ali I. Factors affecting serum ferritin level in school going children of District Bannu. *J Postgrad Med Inst* 2012;26(3):237-241.
 16. Animasahun BA, Itiola AY. Iron deficiency and iron deficiency anaemia in children: physiology, epidemiology, aetiology, clinical effects, laboratory diagnosis and treatment: literature review. *J Xiangya Med* 2021;6:22. doi: 10.21037/jxym-21-6.

Diagnostic Accuracy of Upper Lip Bite Test Versus Modified Mallampati for Predicting Difficult Airway in Patients coming for Surgery in a Tertiary Care Hospital

Diagnostic Accuracy of ULBT Versus MMT for Difficult Airway

Asma Zareen¹, Muhammad Zameer Rajput² and Muhammad Ashraf²

ABSTRACT

Objective: This study aimed to compare the diagnostic accuracy of the ULBT and MMT for predicting difficult laryngoscopy when using the Cormack and Lehane (CL) grading system as the gold standard.

Study Design: Cross-sectional validation study

Place and Duration of Study: This study was conducted at the Shifa International Hospital, Islamabad, during October 2025 till March 2026.

Methods: This study conducted on 211 patients undergoing elective surgery under general anesthesia. Pre-operative airway assessments like MMT and ULBT were performed by the primary investigator. During surgery a second anesthesiologist did laryngoscopy and recorded the CL grade. Diagnostic accuracy indices such as the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy were calculated and compared for both tests.

Results: Difficult airways (CL grade 3) were seen in 22 patients (10.4%). The ULBT had a superior diagnostic performance compared to the MMT across all indices. ULBT had a sensitivity of 86.4%, specificity of 96.3% and accuracy of 95.3% (AUC 0.887). In contrast, the MMT showed lower sensitivity (77.3%), specificity (82.5%) accuracy (82.0%; AUC 0.804), with a notably low PPV (34.0%) indicating a high false-positive rate.

Conclusion: Upper Lip Bite Test proved to be a more accurate and reliable bedside predictor of difficult laryngoscopy than the Modified Mallampati Test.

Key Words: Airway, Intubation, Laryngoscopy, Upper Lip Bite

Citation of article: Zareen A, Rajput MZ, Ashraf M. Diagnostic Accuracy of Upper Lip Bite Test Versus Modified Mallampati for Predicting Difficult Airway in Patients coming for Surgery in a Tertiary Care Hospital. Med Forum 2026;37(9):132-137. doi:10.60110/medforum.370925.

INTRODUCTION

Despite the tremendous advances in anesthesia techniques and equipment, the occurrence of a difficult airway during intubation still leads to serious anesthesia-related injuries and is the most common cause of anesthesia malpractice claims.^{1,2} According to the 2022 American Society of Anesthesiologists Practice Guideline for Management of the Difficult Airway, difficult airways can be further divided into seven types: difficult facemask ventilation, difficult laryngoscope, difficult supraglottic airway ventilation,

difficult or failed tracheal intubation, difficult or failed tracheal extubation, difficult or failed invasive airway, and inadequate ventilation.³ The incidence of difficult intubation as high as 1–18% is still a difficult situation for clinical anesthesiologists.^{4,5} The worst-case situation is "cannot intubate cannot oxygenate (CICO)", and up to one-third of anesthesia-related deaths are due to failure to intubate and ventilate, so this deserves our utmost attention. The occurrence of a difficult airway can lead to tracheal or esophageal injury, aspiration, and severe hypoxemia, which can cause irreversible brain damage and lead to death.⁶ Unfortunately, there is still no consensus on the best method to assess a difficult airway.⁷ Many researchers have attempted to predict difficult intubation by using simple bedside physical examinations, such as the upper lip bite test (ULBT) and the modified mallampati test (MMT).⁵ It has been reported that ULBT appears to be a useful bedside test for predicting difficult airways with moderate sensitivity and high specificity, with higher accuracy than MMT.^{8,9} Any test needs to be proven over and over again. In some studies, assessment of ULTB was a better or moderate predictor to difficult

¹. Resident / Consultant², Department of Anesthesia, Shifa International Hospital, Islamabad.

Correspondence: Dr. Asma Zareen, Resident, Department of Anesthesia, Shifa International Hospital, Islamabad
Contact No: 03365115501
Email: zarigulzar38@gmail.com

Received: May, 2026
Reviewed: June-July, 2026
Accepted: August, 2026

airway than MMT.^{8,10} but in some studies ULBT is not superior to MMT.¹¹ Kar S et al 2022[12] reported the occurrence of difficult intubation was found to be 10.2% (23/225 patients) and the sensitivity, (95.5% vs. 95.4%), specificity (54.8% vs. 50.0%), positive predictive value (91.6% vs. 93.1%), and negative predictive value (39.1% vs. 39.1%) were almost comparable between modified Mallampati test and upper lip bite test. Wang LY 2022 et al¹¹ reported the incidence of difficult airway was 13.5% and the sensitivity, specificity and accuracy for ULBT was 11.59%, 93.96%, 81.33% for MMT 62.32%, 69.29%, 66.2%.

The purpose of this study was to find the simplest and most intuitive strategy for predicting difficult intubation during direct laryngoscopy in patients who did not have risk factors for difficult airways, as well as to evaluate the utility of the modified Mallampati test and upper lip bite tests in this regard.

METHODS

A cross-sectional validation study was conducted in the Department of Anesthesiology, Shifa International Hospital, Islamabad, during October 2025 till March 2026, with the objective of determining the diagnostic accuracy of the Upper Lip Bite Test (ULBT) and Modified Mallampati Test (MMT) for predicting difficult airways in patients undergoing surgery, using the Cormack and Lehane (CL) grading system as the gold standard. Difficult airway was defined as the clinical situation in which a conventionally trained anesthesiologist experienced difficulty with face-mask ventilation of the upper airway, difficulty with tracheal intubation, or both. The sample size was calculated on the basis of a previous study by Kar et al. [18], which reported an occurrence of difficult intubation of 10.2% (23/225 patients), with sensitivity of 95.5% and 95.4% and specificity of 54.8% and 50.0% for MMT and ULBT, respectively, when compared with the Cormack and Lehane classification. A total of 211 patients were required to estimate the diagnostic accuracy of ULBT and MMT with a 9% margin of error and a 95% confidence interval. Consecutive sampling was used for patient recruitment. Patients aged 18 to 70 years, with ASA physical status I to III, who were scheduled for elective surgery requiring general anesthesia with laryngoscopy and endotracheal intubation for orthopedic, thoracic, neurosurgical, or general surgical procedures were included. Patients with limited mouth opening, BMI >35 kg/m², a history of burns or trauma to the airway, inability to stand erect or sit, edentulous patients, tumors or masses involving the cervical or facial regions, limited cervical movement due to conditions such as rheumatoid arthritis or cervical disc disorders, pregnant women, and patients undergoing emergency surgery were excluded. On the day before surgery, the primary investigator, an anesthesiologist, visited the enrolled patients and recorded relevant

information, including type of surgery, age, gender, weight, height, BMI, ASA physical status, medical history, ULBT grade, and MMT grade, on a structured data collection proforma. The Modified Mallampati Test was performed with the patient in a sitting position by asking the patient to open the mouth maximally and protrude the tongue without phonation, and the oropharyngeal structures were visualized using a torch or flashlight. The Upper Lip Bite Test was performed by asking the patient to bite the upper lip with the lower incisors and was graded as Grade I when the lower incisors could bite the upper lip above the vermilion line, Grade II when the lower incisors could bite the upper lip below the vermilion line, and Grade III when the lower incisors could not bite the upper lip. ULBT Grades I and II were considered predictive of easy intubation, whereas Grade III was considered predictive of difficult intubation. On the day of surgery, a second anesthesiologist with more than five years of anesthesia experience performed direct laryngoscopy after induction of anesthesia and sufficient muscle relaxation and assessed the laryngoscopic view using a Macintosh laryngoscope with a size 3 or 4 blade. The CL grade obtained during direct laryngoscopy was considered the gold standard for determining difficult laryngoscopy. In the operating room, routine monitoring, including non-invasive arterial blood pressure, electrocardiography, and oxygen saturation, was applied. Patients were preoxygenated with 100% oxygen for three minutes. General anesthesia was induced according to the discretion of the primary anesthesiologist, and the use of a non-depolarizing muscle relaxant was also determined by the primary anesthesiologist according to routine clinical practice. Anesthesia was maintained using a 50:50 oxygen and nitrous oxide or air mixture with isoflurane or sevoflurane, and ventilation was performed by bag-mask ventilation until the patient was completely paralyzed. The patient's head was placed in the sniffing position before laryngoscopy. For assessment of MMT against the CL classification, MMT Grades III and IV were considered a positive test result for difficult laryngoscopy and CL Grades III and IV were considered positive for difficult laryngoscopy. The collected data were entered and analyzed using appropriate statistical software. Continuous variables were summarized using mean and standard deviation, while categorical variables were presented as frequencies and percentages. The association between the airway assessment tests and CL grades was assessed using the Chi-square test or Fisher's exact test where appropriate, and diagnostic accuracy measures were reported with 95% confidence intervals. A p-value of <0.05 was considered statistically significant.

RESULTS

The sample comprised 130 men (61.6%) and 81 women (38.4%). Most patients were ASA class II (49.3%), followed by ASA I (39.8%) and ASA III (10.9%). On

the Cormack-Lehane gold standard, 22 patients (10.4%) had a difficult airway (grade 3); MMT classified 50 patients (23.7%) as difficult, and ULBT classified 26 patients (12.3%) as difficult.

Table No.1: Frequency and percentage distribution of categorical variables (N = 211).

Category	Frequency	Percent (%)
Gender – Male	130	61.6
Gender – Female	81	38.4
ASA – I	84	39.8
ASA – II	104	49.3
ASA – III	23	10.9
Cormack-Lehane grade 1	137	64.9
Cormack-Lehane grade 2	52	24.6
Cormack-Lehane grade 3	22	10.4
MMT grade 1	105	49.8
MMT grade 2	56	26.5
MMT grade 3	44	20.9
MMT grade 4	6	2.8
ULBT grade 1	111	52.6

ULBT grade 2	74	35.1
ULBT grade 3	26	12.3

Tables 2a and 2b present the 2×2 contingency tables of MMT and ULBT against the Cormack-Lehane gold standard (grade 3 = difficult airway).

Table 2a: 2×2 contingency table: MMT vs Cormack-Lehane gold standard.

	CL Difficult	CL Easy	Total
MMT Difficult	17	33	50
MMT Easy	5	156	161
Total	22	189	211

Table 2b: 2×2 contingency table: ULBT vs Cormack-Lehane gold standard.

	CL Difficult	CL Easy	Total
ULBT Difficult	19	7	26
ULBT Easy	3	182	185
Total	22	189	211

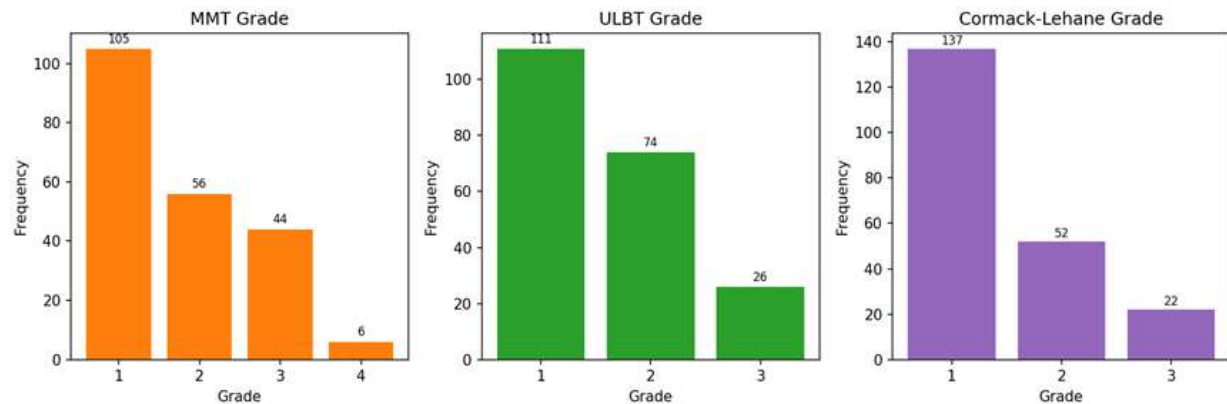


Figure No.1: Frequency distribution of MMT, ULBT and Cormack-Lehane grades.

Table No.3: Diagnostic accuracy of MMT and ULBT against the Cormack-Lehane gold standard (95% CI, Wilson score method).

Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)
MMT	77.3 (56.6–89.9)	82.5 (76.5–87.3)	34 (22.4–47.8)	96.9 (92.9–98.7)	82 (76.2–86.6)
ULBT	86.4 (66.7–95.3)	96.3 (92.6–98.2)	73.1 (53.9–86.3)	98.4 (95.3–99.4)	95.3 (91.5–97.4)

Overall, ULBT outperformed MMT on every diagnostic index. ULBT achieved a sensitivity of 86.4% (95% CI 66.7–95.3), specificity of 96.3% (95% CI

92.6–98.2), PPV of 73.1%, NPV of 98.4% and overall accuracy of 95.3% (95% CI 91.5–97.4). MMT achieved a lower sensitivity of 77.3% (95% CI 56.6–89.9),

specificity of 82.5% (95% CI 76.5–87.3), a notably lower PPV of 34%; reflecting a high false-positive rate (33 of 189 true-easy airways misclassified as difficult) and an overall accuracy of 82% (95% CI 76.2–86.6).

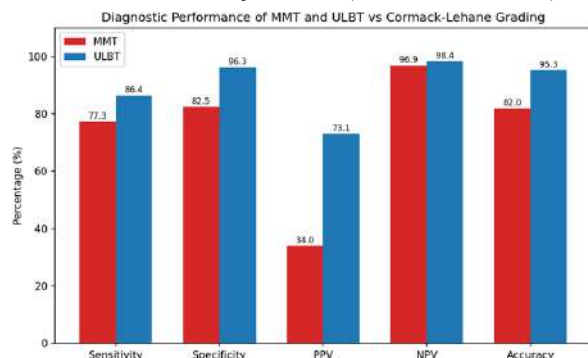


Figure No. 2: Diagnostic performance metrics of MMT vs ULBT (sensitivity, specificity, PPV, NPV, accuracy).

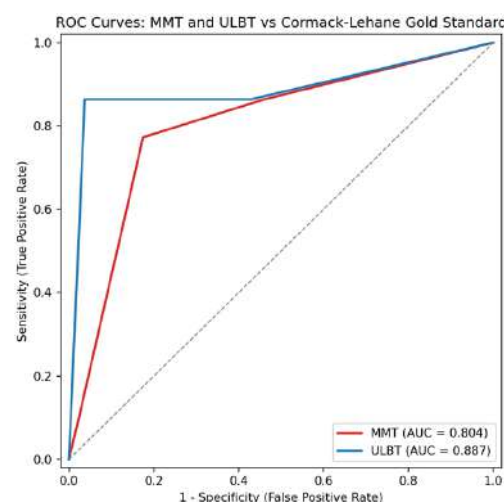


Figure No. 3: ROC curves for MMT and ULBT against the Cormack-Lehane gold standard.

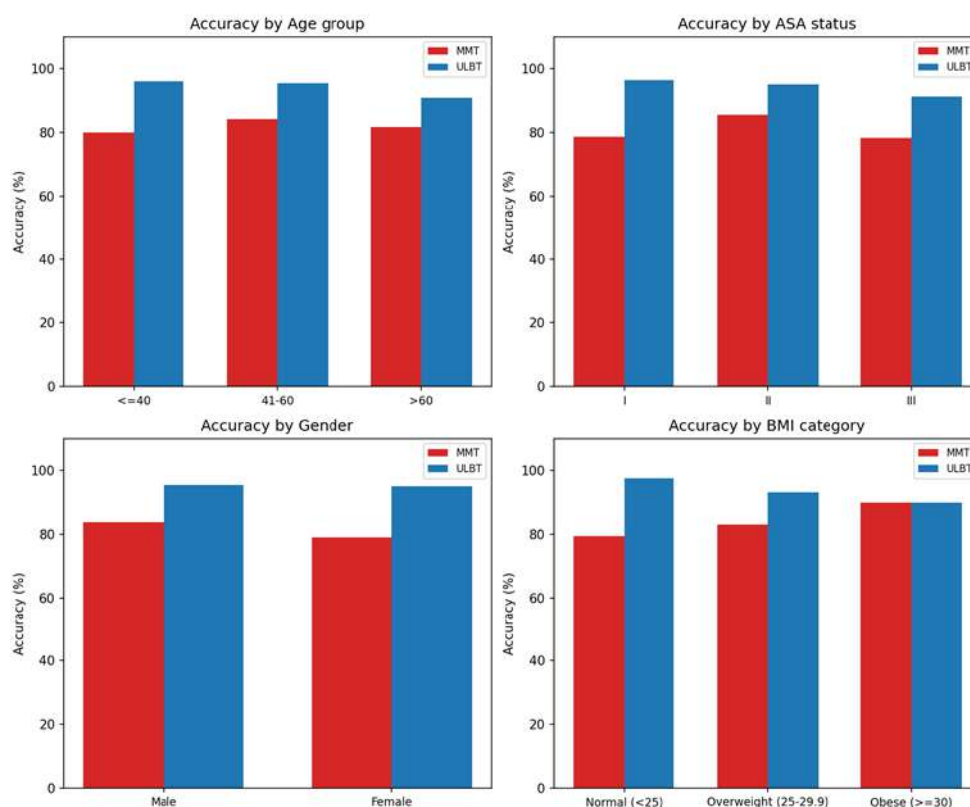


Figure No.4: Stratified accuracy of MMT and ULBT by age group, ASA status, gender and BMI category.

Stratified by Age group

Table No. 4: Diagnostic accuracy of MMT and ULBT vs Cormack-Lehane, stratified by age group.

Subgroup	Test	N	Sens. %	Spec. %	PPV %	NPV %	Acc. %
<=40	MMT	100	77.8	80.2	28	97.3	80
<=40	ULBT	100	77.8	97.8	77.8	97.8	96
41-60	MMT	89	72.7	85.9	42.1	95.7	84.3

41-60	ULBT	89	90.9	96.2	76.9	98.7	95.5
>60	MMT	22	100	80	33.3	100	81.8
>60	ULBT	22	100	90	50	100	90.9

Stratified by ASA status

Table No.5: Diagnostic accuracy of MMT and ULBT vs Cormack-Lehane, stratified by asa status.

Subgroup	Test	N	Sens. %	Spec. %	PPV %	NPV %	Acc. %
I	MMT	84	62.5	80.3	25	95.3	78.6
I	ULBT	84	87.5	97.4	77.8	98.7	96.4
II	MMT	104	91.7	84.8	44	98.7	85.6
II	ULBT	104	91.7	95.7	73.3	98.9	95.2
III	MMT	23	50	81	20	94.4	78.3
III	ULBT	23	50	95.2	50	95.2	91.3

DISCUSSION

The superiority of ULBT over MMT observed in this cohort (accuracy 95.3% vs 82.0%; AUC 0.887 vs 0.804) is in line with the tests' original validation. Khan et al said that the ULBT and reported a accuracy of 88%, sensitivity of 76.5% and specificity of 88.7% for the test. These figures broadly in line with the present results¹⁰.

In a small, blinded comparison, Hester and colleagues found the ULBT outperformed MMT on every index. Sensitivity (55% vs 11%), specificity (97% vs 75%) and accuracy (90% vs 64%) a gap even larger than the one observed here in our study¹³. Within a South Asian population comparable to us, Ali et al. evaluated 324 patients in a Pakistani tertiary-care hospital. They have similarly reported significantly higher accuracy, PPV and NPV for ULBT over MMT but they did not find a significant difference in specificity between the two tests¹⁴.

Taken together, these studies support the general direction of the present findings: ULBT is a more discriminating single bedside test than MMT for anticipating difficult airway. A systematic review done by Moslemi F, et al. pooled 27 prospective studies of the ULBT against Cormack-Lehane. They have found that ULBT accuracy exceeds 85% in 24 of the 27 studies and specificity exceeded 85% in all but one, closely matching the 95.3% accuracy and 96.3% specificity obtained in the present dataset; however, ULBT sensitivity exceeded 70% in only 11 of the 27 pooled studies, indicating that the 86.4% sensitivity found here sits toward the favourable end of the reported range⁸. ULBT has been seen as a good predictor in other study too¹⁵.

Another systematic review and meta-analysis by Wang et al., synthesised 227 studies (686,089 patients) on multiple difficult-airway prediction methods. This was a huge study and reported pooled sensitivity/specificity of 0.39/0.86 for MMT and 0.52/0.84 for ULBT¹⁶.

Against this benchmark, both tests in our study were better on sensitivity (MMT 77.3% vs pooled 39%; ULBT 86.4% vs pooled 52%), while specificity was comparable.

CONCLUSION

Upper Lip Bite Test proved to be a more accurate and reliable bedside predictor of difficult laryngoscopy than the Modified Mallampati Test. While both tests have limitations, the ULBT's higher specificity and lower false-positive rate make it a valuable adjunct for pre-operative airway assessment. Given the variable performance of these tests in broader literature, a combined-test strategy is recommended for enhanced predictive accuracy in clinical practice.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Asma Zareen, Muhammad Zameer Rajput
Drafting or Revising Critically:	Asma Zareen, Muhammad Ashraf
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. CPSP/REU/ANS-2023-043-3121 Dated 29.09.2025

REFERENCES

1. de Santana Lemos C, de Brito Poveda V. Adverse events in anesthesia: an integrative review. J Perianesth Nurs 2019;34(5):978–988.

2. Pimentel MP, Chung S, Ross JM, Wright D, Urman RD. Anesthesia-related closed claims in free-standing ambulatory surgery centers. *Anesth Analg* 2024;139(3):521-531. doi: 10.1213/ANE.0000000000006700. Epub 2024 Apr 19.
3. Apfelbaum JL, Hagberg CA, Connis RT, Abdelmalak BB, Agarkar M, Dutton RP, et al. 2022 American Society of Anesthesiologists practice guidelines for management of the difficult airway. *Anesthesiol* 2022;136(1):31-81.
4. Richa F, Chalhoub V, Gebrayel WB. Upper lip bite test versus modified Mallampati classification in predicting difficult laryngoscopy and/or intubation among morbidly obese patients. *J Clin Anesth* 2020;63:109761.
5. Bai W, Klumpner T, Zhao X, Mentz G, Green G, Riegger LQ, et al. Difficult airway management in children with trisomy 18: a retrospective single-centre study of incidence, outcomes, and complications. *Br J Anaesth* 2023;130(6):e471-e473.
6. Thomas M, Saldanha NM. Comparison of upper lip bite test with modified Mallampati score in predicting difficult intubation. *Int J Sci Health Res* 2022;7(1):1-8.
7. Law JA, Duggan LV, Asselin M, Baker P, Crosby E, Downey A, et al. Canadian Airway Focus Group updated consensus-based recommendations for management of the difficult airway: part 2. Planning and implementing safe management of the patient with an anticipated difficult airway. *Can J Anaesth* 2021;68(9):1405-1436.
8. Faramarzi E, Soleimanpour H, Khan ZH. Upper lip bite test for prediction of difficult airway: a systematic review. *Pak J Med Sci* 2018;34(4):1019-1023.
9. Koirala S, Shakya BM, Marhatta MN. Comparison of upper lip bite test with modified Mallampati test and thyromental distance for prediction of difficult intubation. *Nepal J Med Sci* 2020;5(1):2-9.
10. Khan ZH, Kashfi A, Ebrahimkhani E. A comparison of the upper lip bite test (a simple new technique) with modified Mallampati classification in predicting difficulty in endotracheal intubation: a prospective blinded study. *Anesth Analg* 2003;96(2):595-599.
11. Wang LY, Zhang KD, Zhang ZH, Zhang DX, Wang HL, Qi F. Evaluation of the reliability of the upper lip bite test and the modified Mallampati test in predicting difficult intubation under direct laryngoscopy in apparently normal patients: a prospective observational clinical study. *BMC Anesthesiol* 2022;22(1):314.
12. Kar S, Senapati LK, Samanta P, Satapathy GC. Predictive value of modified Mallampati test and upper lip bite test concerning Cormack and Lehane's laryngoscopy grading in the anticipation of difficult intubation: a cross-sectional study at a tertiary care hospital, Bhubaneswar, India. *Cureus* 2022;14(9):e28754.
13. Hester CE, Dietrich SA, White SW, Secrest JA, Lindgren KR, Smith T. A comparison of preoperative airway assessment techniques: the modified Mallampati and the upper lip bite test. *AANA J* 2007;75(3):177-182.
14. Ali MA, Qamar-ul-Hoda M, Samad K. Comparison of upper lip bite test with Mallampati test in the prediction of difficult intubation at a tertiary care hospital of Pakistan. *J Pak Med Assoc* 2012;62(10):1012-1015.
15. Moslemi F, Khan ZH, Alizadeh E, Khamnian Z, Eftekhari N, Hosseini MS, et al. Upper lip bite test compared to modified Mallampati test in predicting difficult airway in obstetrics: a prospective observational study. *J Perioper Pract* 2023;33(4):116-121.
16. Wang Z, Jin Y, Zheng Y, Chen H, Feng J, Sun J. Evaluation of preoperative difficult airway prediction methods for adult patients without obvious airway abnormalities: a systematic review and meta-analysis. *BMC Anesthesiol* 2024;24:238.

Diagnostic Accuracy of Computed Tomography and Magnetic Resonance Urography in Detection of Bladder Tumors taking Histopathology as Gold Standard in Patients Presenting with Hematuria at Tertiary Care Hospital Karachi

Nida Asad, Sana Shaikh, Muhammad Rehan Alamgir, Kashaf Anwar Arain, Pooja Devi and Munazzah Ibrar

ABSTRACT

Objective: To compare the computed tomography urography and magnetic resonance urography in the diagnosis of bladder tumors in patients who present with hematuria using the histopathology as reference standard.

Study Design: Cross sectional diagnostic accuracy study

Place and Duration of Study: This study was conducted at the Department of Radiology, Sindh Institute of Urology and Transplantation, Karachi from 1st February 2022 to 1st 31st July 2022.

Methods: 356 patients presented to the with hematuria were enrolled. Eligible patients were subjected to a computed tomography ureography and magnetic resonance ureography and the findings compared to the histopathological diagnosis. Sensitivity, specificity, positive predictive value and negative predictive value and overall diagnostic accuracy of both imaging modalities were calculated.

Results: The mean age was 59.23 ± 8.41 years and the mean duration of symptoms was 2.14 ± 0.89 weeks. One hundred and eighty seven (52.5%) were males and 169 (47.5%) were females. Magnetic resonance urography sensitivity was 80.0%, specificity 99.0%, positive predictive value 93.6%, negative predictive value 96.4% and diagnostic accuracy 96.0%. By contrast, computed tomography urography had a sensitivity of 63.6%, specificity of 95.0%, positive predictive value of 70.0%, negative predictive value of 93.4% and diagnostic accuracy of 90.1%. Magnetic resonance urography was thus more sensitive and was overall more accurate to detect bladder tumors than computed tomography urography.

Conclusion: Computed tomography urography was good for detecting bladder tumors in patients with hematuria compared to magnetic resonance urography. Magnetic resonance urography however was more sensitive, specific and had a higher overall diagnostic accuracy than magnetic resonance urography when compared with histopathology as a reference standard.

Key Words: Bladder tumor, Hematuria, Computed tomography urography, Magnetic resonance urography, Diagnostic accuracy, Histopathology

Citation of article: Asad N, Shaikh S, Alamgir MR, Arain KA, Devi P, Ibrar M. Diagnostic Accuracy of Computed Tomography and Magnetic Resonance Urography in Detection of Bladder Tumors taking Histopathology as Gold Standard in Patients Presenting with Hematuria at Tertiary Care Hospital Karachi. Med Forum 2026;37(9):138-143. doi:10.60110/medforum.370926.

INTRODUCTION

Department of Radiology, Sindh Institute of Urology and Transplantation, Karachi, Pakistan.

Correspondence: Dr. Nida Asad, Postgraduate Trainee, Department of Radiology, Sindh Institute of Urology and Transplantation Karachi, Pakistan.

Contact No: 0347-2273263

Email: dr.nida.asad@gmail.com

Received: May, 2026

Reviewed: June-July, 2026

Accepted: August, 2026

Bladder cancer is a substantial tumour of the urinary tract and it is still a challenge in urology. Not only its clinical importance is associated with the disease burden, but it is also a recurrent disease and can be timely detected and managed. Hematuria (gross or microscopic) is the most common symptom of bladder cancer. Painless hematuria is of special importance and is often the initial presentation of a bladder tumour, and urinary frequency, urgency and dysuria may be present, notably in patients with carcinoma in situ, or when the disease is invasive.^{1,2}

There are a number of different ways to diagnose a suspected bladder tumor. Cystoscopy remains a useful tool for bladder evaluation, as well as for direct

visualization of the bladder mucosa and to obtain specimens for a histopathologic diagnosis.³ Cystoscopy, however, is invasive, can be uncomfortable for the patient and can sometimes be technically challenging especially in the presence of gross haematuria, blood clot or poor anatomy.^{3,4}

Computed tomography urography (CTU) is a valuable imaging tool for the evaluation of hematuria due to its ability to evaluate the upper and lower urinary tract, as well as, to characterize urothelial changes and associated urinary tract pathology.^{5,6} CTU will also incorporate cross sectional imaging with contrast enhanced evaluation of the collecting system and will give helpful anatomical information regarding suspected urothelial lesions. It is however not very accurate in diagnosis of bladder tumors, particularly in the case of small and superficial tumors.⁷ In selected patients, the exposure to the ionizing radiation and the use of iodinated contrast material are additional factors that need to be taken into account, and may therefore restrict the use of CTU.⁵

Magnetic resonance urography is an alternative method of imaging the urinary tract, and is important because of its lack of ionizing radiation.⁸ It is a useful modality for situations where CTU is unavailable or not a good choice, and has very good soft-tissue contrast. MR based imaging can also provide additional data about the surrounding soft tissues and bladder wall.^{8,9} In the past, some difference in the diagnostic effectiveness of these modalities. Woo S et al¹⁰ found sensitivity and specificity of 61.5% and 94.9% respectively for CTU as opposed to 76.9% and 93.4% for MRU in the detection of bladder tumors.

Therefore, it will be useful to have the ability to make a precise comparison between CTU and MRU particularly when a patient is referred with hematuria for which bladder malignancy needs to be excluded. Both these modalities are increasingly being used for urinary tract imaging, but there is still a limited amount of locally generated evidence of their comparative diagnostic performance. Hence, the purpose was to assess and compare the diagnostic accuracy of CT urography and MR urography in patients with hematuria for the detection of bladder tumors using the histopathology as the reference standard.

METHODS

This cross-sectional diagnostic accuracy study was conducted at the Department of Radiology, Sindh Institute of Urology and Transplantation Karachi from 1st February 2022 to 31st July 2022 letter No.CPSP/REU/RAD-2017-198-2446 Dated 13.01.2022. Hematuria patients who were referred to the Department of Radiology were targeted for recruitment. The sample size was determined based on a previously reported magnetic resonance urography (MRU) sensitivity of 76.9%, a specificity of 93.4%, the

expected prevalence of bladder tumor of 20.7%, the margin of error of 10% for sensitivity and 7% for specificity, and a 95% confidence interval. The calculated sample size was 356 patients which were obtained using non-probability consecutive sampling.

All patients of either gender, aged between 30–80 years, who presented with a positive urine dipstick for blood and defined operationally as hematuria were included. Patients who were not consenting, and those who had warfarin use, as well as renal and bladder trauma, previous history of malignancy, uncontrolled hypertension, type II diabetes mellitus, pregnant patients, previous stroke, renal impairment, chronic obstructive pulmonary disease, asthma and congestive cardiac failure were excluded. Demographic and clinical data were collected. Computed tomography urography and MRU were performed for evaluation of bladder tumors. Patients were then subjected to flexible cystoscopy and biopsy of suspicious areas were taken and sent for histopathological examination. Histopathological assessment was carried out by a pathologist with extensive experience in bladder pathology for over 10 years, and pathology was deemed as a reference standard to define the presence or absence of bladder tumor.

The data was analysed through SPSS-20. Diagnostic performance measures were computed separately for CTU and MRU. True-positive results were considered to be positive imaging result and positive histopathology, and true-negative results were considered to be negative imaging result and negative histopathology. A false-positive result was defined as positive imaging and negative histopathology and a false-negative result was defined as negative imaging and positive histopathology. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of both CTU and MRU were determined based upon the histopathology reference standard.

RESULTS

The mean age was 59.23±8.41 years, and the mean duration of symptoms was 2.14±0.89 weeks. There were 187 (52.5%) males and 169 (47.5%) females. Histopathological examination confirmed bladder tumor in 55 (15.4%) patients, while 301 (84.6%) had negative histopathological findings (Table 1).

MRU correctly identified 44 of the 55 histopathologically confirmed tumors, with 11 false-negative findings. Among the 301 histopathology-negative patients, MRU correctly classified 298 as negative, with only three false-positive findings. CTU correctly identified 35 of the 55 histopathologically confirmed tumors but missed 20 cases. Among patients without tumor on histopathology, CTU correctly classified 286, while 15 were false positive (Table 2).

Table No. 1: Baseline and histopathological characteristics of study participants (n=356)

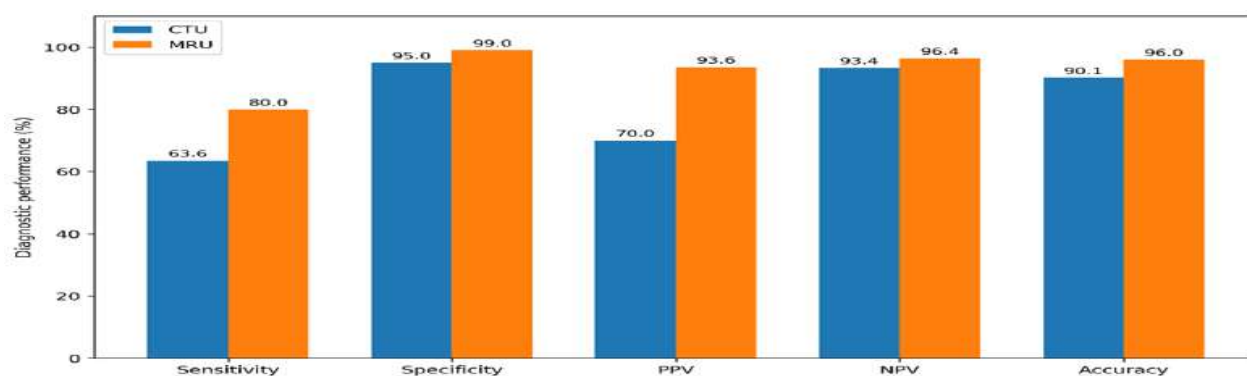
Characteristic	Mean±SD/No.(%)
Age (years)	59.23±8.41
Duration of symptoms (weeks)	2.14±0.89
Male	187 (52.5%)
Female	169 (47.5%)
Histopathology positive	55 (15.4%)
Histopathology negative	301 (84.6%)

These findings translated into a sensitivity of 80.0% for MRU compared with 63.6% for CTU. MRU also demonstrated higher specificity (99.0% vs. 95.0%), PPV (93.6% vs. 70.0%), NPV (96.4% vs. 93.4%), and overall diagnostic accuracy (96.0% vs. 90.1%). The largest difference between the modalities was observed for PPV, followed by sensitivity (Table 3).

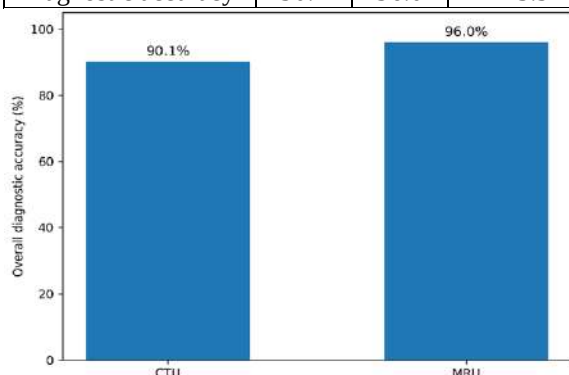
Overall, MRU demonstrated consistently better diagnostic performance than CTU. The improvement was particularly notable for tumor detection, with MRU correctly identifying 44/55 histopathologically positive cases compared with 35/55 using CTU.

Table No. 2: CTU and MRU findings against histopathology

Imaging modality	True positive	False positive	False negative	True negative	Total
CTU	35	15	20	286	356
MRU	44	3	11	298	356

**Figure No. 1: Diagnostic performance of CTU and MRU for bladder tumor detection****Table No. 3: Comparative diagnostic performance of CTU and MRU**

Diagnostic parameter	CTU (%)	MRU (%)	Difference
Sensitivity	63.6	80.0	+16.4
Specificity	95.0	99.0	+4.0
Positive predictive value	70.0	93.6	+23.6
Negative predictive value	93.4	96.4	+3.0
Diagnostic accuracy	90.1	96.0	+5.9

**Figure No. 2: Overall diagnostic accuracy of CTU and MRU**

Furthermore, MRU generated only 3 false-positive results compared with 15 with CTU and reduced false-negative findings from 20 with CTU to 11 with MRU. Thus, MRU demonstrated superior sensitivity and overall diagnostic accuracy while maintaining very high specificity for bladder tumor detection (Figs. 1-2).

DISCUSSION

In the present study, both modalities showed good diagnostic performance with MRU always outperforming CTU. MRU had a sensitivity of 80.0%, specificity of 99.0%, PPV of 93.6%, NPV of 96.4% and overall diagnostic accuracy of 96.0%. By comparison, the sensitivity of CTU was 63.6%, specificity was 95.0%, PPV was 70.0%, NPV was 93.4%, and accuracy was 90.1%. The main advantage of MRU therefore was its high sensitivity and high PPV showing good discrimination of bladder tumors in the population.

The ability to detect blood in the urine (hematuria) is one of the most important presenting signs of bladder malignancy and can often trigger the investigation of the urinary tract.^{1,2} The spectrum of causes of hematuria is from urinary tract disorders with a benign course to urothelial malignancy, clinically significant or not, and current clinical recommendations include risk-based

evaluation.¹¹ Early diagnosis of bladder tumors is especially critical as this may allow the disease to advance and have a negative impact on the management of the disease.^{12,13}

Even with the development of imaging techniques, cystoscopy with histological evaluation is still the cornerstone of the final diagnosis of bladder cancer.^{4,14} Current EAU guidelines would still advocate for cystoscopic evaluation in cases of patients who present with clinical symptoms of bladder cancer and highlight that non-invasive tests are not entirely a substitute for cystoscopy.¹⁴ However, imaging is also useful as an adjunctive test and could detect tumors, upper urinary tract abnormalities, and other candidates for hematuria before invasive investigations.^{5,11} Renal imaging (including CT urography) is therefore currently recommended as part of the investigations of patients with haematuria who are appropriately selected for such investigations.¹⁴

Because of its high spatial resolution, the assessment of the renal parenchyma, collecting systems, ureters and bladder, CTU has emerged as a valuable imaging modality for urinary tract evaluation. In the present study, CTU showed a specificity of 95.0% and a NPV of 93.4% which means that it was good to identify patients without bladder tumors.^{7,15} However, it had a lower sensitivity of 63.6%. Of 55 histopathology proven tumors, CTU correctly diagnosed 35 but not 20. This is a relatively high false-negative rate, as the missed malignant lesion could impede further evaluation and treatment.

There has been a wide range of sensitivity in the ability of CTU to detect bladder cancer as seen in previous investigations.^{15,16} Trinh et al¹⁵ assessed the performance of CT urography in the evaluation of patients with hematuria and in surveillance of patients with bladder cancer, and found an overall accuracy of 91.5%, sensitivity of 86.3%, specificity of 92.4%, PPV of 63.6%, and NPV of 97.8%. Their study also showed that false negative results were often imaging technique related, while false positive results might be due to benign prostatic enlargement, bladder trabeculation, changes caused by treatments, blood clot or inflammatory changes.¹⁵

The previous studies have found a sensitivity of about 79%, specificity of 94%, accuracy of 91%, and a high negative predictive value of 95%, especially in patients investigated due to hematuria.^{15,16} Thus, the overall CTU accuracy in our study (90.1%) is comparable to the previously published results, although the sensitivity is less. Variation between studies may be partly explained by differences in imaging protocols, bladder distention, morphology of the tumor, and size of the lesion as well as patient characteristics and interpretation criteria.¹⁷

The present study showed that MRU had a good diagnostic performance. It correctly identified 44 of the

55 tumours that were histopathologically confirmed, reducing the number of false negative examinations from 20 to 11, compared with CTU. MRU thus realized a sensitivity of 80.0%, which is an absolute improvement of 16.4 percentage points compared to CTU. MRU also made only three false positive examinations, versus 15 with the use of CTU. This led to a specificity of 99.0% and PPV of 93.6% showing the good diagnostic value of MRU.

Biologically and technically, the superiority of MRU is reasonable. MRI has a high soft-tissue contrast and allows multiplanar imaging of the bladder wall and adjacent tissues without the use of ionizing radiation.^{17,18} Multiparametric MRI methods have improved further the characterization of bladder lesions and the evaluation of the local extent of the tumor, with diffusion-weighted MRI and dynamic MRI being the most promising. Vesical Imaging-Reporting and Data System (VI-RADS) has also been developed which offers a structured system of MRI acquisition and interpretation/reporting in the setting of bladder cancer.¹⁹

Multiparametric MRI has recently been shown to have good diagnostic accuracy in bladder tumors and prediction of muscle invasion.²⁰ The current European guidelines are aware of the possibility of multiparametric MRI for local staging, and they also highlight that in the scenario in which MRI is performed for local staging, it should be ideally done before transurethral resection.¹⁹ The present study assessed MR urography for diagnosis of tumor and not for local staging with VI-RADS; however, the cumulative findings in favor of MRI add support to the clinical importance of the better performance of MRU in our population.

The results of present study also agree with Woo S, et al¹⁰ achieved sensitivities and specificities of around 61.5% for CTU and 76.9% for MRU, and 94.9% and 93.4%, respectively. In our study, the sensitivities were 63.6% and 80.0%, respectively. This similarity is remarkable and suggests that, in some clinical and technical scenarios, MR-based assessment might be able to identify a higher percentage of bladder tumors than CTU.

The most marked of the present study was the difference in PPV. The PPV for MRU was 93.6% while that of CTU was 70.0%. The reasons for this difference were the significantly lower number of false positive examinations with MRU. Clinically, better PPV equates to a more positive imaging result being more likely to be histologically-confirmed disease. However, the prevalence of the disease is a factor that affects PPV and NPV and should be considered along with the population.^{20,21}

The NPV was high for both the modalities: 93.4% for CTU and 96.4% for MRU. This finding has application in the assessment of a patient with hematuria because

the higher the NPV, the lower the chance of disease after a negative examination. Nonetheless, there was less than 100% sensitivity in both modalities, and 11 histopathologically confirmed tumors were not detected by MRU. Thus, negative imaging studies should not be used as a substitute for a high suspicion of bladder malignancy. This interpretation is in line with the interpretation of current guidelines, which still consider cystoscopy and histological evaluation as main pillars of bladder cancer diagnosis.^{14,22}

MRU had an overall diagnostic accuracy of 96.0%, while that for CTU was 90.1%. The absolute difference in accuracy was 5.9 percentage points, but when the prevalence of disease is relatively low, there may be clinically important differences that are not reflected in the absolute difference in accuracy. In this study, we were able to confirm the diagnosis by histopathology in only 55 of 356 patients. In such situations, a large number of true negative cases have a significant impact on overall accuracy. As a result, sensitivity, specificity, PPV and NPV offer some extra and clinically beneficial information apart from the overall accuracy estimate.^{20,21}

Another possible benefit of MRU is the lack of ionizing radiation.²² Patients undergo radiation exposure with CTU and typically receive iodinated contrast dye, which is important in patients who need repeat imaging or those who are at risk for iodinated contrast.^{5,22} MR urography is specifically mentioned as an alternative for microhematuria in the updated AUA/SUFU guideline for microhematuria, in which the use of contrast-enhanced CT is contraindicated.¹¹ MRU, however, also has a number of drawbacks, such as longer acquisition times, higher costs, limited availability, and artifacts and contraindications in some patients.²³ Thus, the selection of these two approaches, CTU or MRU, should be personalized and not only determined by their diagnostic accuracy.

The results of this study should therefore not be interpreted as an argument for universally replacing the CTU with the MRU. The fact that CTU is generally abundant, speedy and very useful for extensive evaluation of the urinary tract is still maintained to the present day, and contemporary guidelines continue to give it a strong place in the assessment of hematuria.^{14,22} Our results suggest that MRU might have some extra diagnostic value in the right patient, however, especially when radiation or iodinated contrast are contraindicated or when more soft-tissue characterization is needed.

CONCLUSION

MRU outperformed CTU in detecting bladder tumors in hematuria patients, as evidenced by increased sensitivity, specificity, predictive values and overall diagnostic accuracy. MRU can thus serve as an alternative or a complementary imaging technique in

selected cases, and histopathology will still play a crucial role in diagnosis.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Nida Asad, Sana Shaikh, Muhammad Rehan Alamgir
Drafting or Revising Critically:	Kashaf Anwar Arain, Pooja Devi, Munazzah Ibrar
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.CPSP/REU/RAD-2017-198-2446
Dated 13.01.2022

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71(3):209-49.
2. Teoh JYC, Huang J, Ko WY, Lok V, et al. Global trends of bladder cancer incidence and mortality, and their associations with tobacco use and gross domestic product per capita. *Eur Urol* 2020;78(6):893-906.
3. Barocas DA, Boorjian SA, Alvarez RD, Downs TM, et al. Microhematuria: AUA/SUFU guideline. *J Urol* 2020;204(4):778-86.
4. Barocas DA, Lotan Y, Matulewicz RS, Raman JD, et al. Updates to microhematuria: AUA/SUFU guideline 2025. *J Urol* 2025;213(5):547-57.
5. Waisbrod S, Natsos A, Wettstein MS, Saba K, et al. Assessment of diagnostic yield of cystoscopy and computed tomographic urography for urinary tract cancers in patients evaluated for microhematuria: a systematic review and meta-analysis. *JAMA Netw Open* 2021;4(5):e218409.
6. Sadow CA, Silverman SG, O'Leary MP, et al. Bladder cancer detection with CT urography in an academic medical center. *Radiol* 2008;249(1):195-202.
7. Trinh TW, Glazer DI, Sadow CA, Sahni VA, et al. Bladder cancer diagnosis with CT urography: test characteristics and reasons for false-positive and false-negative results. *AbdomRadiol (NY)*. 2018;43(3):663-71.
8. Helenius M, Brekkan E, Dahlman P, Lönnemark M, et al. Bladder cancer detection in patients with gross haematuria: computed tomography urography with enhancement-triggered scan

- versus flexible cystoscopy. *Scand J Urol* 2015;49(5):377-81.
9. Panebianco V, Narumi Y, Altun E, Bochner BH, et al. Multiparametric magnetic resonance imaging for bladder cancer: development of VI-RADS (Vesical Imaging-Reporting And Data System). *Eur Urol* 2018;74(3):294-306.
 10. Woo S, Panebianco V, Narumi Y, Del Giudice F, et al. Diagnostic performance of Vesical Imaging Reporting and Data System for the prediction of muscle-invasive bladder cancer: a systematic review and meta-analysis. *Eur Urol Oncol* 2020;3(3):306-15.
 11. Luo C, Huang B, Wu Y, Chen J, Chen L. Use of Vesical Imaging-Reporting and Data System (VI-RADS) for detecting the muscle invasion of bladder cancer: a diagnostic meta-analysis. *Eur Radiol* 2020;30(8):4606-14.
 12. Del Giudice F, Pecoraro M, Vargas HA, Cipollari S, et al. Systematic review and meta-analysis of Vesical Imaging-Reporting and Data System (VI-RADS) inter-observer reliability: an added value for muscle invasive bladder cancer detection. *Cancers (Basel)* 2020;12(10):2994.
 13. Yuan B, Cai L, Cao Q, Wu Q, et al. Role of Vesical Imaging-Reporting and Data System in predicting muscle-invasive bladder cancer: a diagnostic meta-analysis. *Int J Urol* 2022;29(3):186-95.
 14. Jazayeri SB, Dehghanbanadaki H, Hosseini M, Taghipour P, et al. Diagnostic accuracy of Vesical Imaging-Reporting and Data System (VI-RADS) in suspected muscle invasive bladder cancer: a systematic review and diagnostic meta-analysis. *Urol Oncol* 2022;40(2):45-55.
 15. Feng Y, Zhong K, Chen R, Zhou W. Diagnostic accuracy of Vesical Imaging-Reporting and Data System (VI-RADS) for the detection of muscle-invasive bladder cancer: a meta-analysis. *Abdom Radiol (NY)* 2022;47(4):1396-1405.
 16. Jazayeri SB, Dehghanbanadaki H, Hosseini M, Taghipour P, et al. Can magnetic resonance imaging differentiate muscle invasion (T2) and lamina propria invasion (T1) urothelial carcinoma of the bladder? A systematic review and meta-analysis of Vesical Imaging-Reporting and Data System accuracy. *Curr Urol* 2023;17(4):262-7.
 17. Witjes JA, Bruins HM, Cathomas R, Compérat EM, et al. European Association of Urology guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2020 guidelines. *Eur Urol* 2021;79(1):82-104.
 18. Babjuk M, Burger M, Capoun O, Cohen D, et al. European Association of Urology guidelines on non-muscle-invasive bladder cancer (Ta, T1, and carcinoma in situ). *Eur Urol* 2022;81(1):75-94.
 19. Richters A, Aben KKH, Kiemeny LALM. The global burden of urinary bladder cancer: an update. *World J Urol* 2020;38(8):1895-1904.
 20. Murad MH, Lin L, Chu H, Hasan B, et al. The association of sensitivity and specificity with disease prevalence: analysis of 6909 studies of diagnostic test accuracy. *CMAJ* 2023;195(27):E925-31.
 21. Trevethan R. Sensitivity, specificity, and predictive values: foundations, pliability, and pitfalls in research and practice. *Front Public Health* 2017;5:307.
 22. Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Paediatr* 2007;96(3):338-41.
 23. Jubber I, Ong S, Bukavina L, Black PC, et al. Epidemiology of bladder cancer in 2023: a systematic review of risk factors. *Eur Urol* 2023;84(2):176-90.

Effects of Objective Structured Clinical Examination on Clinical Competency and Self-Efficacy among Nursing Students

Muhammad Jawad Khan, Azeem Kaleem and Muhammad Saifullah

ABSTRACT

Objective: To compare the effectiveness of Objective Structured Clinical Examination to Traditional Method of Assessment in improving nursing students' clinical competency (clinical judgment and procedural accuracy) and self-efficacy.

Study Design: Comparative study

Place and Duration of Study: This study was conducted at the Rawal College of Nursing and Professional College of Nursing in Islamabad from 1st September 2025 to 31st December 2025.

Methods: 126 bachelors of nursing students were split into two groups of 63 students each. Participants were assessed by Objective Structured Clinical Examination or traditional assessments (written/oral/practical test).

Results: Objective Structured Clinical Examination participants demonstrated clinical competency (68.3% exceeding expectations vs. 20.6%; $p=0.012$) and higher procedural accuracy (3.32 ± 0.569 vs. 2.1 ± 0.871 ; $p=0.003$). Also, both groups showed improvement, but the Objective Structured Clinical Examination group showed a significantly higher increase in self-efficacy ($p=0.000$) than the traditional group. Independent t-tests showed Objective Structured Clinical Examination to be a more effective means for improvement of clinical competence ($p=0.003$) and student confidence.

Conclusion: Objective Structured Clinical Examination method is much more successful than conventional assessment methods in enhancing the clinical performance and confidence of students.

Key Words: Objective Structured Clinical Examination (OSCE), Clinical competency, Self-efficacy, Nursing education, Competency based assessment

Citation of article: Khan MJ, Kaleem A, Muhammad Saifullah, Effects of Objective Structured Clinical Examination on Clinical Competency and Self-Efficacy among Nursing Students. Med Forum 2026;37(9):144-148. doi:10.60110/medforum.370927.

INTRODUCTION

A nurse educator role in assessing practical skills in nursing education is an area of great importance that relates theory and safe and competent care of patients.¹ The nursing student must learn several complex clinical skills for positive outcomes in health and safety to the public.² The status quo of the hands on-tests has been serving as a standard for evaluating these skills for decades. The typical evaluation method is to have the student demonstrate a skill to an evaluator, or ensure that they can do it under evaluation conditions.³ However, in the recent years, the practical exam model has been found to be restrictive in the context of changing demands of healthcare.

¹. MSN Scholar / Assistant Professor², Lahore School of Nursing, The University of Lahore.

Correspondence: Muhammad Jawad Khan, MSN Scholar, Lahore School of Nursing, The University of Lahore.
Contact No: 0345-0949440
Email: jawadkhanmrt@gmail.com

Received: May, 2026
Reviewed: June-July, 2026
Accepted: August, 2026

The formats are accused by critics of being subjective and that there is a great variation with regard to how examiners use them.⁴

Furthermore, conventional strategies rarely include soft, but important skills, such as effective communication and spontaneous problem solving.⁵ Traditional exams can set up an artificial exam setting and approach, perhaps resulting in memorization or rote learning instead of the extensive clinical judgment required in practice.⁶ The one size fits all approach may also disadvantage those who perform best in an iterative or project learning environment, as it may provide undue performance pressure for pupils who are not adequately assessed with regard to their actual ability.⁷ Given these limitations, there has been recognition of a need for a more complex alternative, the Objective Structured Clinical Examination.⁸ During an OSCE, students move to different stations that are all time limited and have been carefully set up to assess a particular clinical skill or competency.

The OSCE consistently has been shown to be an objective and more uniform and equitable way to assess performance.⁹ The OSCE emphasizes practical decision making over mere recall, and provides students with a simulation of a variety of clinical scenarios to prepare

them for the complexities of real-world practice.¹⁰ The OSCE can measure a wide range of professional skills, whereas conventional assessments may focus on just one.¹¹ The implementation of OSCE-based assessment is not without its difficulties, however, even with the advantages.¹² Implementing a high-quality OSCE is costly, and requires specific equipment, space for stations, and extensive faculty training. It continues to be a topic of contention as to its cost effectiveness, especially in contexts of limited resources.¹³ Considering these controversies, this study will compare the efficiency of OSCE and conventional practical examinations in assessing the competencies of the nursing students in relative terms.¹⁴ The research uses an interventional design to assess the effect of both methods on student's clinical skills, communication and confidence.¹⁵

METHODS

This quasi-experimental pre-test/post-test was conducted at Rawal College of Nursing and Professional College of Nursing in Islamabad from 1st September 2025 to 31st December vide letter No. UOL/IREB/25/11/0014 dated August 22, 2025 which included 126 students of 2nd, 3rd and 4th year of BSN program with simple random sampling technique. The researcher used a validated Self-Efficacy Scale to monitor students' technical skills and a standardized 10-item Competency Assessment Rubric to monitor students' confidence levels to measure the impact of the OSCE intervention. To avoid learning effect, the participants were randomly allocated to taking both examination formats one week apart. Informed consent and anonymity were maintained in the process,

ensuring adherence to ethical standards. The data was analyzed using SPSS-25 and inferential tests such as paired and independent t-tests to assess whether the structured OSCE had a significant effect on the clinical judgment and procedural accuracy when compared with traditional OSCE.

RESULTS

There were females more than that of males and 26.9% in the 2nd year of their Bachelor of Science in Nursing program, 39.8% in the 3rd year and 42% in the 4th year (Table 1). There was no significant difference between the clinical judgment of the students in the traditional method groups, whereas there was a statistically significant difference between the clinical judgment of the students in the OSCE method groups (11.9±2.245, 18.4±2.374) (p=0.002). The between group analysis (p=0.012) additionally shows that the OSCE intervention is greater than traditional formats (Table 2).

Table No. 1: Demographic data of the study participants (n=126)

Variable	No.	%
What is your age in years?		
18 to 23	83	65.9
24 to 29	43	34.1
What is your gender?		
Male	55	43.7
Female	71	56.3
What year of study are you in?		
2 nd Year	34	26.9
3 rd Year	50	39.8
4 th Year	42	33.3

Table No. 2: Comparative analysis of clinical judgment scores

Assessment Method	Pre-Test	Post-Test	Within-Group (p-value)*	Between-Group (p-value)**
TMOA Method	12.3±2.561	14.1±6.143	0.397	0.012
OSCE Method	11.9±2.245	18.4±2.374	0.002	

Paired t test and independent t test with p<0.05

Table No. 3: Comparative analysis of procedural accuracy scores

Assessment Method	Pre-Test	Post-Test	Within-Group (p-value)*	Between-Group (p-value)**
TMOA Method	1.95±0.771	2.1±0.871	0.110	0.003
OSCE Method	1.91±0.918	3.32±0.569	0.008	

Paired t test and independent t test with p<0.05

Table No. 4: Comparative analysis of self-efficacy scores

Assessment Method	Pre-Test	Post-Test	Within-Group (p-value)*	Between-Group (p-value)**
TMOA Method	22.3±7.081	29.2±7.496	0.041	0.003
OSCE Method	20.6±7.014	39.8±5.700	0.000	

Paired t test and independent t test with p<0.05

The traditional approach yielded a very small and inconsequential improvement in procedural accuracy measures ($p=0.110$). The OSCE method resulted, in contrast, in a substantial increase from 1.91 ± 0.918 to 3.32 ± 0.569 ($p=0.008$), indicating its effectiveness in improving technical skills. The p value of 0.003 between groups is again evidence that OSCE assessment is better at obtaining higher and more consistent procedural competence (Table 3). There was a significant increase in students' confidence using the OSCE method compared with a traditional method ($p=0.000$). The final post test scores for the OSCE group 39.8 ± 5.700 were significantly higher ($P=0.003$) than the traditional group 29.2 ± 7.496 (Table 4).

DISCUSSION

In the present study, female to male ratio was 56.3% indicating that majority of the students were females, consistent with nursing graduates in the region of Pakistan where 62% of graduates were female. The consistency between the regional demographics validates the findings of this study as representative of the young, female dominated workforce that is currently the hallmark of the nursing profession in the region.¹⁶ These results from Pakistan suggest that the shift to nursing for younger students is "volatile" but this may not be true in contexts where older adults make nursing a second career choice. Traditional assessment methods like oral vivas may lack rigor, whereas the structured, time-constrained nature of OSCEs better tests higher-order critical thinking by simulating real-world hospital pressures.¹⁷ The OSCE is superior, as statistical analysis confirmed with a p -value of 0.012 for clinical judgment, and the accuracy rates were significantly higher (66.7% vs 20.6%), with OSCE's standardized checklists more effectively evaluating the technical skills than clinical judgment, and without inter-rater variability.¹⁸ In contrast to conventional examinations that are subject to rater bias, the OSCE requires students to learn all the micro-steps and move from a passive textbook learning mode to an active skill practice mode by Rushforth.¹⁹ This is because the OSCE aims to assess functional and not merely recalled knowledge. The term 'wash back effect' is used to describe OSCE's ability to enhance the pre-exam clinical reasoning, with Scully²⁰ noting that Australian nursing students remembered 30% more clinical protocols than their counterparts on traditional practical tests.

Positional arguments by Jarvis and Harrison²¹ claim the OSCE is "reductionist," as they believe it will focus on technical skills rather than the comprehensive care and stamina needed for a dynamic 8-hour shift in a nursing role. Nursing educators should consider that while the OSCE effectively assesses micro-skills, it is best paired with longitudinal placements to support student self-efficacy and overall professional success.²² Reflecting

Bandura's social cognitive theory, the study found the OSCE approach significantly boosted self-efficacy ($p=0.000$) with 73.0 % of students scoring high versus 25.4% in the traditional group because the simulated "mastery experiences" provide the confidence and proof needed for real-world clinical practice.²³ While abstract exams lack the same impact, the OSCE's high-stress environment can actually diminish self-efficacy for some; therefore, Conde and Clemente²⁴ suggest nursing programs should provide mock OSCEs and ample practice to ensure results reflect clinical ability rather than mere stress management. Lim and colleagues²⁵ suggest that self-efficacy is primarily driven by time spent on hospital wards rather than the assessment method itself. Consequently, while the OSCE is highly effective, it should be viewed as a catalyst that works in conjunction with hands-on clinical experience.

Significant OSCE improvement ($p=0.002$) compared to traditional methods ($p=0.397$) confirms a "wash back effect" where structured assessments actively develop clinical judgment and diagnostic reasoning rather than just measuring them.²⁶ Procedural transparency and the OSCE's structured design act as a scaffold for professional clinical judgment, while standardized checklists as seen in Vincent's research - help "equalize" student skills by narrowing the competency gap and reducing performance variance.²⁷ While traditional observation leads to high variance in technique, the OSCE ensures consistent technical expertise across all students, though Emails and Concannon note that its intense, "unreal" setting can negatively impact cognitive performance for those prone to test anxiety.²⁸ In contrast to the current study, where the OSCE group excelled argue its high-pressure setting may negatively impact cognitive performance ($p < 0.05$), potentially measuring stress resilience rather than pure clinical ability.²⁸

CONCLUSION

The OSCE is more effective in improving nursing students' performance and self-efficacy than traditional teaching. In the area of clinical judgment, all OSCE participants consistently scored in the "Exceeds Expectations" range, whereas the traditional groups were in the basic standard range for the majority. Indeed, students assessed using OSCE scored significantly higher and had a more consistent procedural accuracy, not only this but they also had a greater level of confidence. The OSCE is clearly structured and has a strong focus on feedback, which is an integral part of the development of skills. Thus, OSCE is highly recommended for inclusion in the nursing curriculum since it can develop graduates in clinical practice.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Muhammad Jawad Khan, Azeem Kaleem, Muhammad Saifullah
Drafting or Revising Critically:	Muhammad Jawad Khan, Azeem Kaleem, Muhammad Saifullah
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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

Source of Funding: None

Ethical Approval: No. UOL/IREB/25/11/0014 Dated 22.08.2025

REFERENCES

- Brennan BA. The impact of self-efficacy based prebriefing on nursing student clinical competency and self-efficacy in simulation: An experimental study. *Nurs Educ Today* 2022; 109:105260.
- Chastain A, Schempp A. How Artificial Intelligence Can Affect Physician Assistant Student Self-Efficacy When Preparing for Objective Structured Clinical Examinations. *J Phys Asstt Educ* 2022;10:1097.
- Dedemoğlu ŞB, Ceylan H. The relationship between perceived self-efficacy and perceived stress in physical examination for undergraduate nursing students: A cross-sectional study. *Mehmet Akif Ersoy Univ J Health Sci Inst* 2025;13(1):1-12.
- Hernandez A, Busquets P, Jimenez R, Scanlan J. Mood states and academic performance in the objective structured clinical examination. The mediating effect of self-efficacy. *Nurs Educ Today* 2024; 135:106116.
- Hung CC, Kao HFS, Liu HC, Liang HF, Chu TP, Lee BO. Effects of simulation-based learning on nursing students' perceived competence, self-efficacy, and learning satisfaction: A repeat measurement method. *Nurs Educ Today* 2021;97:104725.
- Liang JC, Huang MC, Yiin SJ. Nursing Learning Self-Efficacy as a Mediator Between Self-Regulated Learning and Academic Self-Efficacy. *J Nurs Educ* 2026:1-12.
- Liu Y, He L, Huang M, Lu Y, Xiao H. Impacts of platform-based CBL on undergraduate nursing students' academic performance, self-efficacy, clinical decision-making and critical thinking abilities: A cluster randomized controlled trial. *Nurs Educ Pract* 2025;82: 104236.
- Martina SE, Purba IE, Sinaga J, Simanjuntak EH. Self-efficacy impact of the Mini-CEX among nursing students in North Sumatera, Indonesia. *J Public Health Res* 2022;11(2): 743.
- Nair MA, Muthu P, Abuijlan IA. The effectiveness of high-fidelity simulation on clinical competence among nursing students. *SAGE Open Nurs* 2024;10:23779608 241249357.
- Vasli P, Shahsavari A, Estebsari F, AsadiParvar-Masouleh H. The predictors of nursing students' clinical competency in pre-internship objective structured clinical examination: the roles of exam anxiety and academic success. *Nurs Educ Today* 2021;107:105148.
- Yeh CH, Yang YP. Comparing the effect of assessment-simulation-animation-based against traditional education on nursing students' anxiety and self-efficacy in maternity clinical practicum. *Nurs Educ Pract* 2024;76:103932.
- Cheraghi F, Hassani P, Yaghmaei F, Alavi-Majed H. Developing a valid and reliable self-efficacy in clinical performance scale. *Int Nurs Rev* 2020;56(2):214-21.
- Khalafi A, Abbasi A, Sarmeydani NS, Albooghobeish M. Investigating the effectiveness of formative OSCE combined with visual feedback in improving clinical competence among Iranian nurse anesthesia students: a quasi-experimental study. *J Adv Med Educ Professionalism* 2024;12(4):251.
- Al Gharibi KA, Schmidt N, Arulappan J. Effect of repeated simulation experience on perceived self-efficacy among undergraduate nursing students. *Nurs Educ Today* 2021;106:105057.
- Grabowski A, Chuisano SA, Strock K, Zielinski R, Anderson OS, Sadovnikova A. A pilot study to evaluate the effect of classroom-based high-fidelity simulation on midwifery students' self-efficacy in clinical lactation and perceived translation of skills to the care of the breastfeeding mother-infant dyad. *Midwifery* 2021;102:103078.
- Maymoun N, Sohail MS. Who wants to be a nurse? Understanding emirati female students' knowledge and attitudes about nursing as a career. *Nurs Educ Perspect* 2020;41(3):E14-9.
- Raziani Y, Nazari A, Raziani S. Nursing students' experiences with the objective structured clinical examination (OSCE): A qualitative study. *Int J Afr Nurs Sci* 2022;16: 100433.
- Mortaz Hejri S, Jalili M, Masoomi R, Shirazi M, Nedjat S, Norcini J. The utility of mini-clinical evaluation exercise in undergraduate and postgraduate medical education: A BEME

- review: BEME Guide No. 59. Medical Teacher 2020;42(2): 125-42.
19. Swift MC, Kohia M, Foley A. Use of the Hypothesis-Oriented Algorithm to Prepare Students for an Objective Structured Clinical Examination in Physical Therapy Education. J All Health 2021; 50(2):117-25.
20. Scully C. Assessor cognition and inter-rater reliability in nursing objective structured clinical examinations 2023;45.
21. Jarvis RI, Harrison NM. Dundee ASSESS: tips on using domain-based grading to enhance the validity, student feedback and educational impact of the OSCE. MedEdPublish 2025;15:43.
22. Deng Y, Liu H. To overcome test anxiety in on-line assessment: unpacking the mediator roles of techno competencies, teacher support, self-efficacy, and autonomy. BMC Psychol 2025; 13(1):192.
23. de la Fuente J, Kauffman DF, Boruchovitch E. Past, present and future contributions from the social cognitive theory (Albert Bandura). Front Media SA 2023; 1258249.
24. Conde PS, Clemente-Suárez VJ. Differences between students and professors in difficulty, stress and performance in a nursing objective structured clinical examination (OSCE). Physiol Behav 2021; 239:113502.
25. Lim JJ, Roberts C, Jauhar Ali SN, Collini P, Burney A, Hughes D. Understanding students' self-efficacy and motivation in sequential OSCE: a qualitative study. BMC Med Educ 2025; 25(1):1736.
26. Guerrero JG, Rosales NS, Castro GMT. Impact of high-fidelity simulation exposure of nursing students with their objective structured clinical examination: A quasi-experimental study. Nursing Open 2023;10(2):765-72.
27. Vincent SC, Arulappan J, Amirtharaj A, Matua GA, Al Hashmi I. Objective structured clinical examination vs traditional clinical examination to evaluate students' clinical competence: A systematic review of nursing faculty and students' perceptions and experiences. Nurs Educ Today 2022;108: 105170.
28. Esmail S, Concannon B. Immersive virtual reality and AI (generative pretrained transformer) to enhance student preparedness for objective structured clinical examinations: mixed methods study. JMIR Serious Games 2025;13: e69428.

Non-Pharmacological and Non-Surgical Options to Combat Obesity

Abdul Qadeer Memon

Non-Pharmacological
and Non-Surgical
Options to Combat
Obesity

ABSTRACT

Objective: To provide a detailed and simplified information about the role of Non-pharmacological and Non-surgical options i.e. lifestyle, exercise and ketogenic diet to combat obesity.

Place and Duration of Study: This study was conducted as narrative review article at King Faisal University College of Medicine, AlAhsa, Kingdom of Saudi Arabia from January 2026 to June 2026.

Google Scholar and PubMed were searched for last ten years up to June 2026. Medical Subject Headings (Mesh) keywords were used. The filters were applied to include original articles, reviews, systematic reviews and meta-analysis, free/open access full text articles in English language.

Results/Review: By modifying the lifestyle and performing regular exercise, 5% to 10% weight can be lost. It helps to reduce obesity and has a positive psychological effect of feeling well. By diet control, there should be a daily deficit of 500 kcal over a period of one year to lose the weight. Ketogenic diet is different from low-carb diet in terms of proportion of fats, protein and carbohydrates and food species to be taken.

Discussion: It may feel difficult to adopt these strategies because the obese people have craving for food and different types of exercises may not be suitable for every patient due to morbid obesity and associated co-morbidities.

Conclusion: The lifestyle modifications, exercise and/or ketogenic diet may be tried to treat obesity. This modality may spare the patients from the side-effects of pharmacological and the complications of the surgical modalities. It may also be helpful to treat the psychological aspect in obese patients, which contributes a major role in gaining weight.

Key Words: Body mass index (BMI), Ketogenic diet, Obesity

Citation of Narrative Review: Memon AQ. Non-Pharmacological and Non-Surgical Options to Combat Obesity. Med Forum 2026;37(9):149-153. doi:10.60110/medforum.370928.

INTRODUCTION

The management of obesity includes a multidisciplinary approach. It starts with change in eating behavior, diet control i.e. low-carb diet or ketogenic diet, faux food, different types of physical exercises like brisk walk, aerobic/anaerobic, Yoga or Tai Chi etc. Pharmacotherapy may be considered as second line of treatment. These oral or injectable drugs may also help to treat co-morbidities like type-2 diabetes mellitus. None of the drugs is without side effects. Bariatric surgery may be suitable when these options fail to work and/or BMI is more than 35 kg/m^{1,2}

Assistant Professor, Department of Surgery, King Faisal University College of Medicine, Al Ahsa, Kingdom of Saudi Arabia.

Correspondence: Abdul Qadeer Memon, Assistant Professor of Surgery, Department of Surgery, College of Medicine, King Faisal University, Al Ahsa, Saudi Arabia.
Contact No: +966-531441959
Email: drqadeermemon@yahoo.com
amuhammad@kfu.edu.sa
ORCID: <https://orcid.org/0000-0002-7071-9489>

Received: June, 2026
Reviewed: August, 2026
Accepted: August, 2026

The bariatric surgery is aimed to reduce the size and capacity of the stomach for food or some intestinal bypass procedures, which may affect the absorption of food ingredients from the intestine. The aim of this narrative review is to discuss the role of non-pharmacological and non-surgical options of management of obesity.

BACKGROUND

Obesity is a heterogeneous disease. Hence, the response to the treatment with different modalities is variable. Obesity results from the primary metabolic as well as endocrine abnormalities. Risk factors of obesity include sedentary lifestyle, over-eating, socioeconomic status, familial/genetic etc. The central nervous system and circulating neuropeptide hormones work together to maintain the body's energy homeostasis. However, genetic inheritance has an impact on it.^{2,3} An important modulator of body weight, food intake and energy homeostasis is the melanocortin 4 receptor (MCR-4). The most prevalent hereditary form of extreme obesity is caused by its mutation or lack.⁴ There is a gut-brain axis. Its response is based on hunger or satiety, which is obtained when peripheral signals from the gastrointestinal tract, adipose tissue, and circulating hormones stimulate or inhibit central neurons.⁵ Dysregulation of this gut-brain axis leads to obesity

either by increased hunger or by decreased satiety. Leptin and ghrelin hormones also play a crucial role in the process by metabolic adaptation, which promotes obesity.⁵ Additionally, larger bites and faster eating are thought to be risk factors for obesity since they reduce feelings of fullness.^{6,7}

METHODS

Google Scholar and PubMed were searched for last ten years up to June 2026. The survey was carried out using combinations of Medical Subject Headings (Mesh) keywords. The filters applied during search of the articles included original articles, reviews, systematic reviews and meta-analysis, free/open access full text articles in English language. Search criteria included keywords such as "Obesity management in adults, Body mass index (BMI), Overweight, Life style, Exercise and Ketogenic diet."

RESULTS / REVIEW

Lifestyle/Exercise: The obese patients, before any medical/surgical interventions, should be referred to psychologists to help them change the behavior in their lifestyle. Evidence-based counselling techniques can assist in starting conversations with patients about therapy. The goal may be achieved through 5As (Assess, Advise, Agree, Assist, Arrange), which can guide shared decision-making with multiple sessions ranging from six to 24 months.⁸ Chronic stress and inadequate sleep can also have a detrimental impact on metabolism and hunger.⁹ The patients who are weight-conscious and regularly perform self-weighing, have been seen to improve weight loss and its maintenance. By this way, 5% to 10% weight can be lost.¹⁰

There is a vital role of physical activity to reduce the body weight and maintain it. The daily calories requirement in diet also depends upon the physical activity. Physical activity reduces adipose tissue in the body and preserves the lean mass. Walking for two minutes per hour, using the stairs, or doing 1800 steps a day on average are examples of moderate physical activity and exercise that may aid in weight loss from 0.5 to 1.5 kg.¹¹ American College of Sports Medicine (ACSM) has classified exercise into aerobic or anaerobic. The aerobic exercise consumes the oxygen present in the muscle to a level that still there is sufficient oxygen in the muscle to supply on demand. The activities in this category include running, cycling, rowing etc. The anaerobic exercise consumes the oxygen that is insufficient to meet the demands. Hence, alternative energy sources present in the muscle must be utilized. These sources preferentially are metabolism of carbohydrates, which produce lactic acid. Such activities include heavy exercise like weight lifting. To advise the intensity of the exercise, the comorbid conditions like cardiovascular disease, asthma, arthritis must be considered.¹² It is advised to engage in 150 to

300 minutes of moderate physical activity or 75 to 150 minutes of strenuous physical activity every week in order to lose weight or prevent weight gain. It is advised to engage in 200–300 minutes of intense activity per week if you want to lose a lot of weight. Initially, the exercise should be of lesser intensity and duration with slow and gradual increase.¹³ Yoga not only helps to reduce obesity but also has a positive psychological effect of feeling well and avoid depression.¹⁴

Diet control and ketogenic diet:

Calories and water intake: Adult males and females should consume 2600–2800 kcal per day and 2000–2200 kcal per day, respectively. Men and women between the ages of 19 and 30 who weigh about 75 kg are advised to consume 3.7 and 2.7 liters of water per day, respectively. Age, sex, weight, level of physical activity, humidity, ambient temperature, and other variables all affect how many calories and water are consumed. Obesity is caused by a positive energy balance.¹⁵ Therefore, in order to achieve weight loss through negative energy balance, energy intake must be reduced and energy expenditure must be increased. The European Food Safety Authority has recommended that there should be a daily deficit of 500 kcal over a period of one year to lose the weight. Fats contain twice energy as compared to carbohydrates and proteins. If a person wants to lose more weight by reducing calorie intake, it must be under supervision of a qualified physician/dietician. No doubt, the diet should be balanced with micro- and macro-nutrients to avoid deficiency and its consequences.¹⁶

Carbohydrates intake: Severe carbohydrates restriction is not necessary during weight loss program. If the total daily food contains 1200 to 1500 kcal, the carbohydrate content may be 135 to 245 g/day and for 1500- to 1800-kcal/day diet, it may be between 170 to 290 g/day.¹⁶

Proteins intake: Higher intake of protein is necessary, particularly in older adults. Inadequate protein consumption can result in significant loss of lean body mass, frailty, edema, hair loss, and skin abnormalities.¹⁶

Fats intake: If the total daily food contains 1200 to 1500 kcal, fats proportion may be 25 to 60 g/day, while if the total daily food contains 1500- to 1800-kcal, fats may be 35 to 70 g/day. Dietary fats will also help to prevent the deficiency of fat-soluble vitamins like A, D, E and K. Unsaturated fatty acids (poly- or mono-) should be preferred in the diet. Such fats are present in soybean, canola, olive and vegetable oils, fish, nuts and seeds.¹⁶ Apart from the macronutrients, the micronutrients (all vitamins and trace elements like iron, copper, zinc) must be considered in the diet.

Ketogenic diet: A ketogenic diet (KD) is a low-carb, high-fat, moderate-protein diet that mimics the breakdown of a fasting state without actually fasting in order to produce ketone bodies (3- β -hydroxybutyrate, acetoacetate, and acetone). The KD is different from the low carbohydrate or calorie-restricted diet. It contains

up to 70% fats, up to 25% proteins and only 5% carbohydrates (Figures 2).

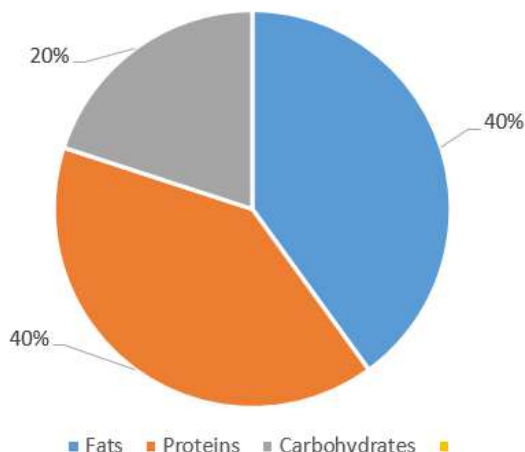


Figure No.1: Low Carbohydrates diet

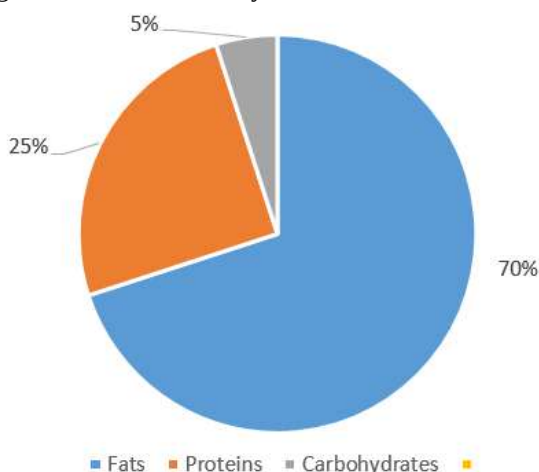


Figure No. 2: Ketogenic Diet

Encouraging fatty acid oxidation without altering protein intake is the aim of implementing a KD. This goal is achieved by reduced intake of carbohydrates, resulting in less insulin release. The metabolic process will have less effect on lean body mass; the fat will be mobilized preferentially for the calories supply (lipolysis) with a net result of caloric deficit.³¹ It provides greater feeling of satiety and controls the appetite better than the diets rich in carbohydrates. There are different varieties of KDs based on low carbohydrates. These include the Medium-Chain Triglyceride Ketogenic Diet, Atkins Diet, Modified Atkins Diet, Classical Ketogenic Diet, and Low Glycaemic Index Therapy.¹⁷ The KD must be started under supervision of a physician/dietician due to potential risks especially if it is for prolonged period. It must be given in phases of few weeks and then evaluated the outcome. Hunger, exhaustion, thirst, poor mood, irritability, constipation, diarrhea, headaches, cramping in the muscles, nausea, dizziness, gastrointestinal discomforts, loss of energy, changes in

heart function, kidney stones, and halitosis are all possible risks of KD. Some of these side effects appear at the beginning of the KD and are so-called 'keto-flu', which may disappear after few weeks (Figures 1&2).¹⁸

Chewing gum: One kind of faux/sham feeding is chewing gum. The faux food promotes gastrointestinal peristalsis and increases the salivary rate by just viewing, smelling, tasting, and chewing food without ingesting it. Like actual feeding, the sham feeding also reduces the hunger.¹⁹ Hence, chewing gum may reduce the appetite and gum mastication without ingestion of food will lead to increased energy expenditure leading to net loss of energy and suppress the craving for high-energy food.²⁰

Deep transcranial magnetic stimulation (dTMS): It is observed that certain areas of the brain are involved in dietary habits and food craving. These include prefrontal cortex, mesolimbic regions, precuneus and other areas. Application of a novel modality, the dTMS has been tried, which reduces weight/BMI by decreasing the leptin levels.^{21,22}

DISCUSSION

It is observed in this narrative review that different types of exercise, behavioral change towards food intake and ketogenic diet play vital role in reducing weight. Initially, it may feel difficult to adopt because the obese people have craving for food and different types of exercises may not be suitable for every patient due to morbid obesity and associated co-morbidities like osteoarthritis, cardiovascular and other problems. Hence, this line of management should be under supervision of a physician.^{23,24} Allice Bellicha et al in their systemic review observed that exercise-training programs and resistance exercise lead to a significant weight loss and fat loss from body and viscera. However, aerobic exercise does not reduce the weight or fat significantly.²⁵ Combined aerobic and resistance training (CART) plays a significant role in reducing weight. It also improves glycemic control in type-2 DM, blood pressure and many inflammatory markers like interleukin-6, TNF-alpha and CRP.²⁶ Moreover, the improvements in the inflammatory markers may help to alleviate the inflammatory conditions in obese patients like osteoarthritis. Different specific chemokines play a vital role in the development of obesity. Exercise has been observed to affect these chemokines and other inflammatory markers.²⁷

In a systemic review and meta-analysis, the role of very low-calorie ketogenic diet (VLCKD) was studied. The VLCKD showed its major role in reducing weight, waist circumference, blood cholesterol and other parameters. However, it has certain contraindications which include type-1 DM, kidney and/or liver failure, recent stroke, unstable angina, myocardial infarction, elderly patients, severe mental illness etc.²⁸⁻³²

Intermittent fasting has been observed to affect the dietary habits by affecting the circadian rhythm of the brain. It is not as effective as other dieting plans, but achieves the weight loss by restricting the energy intake.³³

Behavioral change with or without exercise may reduce weight from 5%-10%. Variety of pharmacological intervention may reduce the weight from 8%-21%, while bariatric surgeries may reduce the weight from 25%-30%.³⁴

It is recommended that every obese patient may be considered individually and the modality of treatment may be tailored accordingly. Among many factors to be considered, the fitness of patients for exercise, comorbidities like DM, heart, kidney, liver problems, pregnancy, breast feeding are few. Exercise programs or ketogenic diet should not be without supervision of a qualified health professional.

CONCLUSION

Obesity has different modalities of treatment. Before going for pharmacological or surgical modalities, the lifestyle modifications, exercise and/or ketogenic diet may be tried to avoid the complications of the above two modalities, which may be cumbersome. Non-pharmacological and non-surgical option may also be helpful to treat the psychological aspect in obese patients, which contributes a major role in gaining weight.

Limitations of the study: The search was limited to Pubmed/Medline and Google Scholar. The wider search may include more information.

Grant support & financial disclosures: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies, King Faisal University, AlAhsa, Kingdom of Saudi Arabia. Grant number: KFU264740

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

Source of Funding: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies, King Faisal University, AlAhsa, Kingdom of Saudi Arabia. Grant number: KFU264740.

REFERENCES

1. Semlitsch T, Stigler FL, Jeitler K, Horvath K, Siebenhofer A. Management of overweight and obesity in primary care—A systematic overview of international evidence-based guidelines. *Obesity Reviews* 2019;20:1218–1230. <https://doi.org/10.1111/obr.12889>
2. Roomy MA, Hussain K, Behbehani HM, Abu-Farha J, Al-Harris R, Ambi AM, et al. Therapeutic advances in obesity management: an overview of the therapeutic interventions. *Front. Endocrinol* 2024;15:1364503. doi: 10.3389/fendo.2024.1364503
3. Loos RJF, Yeo GSH. The genetics of obesity: from discovery to biology. *Nat Rev Genet* 2022;23(2):120–133. doi:10.1038/s41576-021-00414-z
4. Imangaliyeva A, Sikhayeva N, Bolatov A, Utupov T, Romanova A, Akhmetollayev I, et al. Genetic Insights into Severe Obesity: A Case Study of MC4R Variant Identification and Clinical Implications. *Genes* 2025;16:508. <https://doi.org/10.3390/genes16050508>
5. Kessler C. Pathophysiology of obesity. *Nuts Clin North Am* 2021;56(4):465–478. doi:10.1016/j.cnur.2021.08.001
6. Lasschuijt MP, de Graaf K, Mars M. Effects of Oro-Sensory Exposure on Satiety and Underlying Neurophysiological Mechanisms-What Do We Know So Far? *Nutrients* 2021;13:1391
7. Slyper A. Oral Processing, Satiety and Obesity: Overview and Hypotheses. *Diabetes Metab Syndr Obes* 2021;14:3399–3415.
8. Wiechert M, Holzapfel C. Nutrition Concepts for the Treatment of Obesity in Adults. *Nutrients* 2021;14(1):169. doi: 10.3390/nu14010169.
9. Duan D, Kim LJ, Jun JC, Polotsky VY. Connecting insufficient sleep and insomnia with metabolic dysfunction. *Ann N Y Acad Sci* 2023;1519(1):94–117. doi:10.1111/nyas.14926
10. Varkevisser RDM, van Stralen MM, Kroeze W, Ket JCF, Steenhuis IHM. Determinants of weight loss maintenance: a systematic review. *Obes Rev* 2019;20(2):171–211. doi:10.1111/obr.12772
11. Ferguson T, Olds T, Curtis R, Blake H, Crozier AJ, Dankiw K, et al. Effectiveness of wearable activity trackers to increase physical activity and improve health: a systematic review of systematic reviews and meta-analyses. *Lancet Digit Health* 2022;4(8):e615–e626. doi: 10.1016/S2589-7500(22)00111-X.
12. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc* 2009;41(2):459–71.
13. Niemi GM, Rewane A, Algotar AM. Exercise and Fitness Effect on Obesity. 2023 Nov 17. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–.
14. Inbaraj G, Bajaj S, Misra P, Kandimalla N, Thapa A, Ghosal A, et al. Yoga in Obesity Management: Reducing cardiovascular risk and enhancing well-being- A review of the current literature. *Curr Probl Cardiol* 2025;50(6):103036. doi: 10.1016/j.cpcardiol.2025.103036.

15. Faizan U, Rouster AS. Nutrition and Hydration Requirements In Children and Adults. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan
16. Almandoz JP, Wadden TA, Tewksbury C, Apovian CM, Fitch A, Ard JD, et al. Nutritional considerations with antiobesity medications. *Obesity* (Silver Spring) 2024;32(9):1613-1631. doi: 10.1002/oby.24067.
17. Baylie T, Ayelgn T, Tiruneh M, Tesfa KH. Effect of Ketogenic Diet on Obesity and Other Metabolic Disorders: Narrative Review. *Diabetes Metab Syndr Obes* 2024;17:1391-1401 <https://doi.org/10.2147/DMSO.S447659>
18. Natalia Drabińska, Wiesław Wiczowski, Mariusz Konrad Piskula, Recent advances in the application of a ketogenic diet for obesity management, *Trends in Food Science Technol* 2021; 110:28-38. doi: 10.1016/j.tifs.2021.01.080.
19. Hsu YC. Szu SY. Effects of Gum Chewing on Recovery From Postoperative Ileus: A Randomized Clinical Trail. *J Nurs Res* 2022;30:E233.
20. Jimenez-ten Hoevel C, Llauro E, Valls RM, Besora-Moreno M, Queral J, et al. Effects of Chewing Gum on Satiety, Appetite Regulation, Energy Intake, and Weight Loss: A Systematic Review. *Nutrients* 2025;17:435. <https://doi.org/10.3390/nu17030435>
21. Li G, Hu Y, Zhang W, Wang J, Weibin JI, Manza P, et al. Brain functional and structural magnetic resonance imaging of obesity and weight loss interventions. *Mol Psychiatr* 2023;28:1466–1479. <https://doi.org/10.1038/s41380-023-02025-y>
22. Luzi L, Gandini S, Massarini S, Bellerba F, Terruzzi I, Senesi P, et al. Reduction of impulsivity in patients receiving deep transcranial magnetic stimulation treatment for obesity. *Endocrine* 2021; 74(3):559-570. doi: 10.1007/s12020-021-02802-1.
23. Wadden TA, Chao AM, Moore M, Tronieri JS, Gilden A, Amaro A, et al. The Role of Lifestyle Modification with Second-Generation Anti-obesity Medications: Comparisons, Questions, and Clinical Opportunities. *Curr Obes Rep* 2023;12:453–473. <https://doi.org/10.1007/s13679-023-00534-z>
24. Mesinovic J, Hurst C, Leung GKW, Ryan JR, Daly RM, Scott D. Exercise and dietary recommendations to preserve musculoskeletal health during weight loss in adults with obesity: A practical guide. *Rev Endocr Metab Disord* 2025; 26:785–803. <https://doi.org/10.1007/s11154-025-09968-3>
25. Bellicha A, van Baak MA, Battista F, Beaulieu K, Blundell JE, Busetto L, et al. Effect of exercise training on weight loss, body composition changes, and weight maintenance in adults with overweight or obesity: An overview of 12 systematic reviews and 149 studies. *Obesity Reviews* 2021;22(S4): e13256. <https://doi.org/10.1111/obr.13256>
26. AL-Mhanna SB, Batrakoulis A, Wan Ghazali WS, Mohamed M, Aldayel A, Alhussain MH, et al. Effects of combined aerobic and resistance training on glycemic control, blood pressure, inflammation, cardiorespiratory fitness and quality of life in patients with type 2 diabetes and overweight/obesity: a systematic review and meta-analysis. *Peer J* 2024;12:e17525 DOI 10.7717/peerj.17525
27. He W, Wang H, Yang G, Zhu L, Liu X. The Role of Chemokines in Obesity and Exercise-Induced Weight Loss. *Biomolecules* 2024;14:1121. <https://doi.org/10.3390/biom14091121>
28. Muscogiuri G, El Ghoch M, Colao A, Hassapidou M, Yumuk V, Busetto L. Obesity Management TaskForce (OMTF) of the European Association for the Study of Obesity (EASO). European Guidelines for Obesity Management in Adults with a Very Low-Calorie Ketogenic Diet: A Systematic Review and Meta-Analysis. *Obes Facts* 2021; 14(2):222-245. doi: 10.1159/000515381.
29. Barrea L, Verde L, Di Lorenzo C, Savastano S, Colao A, Muscogiuri G. Can the ketogenic diet improve our dreams? Effect of very low-calorie ketogenic diet (VLCKD) on sleep quality. *J Transl Med* 2023;21:479. <https://doi.org/10.1186/s12967-023-04280-7>
30. Lim SL, Tay M, Ang SM, Wai SN, Ong KW, Neo WJ, et al. Development and Pragmatic Randomized Controlled Trial of Healthy Ketogenic Diet Versus Energy-Restricted Diet on Weight Loss in Adults with Obesity. *Nutrients* 2024;16:4380. <https://doi.org/10.3390/nu16244380>
31. Dyńska D, Rodzeń NŁ, Rodzeń N M, Pacholak-Klimas A, Ede G, Sethi S, et al. Ketogenic Diets for Body Weight Loss: A Comparison with Other Diets. *Nutrients* 2025;17:965. <https://doi.org/10.3390/nu17060965>
32. Paoli A, Bianco A, Moro T, Mota JF, Coelho-Ravagnani CF. The Effects of Ketogenic Diet on Insulin Sensitivity and Weight Loss, Which Came First: The Chicken or the Egg? *Nutrients* 2023;15: 3120. <https://doi.org/10.3390/nu15143120>
33. Santos HO, Genario R, Tinsley GM, Ribeiro P, Carteri RB, Coelho-Ravagnani CF, et al. A scoping review of intermittent fasting, chronobiology, and metabolism. *Am J Clin Nutr* 2022;115(4):991-1004. doi: 10.1093/ajcn/nqab433.
34. Elmaleh-Sachs A, Schwartz JL, Bramante CT, Nicklas JM, Gudzone KA, Jay M. Obesity Management in Adults: A Review. *JAMA* 2023; 330(20):2000-2015. doi: 10.1001/jama.2023.19897.