

# NIU Journal of Health Sciences



**Nexus International University, Uganda.**

***Copyright © 2026 Nexus International University, Uganda.***

***All rights reserved.***

***Apart from fair dealing for the purpose of research or private study, or criticism or review, and only as permitted under the Copyright Art, this publication may only be produced, stored or transmitted, in any form or by any means, with prior written permission of the Copyright Holder.***

**Published in October, 2026**

**ISSN: 3136-6457 (Print)**

**ISSN: 3136-6449 (Online)**

**Published by:**

***Nexus International University,  
Kampala, Uganda.***



## Editorial

This edition of *NIU Journal of Health Sciences* touches on health and medically related issues such as Disease Control, Harmful Traditional Practices, Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) among admitted patients at Baqubah Teaching Hospital in Diyala, among others.

One of the papers, in this issue, reveals that that despite relatively high educational levels among participants, some harmful traditional health practices are still believed and practiced. It therefore, recommends that increasing awareness and providing accurate health education are essential to reduce the spread of these misconceptions.

Another paper also reveals that a high prevalence of multidrug-resistant *S. aureus*, including a substantial proportion of methicillin-resistant (MRSA) strains, at Baqubah Teaching Hospital. The significant variations in susceptibility patterns across different antibiotics and anatomical infection sites emphasize the necessity of tailored empirical therapy. These alarming resistance rates warrant the immediate implementation of strict antimicrobial stewardship programs and regular local surveillance to curb the dissemination of these refractory pathogens.

On the whole, this edition of *NIU Journal of Health Sciences* features many empirical and theoretical based articles which can be of great benefit to every reader.

**Professor Oyetola O. Oniwide**

Nexus International University,  
P.O. Box 70773,  
Kampala, Uganda.  
[editor@niu-journals.ac.ug](mailto:editor@niu-journals.ac.ug)

**October, 2026.**





## Apoptosis as the Primary Elimination Mechanism for Cancer Cells: Mechanisms, Evidence, and Therapeutic Implications

QUTAIBA HOMMADI MAHMOOD AL-SAMARRAIE  
College of Applied Science, University of Samarra, Samarra, Iraq.  
[gutaiba.h@uosamarra.edu.iq](mailto:gutaiba.h@uosamarra.edu.iq)  
<https://orcid.org/0000-0001-7032-1561>

**Abstract.** Apoptosis constitutes the principal mechanism controlling the elimination of cancer cells. Apoptosis arises as a fundamental capability of multicellular organisms regulating cell number and maintaining homeostasis. It is the best-studied form of programmed cell death and broadly participates in multiple physiological processes embryogenesis, tissue remodeling, and immune cell maturation, among others. According to accumulated evidence, apoptosis acts as a potent tumor-suppressor mechanism curtailing cancer cell expansion. Cancer cells are subjected to multiple biological insults continuously exerted by their micro- and macro-environment and, when left unresolved, such detrimental events drive cells toward a neoplastic transformation. Cancer cells often acquire opposing alterations that foster adaptation to these events, evade replication barriers, and expand into tumors. Recent studies have argued that when cancer clones develop resistance, pro-apoptotic signals become a prominent component of antagonistic programs, reinforcing the notion that a majority of cancer clones are still susceptible to apoptotic removal during tumor establishment.

At least three forms of terminal cancer-cell elimination have been identified: apoptosis, senescence, and necrosis. Apoptosis distinctively leads to the generation of an immunogenic signal that facilitates the elimination of cancer-cell clones even from resistant tumors. Available genetic, molecular, and pharmacological evidence supports the notion that apoptosis operates as a major mode for the elimination of cancer cells during malignant transformation. Importantly, a wide variety of therapeutic modalities induce death predominantly through apoptosis. Because apoptosis appears to still remain a critical backup option during cancer prevention, continued further investigation into the mechanisms of apoptosis and the establishment of new therapeutics for its induction holds considerable promise for improving cancer treatment.

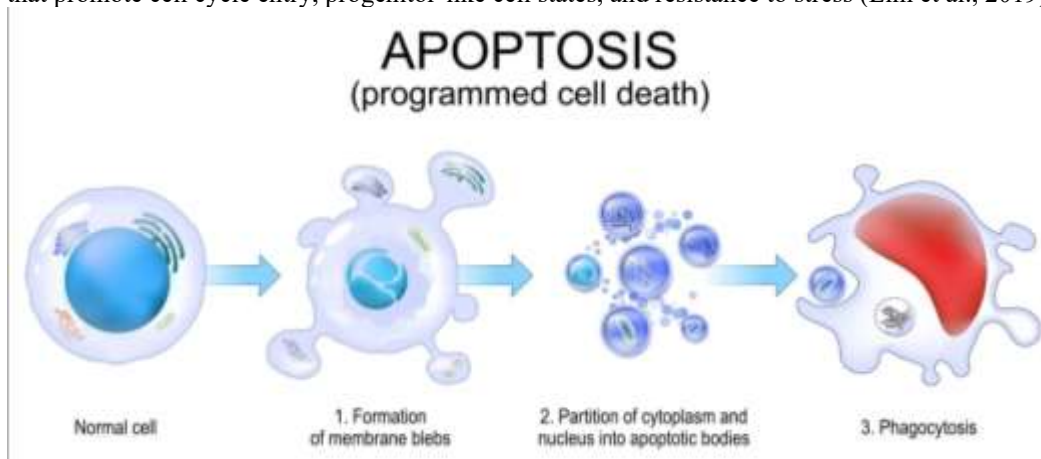
A multitude of stresses imparted by tumor microenvironments destabilizes normal tissues and triggers apoptosis. Treatments that cause further stresses, including DNA lesions, directly promote massive apoptosis, underscoring the still-latent vulnerability of cancer cells. Collectively, compounds that elicit extensive, unmanageable apoptosis in cancer cells, either through direct activation or relay of biochemical signals, can become new agents or be combined with existing ones for more effective therapy.

### 1. Introduction

Apoptosis is widely recognized as a central elimination mechanism for cancer cells. Most anti-cancer treatments induce cancer cell death, primarily because damage inflicted on malignant cells activates apoptotic pathways, which are often dysregulated in tumors. Thus, fading of these damage signals or the acquisition of pro-survival mutations allows tumor cells to escape the primary elimination mechanism that therapies are designed to engage. This pivotal role of apoptosis in tumor eradication arguably positions it among the most important hallmarks of cancer, especially when discussing therapeutic options. This work compiles the existing evidence that links apoptotic execution to the clearance of tumor cells and considers the implications for the development of therapeutics that target these pathways toward the elimination of cancer (Morana *et al.*, 2022). There are some types of cancer that result from chromosomal aberration, and apoptosis plays a major role in their treatment (Al-Samarraie, 2026).

Methods have been developed to induce apoptosis in cancer cells, aiming to restore pathway engagement and tumor-cell elimination. These methods are classified as pro-apoptotic agents and BH3 mimetics. Pro-apoptotic agents activate the apoptotic machinery by targeting specific players at various stages of the core pathway. The rapidly evolving landscape of BH3 mimetics directly targets pro-survival proteins of the BCL-2 family, freeing pro-apoptotic proteins from tight control and unleashing apoptosis upon cells still possessing functional executioners. Both classes have potential for improved cancer therapy, yet ensuring selective action on tumor cells remains critical to safe application (Franzese *et al.*, 2024).

Apoptosis is a well-defined form of programmed cell death involving a highly regulated series of biochemical events leading to the characteristic morphological and molecular features of cellular disassembly. Apoptosis is triggered by a variety of stimuli, including developmental signals or pathogen-derived signals, and plays a crucial role in normal biological processes such as embryogenesis, tissue homeostasis, the immune response, and the response to injury (Morana *et al.*, 2022). Apoptosis also acts as a key tumor-suppressive mechanism, faithfully executing developmental and homeostatic cell-death programs. Cellular transformation typically involves alterations in apoptosis control—such as the emergence of oncogenic signalling or the disruption of p53 activity—that promote cell cycle entry, progenitor-like cell states, and resistance to stress (Lim *et al.*, 2019).



**Figure (1)** Stages of Apoptosis

## 2. Historical Perspective on Apoptosis in Cancer

Cell death has been an object of biological curiosity since the 1850s, when histologists began documenting the microanatomical pathways leading to the demise of individual cells in cultured tissues (Jacobson *et al.*, 1997). Initial concepts of cell death were simplistic on both mechanistic and teleological levels, but advances in light microscopy and biochemistry in the 1960s prompted speculation that physiological apoptosis had evolved to facilitate selective removal of cells during development and tissue homeostasis (Boehm & Bock, 2019). Following these developments, British zoologist Sir Victor Charles Tcharles Huxley undertook pioneering, sophisticated, and painstaking studies on avian red blood cell development in the 1920s—able to distinguish and name “decidual corpuscles” of abortive red blood cells (Huxley, 1922). The discovery that excessive Huxley pathology also accompanied oncogenesis came only later (Huxley, 1949).

Apoptosis (programmed cell death) acts as a primary mechanism responsible for initiating cancer cells; it occurs in physiologically discrete stages, in both intrinsically (mitochondrially) and extrinsically (death receptor-initiated) activated forms. Current evidence substantiates the crucial function of apoptotic clearance in cancer cell initiation, instructing robust therapeutic approaches aimed at reinstating apoptosis to address the pervasive resistance to this death program within tumors. Cancer is a leading global cause of morbidity and mortality, necessitating urgent efforts to prevent and treat rampant tumor proliferation. Although exciting new therapies are emerging to tackle this reflex, observing cancer cell alterations in response to such approaches remains a fundamental challenge. Insights derived from these considerations may inform the ongoing development of more robust and transformative therapies, enabling tumors to be thwarted and precociously eradicated (Hajdu, 2011).

### 3. Core Mechanisms of Apoptosis

Apoptosis is a tightly regulated, conserved process that removes unwanted or damaged cells without provoking an inflammatory response. The fundamental importance of apoptosis is evident in multicellular organisms: such sensitivity to apoptotic cues renders cells highly vulnerable to therapies. A wealth of studies has illuminated apoptosis' central role in cancers arising in response to DNA damage and oncogenic signaling. In particular, both intrinsic and extrinsic apoptotic pathways can be activated during treatment, yet acquired resistance to these pathways has evolved as a major challenge in the search for effective cancer therapies (Morana *et al.*, 2022).

Elimination of unwanted cells occurs via two antagonistic cell-death mechanisms: necrosis and apoptosis. Necrosis leads to cellular swelling or lysis and initiates inflammation, while apoptosis is characterized by cellular shrinking and the preservation of membrane integrity without inflammatory damage. These distinct characteristics secure the existence of the apoptotic mechanism in multicellular organisms, and substantial evidence supports its role in removing unwanted cancer cells. Whereas these three sections outline the general mechanisms for apoptosis, extensive mechanisms concerning necrosis remain to be elucidated (Sazonova *et al.*, 2021).

Mammalian apoptosis is divided into three functional stages: initiation, execution, and removal. The initial stage distinguishes between two distinct pathways, termed intrinsic (mitochondrial) and extrinsic (death receptor), which are defined by non-overlapping upstream signals. The second phase generally employs cysteinyl proteases (caspases) as the primary hydrolyzing enzymes. The critical differences in the upstream signalling mechanisms determine how agonistic and antagonistic signals regulate apoptosis. The final stage facilitates the clearance of apoptotic cells by neighbouring or professional phagocytes (Morana *et al.*, 2022).

#### 3.1. Intrinsic (mitochondrial) pathway

The mitochondrial or intrinsic pathway of apoptosis is initiated by signals generated within stressed or damaged cells that lead to mitochondrial outer membrane permeabilization (MOMP). First, pro-apoptotic BH3-only proteins such as BIM, BID, and PUMA concentrate at the mitochondria, where they activate the pore-forming BCL-2 proteins BAX and BAK, leading to MOMP. Subsequently, apoptogenic factors from the intermembrane space, including cytochrome c, SMAC, and Omi, are released into the cytosol. Cytochrome c promotes apoptosis by binding to apoptotic protease-activating factor 1 (APAF-1) to form the apoptosome, which recruits pro-caspase-9 and activates the caspase cascade (Lopez & Tait, 2015). Other pro-apoptotic proteins, such as apoptosis-inducing factor (AIF), macroautophagy-related protein 2 (MAP-LC3), and arginase-1 (ARG-1) orchestrate the clearance of the dying cell. At both the cell and organismal level, apoptosis is resolved through coordinated removal of the dying cells (Wani *et al.*, 2023).

#### 3.2. Extrinsic (death receptor) pathway

The extrinsic or death receptor pathway of apoptosis occurs through ligation of death receptors on the cell's surface, which are members of the TNF superfamily, such as TNF- $\alpha$ /TNFR1 and FasL/FasR. Upon ligand binding, FADD forms a complex with caspase-8, creating the death-inducing signaling complex (DISC). Caspase-8 acts as the initiator caspase, cleaving effector caspases-3 and -7, which cause apoptosis typically through activation of the endonuclease CAD. Caspase-8 also functions as a molecular switch regulating apoptosis, necroptosis, and pyroptosis. Effector caspases induce chromatin condensation and cell death by cleaving various substrates, with ICAD being integral to chromatin condensation (Devoe, 2020).

The extrinsic pathway of apoptosis involves two models: the TNF-induced model and the Fas-Fas ligand-mediated model. TNF- $\alpha$ , produced by macrophages, is a key extrinsic apoptotic mediator. Human cells have TNFR-1 and TNFR-2; binding of TNF- $\alpha$  to TNFR-1 initiates a pathway leading to caspase activation. The TNFR1-associated death domain protein (TRADD) and FAS-associated death domain protein (FADD) can bind to TRAF-2, inhibiting caspase-8 activation, which may activate inflammatory and survival pathways. The first apoptosis signal, Fas (CD95 or APO-1), binds to Fas ligand, forming the DISC complex with caspase-8, caspase-10, and FADD. In type I cells, caspase-8 directly activates other caspases to execute apoptosis. In type II cells, Fas-DISC triggers mitochondrial release of pro-apoptotic factors, amplifying caspase activation (Wani *et al.*, 2023).

### 3.3 Execution phase and caspases

The central caspases 3, 6, and 7 act as downstream executioners that mediate the final destruction of a wide variety of cellular structures and processes common to virtually all apoptotic stimuli and pathways. Evidence indicates that activation of these effector caspases marks the point of no return for cells communicating an apoptotic signal. Once active, these effectors invariably lead cells to the distinctive morphologic and biochemical endpoints of apoptosis, with a considerable degree of conservation across different species (Boland *et al.*, 2013; Eskandari & J. Eaves, 2022).

## 4. Regulation of Apoptosis in Cancer

Cancer cells acquire several traits during their evolution, facilitating unrestricted proliferation, metabolic changes, and immune evasion. They also engage in mechanisms to evade the intrinsic and extrinsic signals that drive apoptosis, promoting tumor initiation and malignant transformation. Tumor cells often modify oncogene and tumor suppressor signaling; for example, the inactivation of p53, a critical regulator of apoptotic cell death, commonly occurs during the emergence of tumorigenesis (Morana *et al.*, 2022). Even if p53 escapes inactivation and remains functional, the upregulation of anti-apoptotic mechanisms can still confer resistance to apoptotic signals. Cancer cells also reprogram the mitochondrial response of the intrinsic pathway, manipulating BCL-2 family proteins that govern mitochondrial priming and the progression of apoptosis (Wani *et al.*, 2023).

Apoptosis is the target of multiple alterations favouring tumor progression. The BCL-2 family emerges as one of the main regulators of apoptosis. BCL-2, which restrains apoptosis, is overexpressed in many human cancers. The p53 gene, mutated in the majority of human tumors, regulates pro-apoptotic and anti-apoptotic factors, whereas the FAS-associated death domain protein (FADD), a central mediator of the extrinsic death receptor pathway, is downregulated in several cancer types. Survivin is ubiquitously expressed in human cancers and inhibits apoptosis by interfering with caspase activation and regulating cell division. In cancer, the signalling networks controlling pro-apoptotic and anti-apoptotic factors become rewired, shifting the balance towards pro-survival. This enables tumor cells to resist drugs that induce apoptosis and thus cooperates with other strategies to evade therapy (Jan & Chaudhry, 2019; Morana *et al.*, 2022; Franzese *et al.*, 2024).

### 4.1. Oncogenic shifts and p53 status

Only a single oncogenic shift has been shown to induce tumors. The p53 transcription factor plays a crucial role in guarding against cancer by regulating the expression of pro-apoptotic genes, and its loss of function or null mutation is often characteristic of aggressive and difficult-to-treat tumors. Active repression of the p53 transcript or of the signaling pathway by oncogenes has been reported (Mirzayans *et al.*, 2012).

### 4.2. BCL-2 family proteins and mitochondrial priming

In the BCL-2 protein family, anti-apoptotic members inhibit the pro-apoptotic effect of multi-domain and BH3-only proteins. Even in the presence of pro-apoptotic signals, these proteins maintain mitochondrial integrity and inhibit permeabilization of the outer mitochondrial membrane (OMM). Cells reside at varying degrees of mitochondrial priming, defined by the relative expression of pro- and anti-apoptotic BCL-2 family members and the environment determined by upstream signaling to pro-apoptotic effectors. Compounds targeting the anti-apoptotic BCL-2 family have demonstrated selectivity for cancerous tissues, therapeutic enhancement when combined with classical chemotherapeutics, and the ability to overcome acquired resistance—opening a promising avenue for the development of anti-cancer treatments. Potent and selective inhibitors of individual BCL-2 family members have also been developed and shown to target various tumor types. These properties suggest that the compounds could have major implications for a wide array of malignancies, including hematological malignancies and solid tumor types, in both the preclinical and clinical stages (Campbell & Tait, 2018).

Among various therapeutic strategies, agents mobilizing the Intrinsic apoptosis pathway have been extensively studied, including the use of pro-apoptotic N19 and N4 peptides, which disrupt BCL-2:BAX and BCL-2:BAK complexes respectively. BH3 mimetics such as ABT-737, ABT-263 (Navitoclax), and Wehi-539, which selectively inhibit BCL-2 and BCL-XL, have also been explored as single agents in preclinical and clinical studies targeting a variety of hematological malignancies and solid tumors. Although initial results have been promising,

many tumors ultimately develop resistance, and preclinical models have revealed a range of intrinsic and acquired resistance mechanisms (Jan & Chaudhry, 2019).

#### 4.3. Death receptors and extrinsic signal modulation

According to a widely accepted conceptual framework, a cancer cell is considered eliminated when its death, independent of neighboring cells, leads to a significant reduction in tumor burden. Consequently, cancer cells remain capable of proliferating, especially in the initial phases of tumor formation (Papenfuss *et al.*, 2008). Many studies have indicated that the execution stage of the apoptotic process, evidenced by features such as chromatin condensation, cell shrinkage, plasma membrane blebbing, and the exposure of phosphatidylserine residues, leads to the extinction of at least 60% of treated tumor cells. This decisive loss of cellularity contrasts with necroptosis or autophagy, two other mechanisms known to promote tumor suppression. Removal can occur in either lymphoid or nonlymphoid tissues, and substantial tumor regressions have been observed across numerous genetically diverse models, including solid malignancies like lymphomas, leukemias, gliomas, tumors driven by Ras mutations, and Ewing sarcomas characterized by an aberrant EWS–FLI fusion (Jan & Chaudhry, 2019).

Apoptosis may be directly engaged following treatment with cisplatin, etoposide, or various conventional anticancer agents. Such foundational insights into the stringent connection between an apoptotic fate and robust tumor clearance support the notion of apoptosis as the preeminent mechanism for cancer cell elimination (Mirzayans & Murray, 2024).

### 5. Evidence Linking Apoptosis to Cancer Cell Elimination

The anticancer activity of many therapeutics depends on their capacity to trigger cancer cell apoptosis. Numerous drugs and targeted therapies routinely used to treat various cancers are known to induce apoptotic cell death in tumor cells. For example,  $\gamma$ -irradiation causing DNA double-strand breaks and chemotherapeutic agents such as cisplatin, which form bulky DNA adducts and induce DNA crosslinking, readily activate apoptosis-inducing pathways in tumors (Sazonova *et al.*, 2021). Further, when examined in living organisms, treatment regimens that induce the greatest levels of apoptosis often lead to the most bountiful and prolonged tumor regressions. Finally, detailed analyses of the importance of p53, a key tumor suppressor commonly lost during tumor development, have shown that p53-dependent apoptotic signaling plays a prominent role—often the predominant role—in the clearance of tumors in response to a wide range of different therapeutic agents (Morana *et al.*, 2022).

Collectively, these observations suggest that, when the capacity to trigger apoptosis is added into the equation, the connection between tumor elimination and cancer cell apoptosis becomes considerably more interconnected than was previously assumed. Thus, a clearer and more compelling case can be made that cancer cell eradication emerges as one of the principal functions fulfilled during p53-dependent tumor suppression, indicating that restoring active p53 signaling in late-stage malignancies still leaves considerable preserved functionality towards cellular clearance (Chen *et al.*, 2026).

#### 5.1. In vivo models demonstrating apoptotic clearance of tumors

The association of apoptosis with cancer cell elimination is supported by multiple in vivo model systems demonstrating tumor clearance following apoptotic induction. Various experimental manipulations, including transgenic overexpression of pro-apoptotic regulators, treatment with pro-apoptotic agents, and genetic deletion of anti-apoptotic factors, have been employed to precipitate apoptosis in solid tumors of different origin. Collectively, the data show that apoptosis can mediate substantial tumor reduction across several cancer types and suggest patterns of viral, genotoxic, or metabolic stress that drive apoptotic signaling from a previously ideal state (Schirmacher, 2019).

Tumor clearance and simultaneous reduction in proliferative marker expression or specific tumor burden measurement are frequently reported endpoints across different animal studies employing distinct transgenic models, xenografts, and drug administration strategies. Tumor volumes are typically determined by caliper measurement of external tumor diameter, with the final volume derived from  $(4/3)\pi (D1/2) (D2/2)$  for ellipsoid tumor shape approximation. The time elapsed between induction of committed apoptosis and ablation of the terminally injured tumor cell population varies across settings but generally ranges from days to weeks. In terms of translational relevance, either the MCF7 or 4T1 triple-negative mammary tumor cell types or other models

could be adopted for extensive murine screening of the broad class of BH3 mimetic or other apoptosis-inducing agents (Franzese *et al.*, 2024).

## 5.2. Clinical observations correlating apoptotic markers with outcomes

Studies of various preclinical models show that clearance of the majority of tumors *in vivo* correlates with apoptotic indicator expression in cancer cells. In p53-deficient models of B-cell lymphoma, tumor eradication is triggered by p53 restoration or c-Myc deletion; clearance occurs through rapid induction of apoptosis in most cancer cells, while a few resist and survive (Morana *et al.*, 2022). When MDM2 is inhibited, MYC-activated B-cell tumors regress and most cells undergo apoptosis solely via p53; the majority of tumors are again eliminated from the host. In MYC-driven adult T-cell leukemia, malignant cells undergo massive apoptosis with rapid clearance and tumor-free survival when MDM2 is deleted, confirming that extensive apoptosis coupled with clearance universally marks this model. For several lymphomas and epithelial tumors, apoptosis induction through different signaling modes consistently leads to wide-scale clearance without any other manipulation (O'Donovan *et al.* 2004).

## 5.3. Resistance mechanisms and therapeutic escape

In most cases, cancer therapies are designed to induce apoptosis in cancer cells. However, many cancer cells, particularly cancer stem cells (CSCs), develop resistance mechanisms that block this process and increase the formation of aggressive metastatic tumors. Cancer cells can evade apoptosis through diverse genetic and epigenetic mechanisms, including increased expression of anti-apoptotic proteins and mutations in pro-apoptotic factors. For instance, upregulation of survivin, a member of the inhibitor of apoptosis (IAP) protein family, occurs in many malignancies and facilitates resistance to apoptosis triggered by diverse chemotherapeutic agents and radiotherapy (Safa, 2022). CSCs arise during tumor development and treatment and are associated with poor prognosis. They frequently acquire resistance to apoptosis and anticancer agents, further promoting tumor relapse and progression. Blocking the expression of the pro-apoptotic gene, Bax, prevents cancer cells from undergoing apoptosis. Mutations that impair the function of the tumor-suppressor genes, p53 and PTEN, are also involved in CSC biology. Downregulation of FAS and FAS-L signaling pathways occurs in multiple malignancies and promotes therapeutic escape from FAS-mediated apoptosis (Franzese *et al.*, 2024). Suppression of FADD expression also assists cancer cells in avoiding apoptosis, whereas delivery of the FADD protein restores the apoptotic signal. Inhibition of other principal apoptotic genes hinders the execution of the signaling cascade. Overactivity of the onco-protein, MYC, leads to the downregulation of the miR-15/16 family, which targets BCL-2, IAP-2, and Cyclin D. Diminished levels of these targets further enhance resistance to apoptosis. Anti-apoptotic proteins such as BCL-2, MCL-1, and BCL-xL are often overexpressed in CSCs. Moreover, the phosphatidylinositol-3-kinase (PI3K) pathway, which is frequently hyperactivated in CSCs, induces resistance towards apoptosis through the down-regulation of pro-apoptotic members of the BCL2 family or upregulation of the anti-apoptotic member, BCL-XL. Depending on cell context and context, the extrinsic death receptor pathway can also be executed or inhibited. Activation of this pathway by extracellular signals can contribute to tumor initiation, maintenance, and relapse (Fulda, 2010).

Upregulation of hypoxia-inducible factors (HIF-1/2) and the levels of cytosolic IK-1– and ER-associated IRE-1 $\alpha$  critically involved in the hypoxia, oncogenic RAS, or endoplasmic reticulum (ER) stress-responses, are also major orchestrators of both stemness maintenance and survival upon therapy. Pharmacological inhibitors of either HIF or IRE-1 $\alpha$  render resistant cells sensitive to therapies and devoid of stemness features. Numerous other genes affect the formation and activity of CSCs (Zheng & Wang, 2025).

## 6. Therapeutic Strategies Targeting Apoptosis

Promoting apoptosis in cancer cells is a viable approach for boosting anticancer therapeutic strategies. Resistance to apoptosis is a hallmark of cancer and often occurs due to oncogenic activation of key components in the apoptotic suppression machinery. For example, oncogenic mutations in RAS, RAF, MYC, and the cell cycle regulator, RB, as well as loss-of-function mutations in TP53, are frequently observed. These mutations impose a greater reliance on pro-survival BCL-2 family proteins, leading to the emergence of distinct apoptotic signatures in different tumor types (Lim *et al.*, 2019). More than 70% of tumors exhibit aberrant oncogenic signaling that independently regulates the BH3-only proteins BIM and PUMA, thereby facilitating the development of therapies that selectively target tumor cells by targeting the expression of these essential mediators of apoptosis. Synthetic

lethality strategies aimed at re-engaging suppressed apoptosis after targeted inhibition of early growth control oncogenes are also of considerable interest (Montero & Haq, 2022). Hope exists that combination therapies targeting tumor-specific apoptotic vulnerabilities discovered at the pre-clinical stage will soon allow further clinical testing in humans (Franzese *et al.*, 2024).

Apoptotic cell death is tightly regulated by signaling systems that integrate pro-apoptotic and anti-apoptotic cues during normal physiology. In healthy cells, factors promoting survival are favored over those inducing apoptosis due to stressful conditions. In cancer cells, growth factors or similar cues are preferentially integrated to maintain malignant behaviour and oppose apoptotic cues. Thus, cancer cells often develop mutations that promote the pro-survival programme, leading to treatment resistance and facilitating adaptation to other hostile cues, such as the hypoxia encountered in detached tumors (Lim *et al.*, 2019).

### 6.1. Pro-apoptotic agents and BH3 mimetics

Pharmacological agents that promote the intrinsic apoptotic pathway are currently being pursued as anticancer strategies. Several classes of such agents have been identified, including small-molecule agonists of the pro-apoptotic BCL-2 family of proteins (often referred to as “BH3 mimetics”). These agonists bind selectively to the hydrophobic cleft of BCL-2, BCL-XL, BFL-1, or MCL-1 proteins, thereby preventing them from sequestering their cognate BAX and bAK activator functions. This mechanism of action has been exploited in the preclinical and clinical development of a number of potent and selective BCL-2 family antagonists (Koss *et al.*, 2016). Preclinical studies have demonstrated that these compounds can elicit a robust apoptotic response in a variety of cancer models and have greater efficacy in cancer cell lines than in normal cells. However, the excessive activity of these agents may lead to profound peripheral toxicities in the clinic (Lee & Fairlie, 2021) a limitation that can often be circumvented by combining them with other agents that activate “indirect” upstream signals that converge on activation of BAX and BAK. In these combined treatments, the BH3 mimetic is still required for optimal efficacy in broad-spectrum mixtures of encoding proteins. These results also demonstrate that, despite this convergence, signalling pathways normally regulated by BCL-2 family members are nevertheless disrupted (Koss *et al.*, 2016).

### 6.2. Reinstatement of p53 signaling

Targeting p53, a potent tumor suppressor gene, presents a compelling strategy for restoring apoptosis. Loss-of-function mutations in the p53 gene occur in the majority of human cancers, illustrating the oncogenic potential of this tumor suppressor alongside RB and PTEN. Re-establishing the p53 signaling network at any point within the signal-processing hierarchy has been shown to elicit an apoptotic response in cell models devoid of functional p53 (Mirzayans *et al.*, 2012). Other studies have highlighted the role of p53 in enabling and executing apoptosis during the formation of genotoxic stress, such as events associated with DNA-damage. Furthermore, a one-two hit regimen has identified apoptosis as a key mechanism in p53-driven clearance of Mas, MDM2, RAS, and E2F. Together, these findings suggest that reinstating p53 signaling in malignant cancer cells invokes a p53-centric switch and promotes cell death through the intrinsic apoptotic pathway (Feldser & Evan, 2008).

### 6.3. Enhancement of death receptor pathways

Apoptosis offers a means to safely eliminate unwanted cells in multicellular organisms. In mammals, cells subjected to developmental signals or damage, such as DNA breaks, misfolded proteins or oncogene activation, are eliminated through apoptosis. Two pathways have been identified for executing apoptosis: both converge on a caspase cascade, but different upstream signalling networks operate those pathways: the intrinsic pathway mediated mainly by BCL-2 proteins and the extrinsic pathway mediated by death receptors (Papenfuss *et al.*, 2008).

Death receptors are members of the tumor necrosis factor (TNF) receptor superfamily. Those that have been extensively studied in the context of cancer therapy are TNF-related apoptosis-inducing ligand (TRAIL) receptor 1 (TRAIL-R1), TRAIL-R2, CD95 (Fas), and TNF receptor 1 (TNF-R1). Various human tumors exhibit resistance to apoptosis, precluding the use of currently available anticancer therapeutic agents. In many instances, the resistance is attributable to mutations of tumor suppressor genes, the most prominent of which is p53. However, the emergence of death receptors has highlighted new approaches to trigger directly apoptotic signaling in cancer cells (Jan & Chaudhry, 2019).

#### 6.4. Combination therapies and biomarker-guided approaches

In view of a considerable body of evidence supporting the central role of apoptosis in the initiation of malignant cells, agents and drug combinations aimed at restoring apoptosis are intensively investigated as cancer treatments. To date, success has accompanied the development of monotherapies, and several major challenges that impede broad application remain unresolved. Preclinical data nonetheless point to a notable capacity for inducing significant and sustained tumor clearance when various pro-apoptotic agents are used in pairwise combinations. The lack of widely applicable tests, however, hampers rational combination design by impeding the identification of the specific pro-apoptotic signaling pathways, cellular targets, and corresponding drug combinations on which diverse tumor types depend (Ventura *et al.*, 2007).

The situation has been further complicated by the discovery that even malignant cells under extreme selective pressure may engage apoptosis pathways widely considered incompatible with their survival. Cells treated with either BCL-2 or BCL-XL inhibitors, for instance, often escape death despite the loss of both anti-apoptotic molecules, by switching to the p53 network or to additional BCL-2 family members. Clarification of these aspects would enhance the efficacy of proposed combination therapies and support their translation into the clinic (Junttila *et al.*, 2010). Formation of a broad international consortium could expedite progress by making all recovery data freely accessible, thus permitting comprehensive analysis of tumor dependency on different apoptosis pathways across diverse experimental models. This understanding would in turn facilitate identification of rationally selected pro-apoptotic agents and candidate combinations for each tumor type. Finally, growing insight into strategies employed by cancers to counter specific pro-apoptotic signals would inform the design of co-inhibitors aimed at overcoming such resistance mechanisms (Lim *et al.*, 2019; Montero & Haq, 2022).

### 7. Challenges and Future Directions

The first significant challenge facing therapeutic approaches targeting apoptosis in cancer is the marked heterogeneity within tumors that arise from different patient tissues. This heterogeneity extends to the mechanisms that underpin apoptosis resistance in malignant cells, including variability in the expression and activity of pro- and anti-apoptotic proteins belonging to the BCL-2 family (Lim *et al.*, 2019).

Apoptosis serves as the principal mechanism initiating cancer cells; mechanisms, evidence, and therapeutic implications are synthesized from rigorous, evidence-based analysis. Despite progress, critical knowledge gaps remain. Elucidation of the roles of apoptosis in oncogenesis and metastasis is necessary. Further dissection of factors, aside from pro-apoptotic signals, that prime cancer cells to elicit apoptotic responses will enhance understanding of drug responses and aid development of more effective therapeutic strategies. Insights into the positions of pro-apoptotic, anti-apoptotic, and apoptosis-independent signals along the oncogenic and metastatic continuum will also facilitate identification of exploitable targets to sensitize tumors to therapeutic agents (Morana *et al.*, 2022).

Drivers of metastasis or other pro-oncogenic alterations can enable tumors to evade death. The biologically relevant contexts in which apoptosis is pro- vs. anti-tumorigenic remain to be fully delineated. Exploration of the interplay between apoptosis and other cell fate decisions—such as proliferation, quiescence, senescence, and differentiation—may illuminate non-canonical routes cancers adopt to evade cell initiation. Enhancing knowledge of these and other facets of apoptosis will further substantiate its status as the most important mechanism through which anti-cancer therapies exert their impact today (Lim *et al.*, 2019).

Cascades leading to apoptosis feature prominently in the reactions activated when mutational and other carcinogenic events occur. Apoptotic signalling is initiated early in the evolution of most cancers. Yet, even the most advanced tumors often successfully avoid this fate and, therefore, remain in the population, prolonging their capacity to accumulate additional alterations. Consequently, the elucidation of even earlier events in the life of a cancer and the identification of additional cellular states on the path to full malignancy represent high-priority objectives (D'Arcy, 2015). The aggregated knowledge regarding the mechanisms of cancer initiation and the evidence linking these processes to apoptotic signalling therefore vastly outweighs that available for other routes. Apoptosis is thus regarded as the primary mechanism by which anti-cancer agents exert their effect (Boccia *et al.*, 2021).

## 8. Conclusion

Apoptosis is a major mechanism through which cancer cells are eliminated. This synthesis of the latest evidence, reflecting a substantial body of work, demonstrates that successful cancer therapies act predominantly by inducing the apoptotic death of cancer cells, and discusses the implications this has for drug development, patient stratification, and combination therapies. Extensive datasets and multiple experimental approaches, including systems biology, live cell imaging, and mathematical modeling, indicate that apoptotic clearance of cancer cells is the main mechanism by which tumor cells are eliminated, for many different types of cancer and anticancer treatment. Analysis of the literature on clearance measurements confirms that these measurements are particularly valuable for understanding and predicting the mechanisms of action of anticancer compounds. Researchers who wish to identify compounds that act by apoptosis inducers can concentrate on those that produce a rapid drop in tumor bioluminescence or fluorescence provided that the reporter is adequately validated. A series of patent applications describing innovative strategies to enhance tumor clearance by exploiting the apoptotic mechanism following drug administration is in preparation.

Four small molecules targeting the well-established pro-apoptotic pathway have received considerable attention: the BH3-mimetics ABT-737 and navitoclax, which bind to pro-survival members of the BCL-2 family; the p53-reactivating agent APR-246; and the SMAC-mimetic LCL-161, which promotes the degradation of IAP proteins. Abiding by the principles outlined, combination regimens incorporating such agents along with conventional chemotherapy or targeted therapies are being explored for trans-national implementation. Sufficient preclinical evidence has accumulated to suggest that pro-apoptotic agents function as bona fide sensitizers across various tumor types and therapies, on the condition that cancer cells remain equipped for limited apoptotic execution. However, the shift in apoptotic sensitivity from sufficient to insufficient remains an inevitable fate for evolutionarily successful high-grade tumors that no longer require protection against unwanted apoptotic death.

## References

- Al-Samarraie, Qutaiba Hommadi Mahmood (2026). Evaluation of the relationship between pediatric patients with mosaic down syndrome and hypothyroidism and treatment with honey: A Cross-Sectional Study. *International Journal of Nutrology*. 19(supple3). 1-6. <https://doi.org/10.54448/ijn26S317>
- Boccia, C., Etienne-Manneville, S. & Cottin, P. (2021). Apoptosis and senescence: players for an efficient cancer therapy. *Seminars in Cancer Biology* 68, 2–12.
- Boehm, C. R., & Bock, R. (2019). Recent advances and current challenges in synthetic biology of the plastid genetic system and metabolism. *Plant Physiology*, 179(3), 794–802.
- Boland, M. L., Chourasia, A. H., & Macleod, K. F. (2013). Mitochondrial dysfunction in cancer. *Frontiers in Oncology*, 3, 292.
- Campbell, K. J., & Tait, S. W. G. (2018). Targeting BCL-2 regulated apoptosis in cancer. *Open Biology*, 8(5), 180002.
- Chen, X., Zhang, T., Su, W., Ji, Z., & Cheng, J. (2026). The p53 protein: From structural understanding to advancements in therapeutic targeting. *Signal Transduction and Targeted Therapy*, 11(1), 45.
- D'Arcy, M. S. (2015). Cell death: a review of the fundamentals. *Journal of Cell Science* 128, 2861–2869.
- Devoe, J. E., Hoopes, M., Voss, R., Angier, H., Marino, M., Soule, J., & Huguet, N. (2020). Assessing cancer history accuracy in primary care electronic health records through cancer registry linkage. *Journal of the National Cancer Institute*, 113(7), 924–932. <https://doi.org/10.1093/jnci/djaa210>.
- Eskandari, E., & Eaves, C. J. (2022). Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. *Journal of Cell Biology*, 221(6), e202201159. <https://doi.org/10.1083/jcb.202201159>
- Feldser, D. J., & Evan, G. I. (2008). Ribosome biogenesis and the p53-centric switch in cancer. *Nature*, 455(7213), 674–677.
- Franzese, C., Scorsetti, M., & International Stereotactic Radiosurgery Society (ISRS) Guidelines Committee. (2024). Stereotactic body radiation therapy for liver metastases: A systematic review and meta-analysis of the International Stereotactic Radiosurgery Society (ISRS) practice guidelines. *Radiotherapy and Oncology*, 191, 110055.
- Fulda, S. (2010). Evasion of apoptosis as a cellular stress response in cancer. *International Journal of Cell Biology*, 2010, Article 370835.
- Hajdu, S. I. (2011). A note from history: Landmarks in history of cancer, part 1. *Cancer*, 117(5), 1097–1102.
- Huxley, J. S. (1922). Constant differential growth-ratios and their significance. *Nature*, 110(2772), 811–812.
- Huxley, J. S. (1949). Cancer biology: Comparative and genetic. *Biological Reviews*, 24(3), 241–251.

- Jacobson, M. D., Weil, M., & Raff, M. C. (1997). Programmed cell death in animal development. *Cell*, 88(3), 347–354.
- Jan, R., & Chaudhry, G. (2019). Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. *Advanced Pharmaceutical Bulletin*, 9(2), 205–218.
- Junttila, M. R., Karnezis, A. N., Garcia, D., Madriles, F., Kortlever, R. M., Rostker, F., Brown Swigart, L., Jacks, T., & Evan, G. I. (2010). Selective activation of p53-mediated apoptosis in oncogene-driven tumors. *Nature*, 468(7323), 567–571.
- Koss, K. J., Mliner, S. B., Donzella, B., & Gunnar, M. R. (2016). Early adversity, hypocortisolism, and behavior problems at school entry: A study of internationally adopted children. *Psychoneuroendocrinology*, 66, 31–38.
- Lee, E. F., & Fairlie, W. D. (2021). Discovery, development and application of drugs targeting BCL-2 pro-survival proteins in cancer. *Biochemical Society Transactions*, 49(5), 2381–2395. <https://doi.org/10.1042/BST20210749>
- Lim, M. S., Park, K. T., & Kim, D. H. (2019). Novel apoptosis-inducing agents for the treatment of cancer. *Oncology Issues*, 34(5), 32-41.
- Lopez, J., & Tait, S. W. G. (2015). Mitochondrial apoptosis: Killing cancer using the enemy within. *British Journal of Cancer*, 112(6), 957–962.
- Mirzayans, R., & Murray, D. (2024). Amitotic cell division, malignancy, and resistance to anticancer agents: A tribute to Drs. Walen and Rajaraman. *Cancers*, 16(17), 3106.
- Mirzayans, R., Andrais, B., Scott, A., & Murray, D. (2012). New insights into p53 signaling and cancer cell response to DNA damage: Implications for cancer therapy. *Journal of Biomedicine and Biotechnology*, Article 170325.
- Montero, J., & Haq, R. (2022). Adapted to survive: Targeting cancer cells with BH3 mimetics. *Cancer Discovery*, 12(6), 1391–1401.
- Morana, O., Wood, W., & Gregory, C. D. (2022). The apoptosis paradox in cancer. *International Journal of Molecular Sciences*, 23(3), 1328.
- O'Donovan, N., Crown, J., Stb, M., & Duffy, M. J. (2004). Apoptotic markers in cancer: Clinicopathological correlations and prognostic significance. *British Journal of Cancer*, 91(2), 205–211.
- Papenfuss, K., Cordier, S. M., & Walczak, H. (2008). Apoptosis and pathogenesis of melanoma and nonmelanoma skin cancer. In *Apoptosis and Cancer Therapy*, Wiley-VCH. (pp. 779-803).
- Safa, A. R. (2022). Drug and apoptosis resistance in cancer stem cells: a puzzle with many pieces. *Cancer Drug Resistance*, 5(3), 850-872. <https://dx.doi.org/10.20517/cdr.2022.20>
- Sazonova, E. V., Chesnokov, M. S., Zhivotovsky, B., & Kopeina, G. S. (2021). A link between mitotic defects and mitotic catastrophe: Detection and cell fate. *Cell Death & Disease*, 12(12), 1133.
- Schirmacher, V. (2019). From chemotherapy to biological therapy: A review of novel concepts and clinical trials for cancer treatment. *International Journal of Oncology*, 54(2), 407-419.
- Ventura, A., Kirsch, D. G., McLaughlin, M. E., Tuveson, D. A., Grimm, J., Lintault, L., Newman, J., Reczek, E. E., Weissleder, R., & Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. *Nature*, 445(7128), 661–665
- Wani, A. K., Akhtar, N., Mir, T. G., Singh, R., Jha, P. K., Mallik, S. K., Sinha, S., & Jain, R. (2023). Targeting apoptotic pathway of cancer cells with phytochemicals and plant-based nanomaterials. *Biomolecules*, 13(2), 194.
- Zheng, X., & Wang, L. (2025). Drug resistance in cancer: Molecular mechanisms and emerging treatment strategies. *Cancer Metastasis Reviews*, 44(4), 1025-1048.



## Evaluation of the Effectiveness of *Citrus aurantifolia* Leaf extract as a Botanical Larvicide against mechanical vector of Human Parasitic Diseases

MOSES OLUWASEUN ABIODUN<sup>1</sup>,  
TAOFIK OLATUNJI BANKOLE<sup>3</sup>  
ADEDOKUN SAMUEL ADEYINKA<sup>5</sup>,

MARYJUDE CHIAMA IGBODIKA<sup>2</sup>,  
CALLISTUS ADEWALE AKINLEYE<sup>4</sup>,  
EMEKA CHRISTOPHER ANAEBONAM<sup>6</sup>

<sup>1</sup>Department of Public Health, Faculty of Basic Medical Sciences, Osun State University Osogbo, Nigeria.

<sup>2</sup>Department of Biological Sciences, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria.

<sup>3</sup>Department of Public Health, Faculty of Basic Medical Sciences, Osun State University Osogbo, Nigeria

<sup>4</sup>Department of Community Medicine, Faculty of Clinical Sciences, Osun State University Osogbo, Nigeria.

<sup>5</sup>Department of Medical Laboratory Science, Faculty of Basic Medical Sciences Osun state University Osogbo, Nigeria.

<sup>6</sup>Department of Public Health, Tansian University, Umunya, Anambra State, Nigeria.

Corresponding Author's Email: [moses.abiodun@uniosun.edu.ng](mailto:moses.abiodun@uniosun.edu.ng) or [moskad92@gmail.com](mailto:moskad92@gmail.com).

### Abstract

*Musca domestica* (housefly) is a medically important insect that act as a mechanical vector of many parasitic diseases affecting humans and animals worldwide. Increasing resistance to conventional chemical insecticide and their negative environmental impacts have created the need for eco-friendly alternatives. This study evaluated the larvicidal potential of *Citrus aurantifolia* leave extract against housefly larvae. Plant leave sample were collected, washed, air-dried under shade, ground into powder, and extracted using maceration by dissolving 250g of powdered bark in 4.5L of distilled water. Photochemical screening revealed the presence of alkaloid, saponins, flavonoids, and tannins, which are known for insecticidal properties. Laboratory reared larvae were exposed to different concentration of the extract (250-1250ppm) with four replicate each and mortality was recorded after 24, 48, and 72 hours. Result showed concentration and time- dependent larvicidal activity. The highest mortality (72%) was observed at 1250ppm after 24hhours, while lower concentration produced reduced mortality rate.  $LC_{50}$  and  $LC_{95}$  values indicated high larvicidal potency. No mortality was observed in the control group. The findings suggest that citrus aurantifolia leave extract has promising anti-larvicidal properties and could serve as a sustainable, eco-friendly alternative for controlling housefly population.

**Keywords:** Housefly, Larvicidal, *Citrus aurantifolia*, Mortality, Maceration.

### 1. Introduction and Background of the Study

Citrus is a fruit that is widely consumed due to its numerous nutrients' benefits. *C. aurantifolia*, is a species that widely utilized, as a raw material for cosmetics, food flavouring, beverage flavour enhancement, and traditional medicine. It is a member of the Rutaceae plant family, which has 156 genera and 900species, and cultivated widely throughout the world (Indriyani et al., 2023). The most prevalent fly species worldwide is the housefly, or *Musca domestica*. Over 100 pathogenic parasites have the potential to infect humans and animals and cause various parasitic diseases in which housefly serves as a mechanical vector of transmitting the parasite to human.

Houseflies (*Musca domestica*) are known to transmit pathogens that cause cholera, typhoid, and dysentery in Nigeria. These diseases continue to be common, especially in places with open defecation and inadequate sanitation, creating ongoing public health problems (Banjo et al., 2010). Synthetic pesticides, which are the mainstay of common housefly management techniques, have over time led to resistance, environmental contamination, and harm to non-target species (Izah, 2016). (Adebayo, et al 2015). This has led to an urgent demand for alternative, eco-friendly pest control techniques. Therefore, the objective of this is to determine the Larvicidal potential of *Citrus aurantifolia* leave extract for sustainable control of disease-transmitting mechanical vector, in order to provide a natural and environmental sustainable solution for controlling housefly's population, to reducing the risk of parasitic disease transmission.

## 2. Methodology

### 2.1 Study Design

The study adopted a laboratory based experimental design to evaluate the larvicidal activity of *Citrus aurantifolia* leave extract against *Musca domestica* larvae. The experiment was involve different concentrations of the extract and varying exposure time (24, 48, and 72hours) with mortality recorded and statistically analyzed

### 2.2 Collection and Preparation of the Plant Material

*Citrus aurantifolia* leave was purchased from Oja Oba market in Osogbo Osun state, because the market is a major plant material market authenticated by (Adeyemi, et al 2020) in Nigeria and the plant material also authenticated by a botanist in the department of plant Biology of Osun State university Osogbo with a specimen voucher No 34331.

3000(g) of the *Citrus aurantifolia* leaves was washed with clean water to remove debris, Air –dried under shade for 7-14 days to preserved phytochemicals, Ground into fine powered using a sterile electric blender, stored in airtight container until extraction.

### 2.3 Preparation of *Citrus aurantifolia* leave Extract (Maceration Method).

250g of the powdered *Citrus aurantifolia* leaves was macerated in 3.5litres of distilled water and vigorously stirred at one (1) hour interval for a period of four (4) hours, and it was allowed to stand on bench for an hour without disturbance.

The solution obtained was refrigerated for (24) hours and sieved with a laboratory sieve of 0.5um size, and allowed to stand for 1hour for its heavy particles to settle down then the supernatant was decanted and filtered using whatman filter paper and the residue was transferred into an open tray and dried in the oven at 100 degree for 2 days scraped with spatula and grinded into fine powder using laboratory pestle and mortar (Abiodun et al., 2021) Percentage yield was calculated using this formula.

$$\% \text{ yield} = \frac{\text{weight of the extract}}{\text{weight of powdered seed}} \times \frac{100}{1}$$

#### Phytochemical Screening of Extract

Qualitative phytochemical analysis was conducted using standard procedure to detect major bioactive compound

#### Test of Major Phytochemicals

##### Alkaloids (Mayer Test)

Extract + Mayer reagent

Cream precipitate indicates presence of alkaloid

##### Flavonoids (Alkaline Test)

Extract + NaOH solution

Yellow coloration that disappears with acid confirm flavonoids

##### Tannins (Ferric Chloride Test)

Extract + 1%  $\text{FeCl}_2$

Blue- black coloration indicated tannins

##### Saponins (Froth Test)

The extract was shaking with distilled water

Persistent froth indicated saponins

##### Terpenoids (Salkowski Test)

Extract + Chloroform + Concentrated  $\text{H}_2\text{SO}_4$

Reddish brown interface indicated terpenoids  
Phenols (Ferric Chloride Test)  
Dark coloration indicated presence of phenolic compound

**My scientific note:** These tests provide preliminary qualitative confirmation of bioactive compounds responsible for larvicidal activity, although they do not concentration quantified

### Rearing of *Musca domestica* Larvae

The housefly larvae was collected from poultry decomposed manure sample and it was maintained under laboratory conditions at a temperature of 28±2 °C and 60-70% relative humidity feed standard larvae diet (Wheat bran+ yeast+ water) in a sack covered with cheese cloth, The third instars larvae was used for bioassay due to their high sensitivity and uniform development stage. (Ahmed, et al., 2018).

### Larvicidal bioassay Procedure

The Larvicidal bioassay ones were carried out by following the method of Biovain (2011) and Palacin et al (2019) respectively with little modification. The aqueous extract was dissolved in 1000ml of distilled water to form different concentrations such as 250ppm, 500ppm, 750ppm, 1000ppm; 1250ppm for plant extract in the plastic cup, 25 larvae was transferred into each test solution prepared. Four replicates were maintained for different. Concentrations and mortality of the larva was recorded after 24, 48 and 72 hours for the plant extract. Dead larvae were detected when appendages did not move when probed with needle. The mortality rate of the tested larva was then calculated after 72 hours by using the below formula.

$$\text{mortality percentage} = \frac{\text{No of dead larvae}}{\text{No of larvae tested}} \times 100$$

$$\text{Corrected mortality \%} = \frac{\text{observed mortality rate} - \text{control mortality rate}}{100 - \text{control mortality rate}}$$

$$\text{Observed mortality rate \%} = \frac{\text{control mortality \%} \times 100}{100 - \text{control mortality \%}} \times 10 \quad \text{Eneojor, (2015)}$$

### Determination of LC<sub>50</sub> and LC<sub>95</sub>

#### Data preparation

Percentage mortality was corrected using Abbott's formula

$$\text{Corrected mortality (\%)} = \frac{\text{Expected} - \text{Control}}{(100 - \text{Control})} \times 100$$

### Probit Analysis

LC<sub>50</sub> (Lather concentration for 50% mortality) and LC<sub>95</sub> value was determined using probit regression analysis  
Scientific note: LC<sub>50</sub> is the most reliable indicator of larvicidal potency where lower LC<sub>50</sub> indicate higher toxicity.

### Statistical Analysis

A Two-way Analysis of variance (Two-Way ANOVA) was used

Factors:

Factor A: Extract concentration 250ppm, 500ppm, 750ppm, 1000ppm, 1250ppm

Factor B: Exposure time: (24, 48, 72 hours)

Dependent Variable:

Larvae Mortality percentage

### Model Structure

The ANOVA model

$$Y_{ijk} = \mu + C_1 + T_j + (CT)_{ij} + \Sigma_{ijk}$$

$$Y_{ijk} = \text{Observed mortality}$$

$$\mu = \text{Overall mean}$$

$$C_1 = \text{Effect of concentration}$$

$$T_j = \text{Effect of Time}$$

$$(CT)_{ij} = \text{Interaction effect}$$

$$\Sigma_{ijk} = \text{Error term}$$

Post Hoc Test

Turkey's HSD test was used to separate the means when the significant difference are observed at P<0.05)

Significant Level

Statistical significance was set at  $P < 0.05$ )

### Ethical and Safety consideration

Proper handling of biological specimen was ensured

Waste disposal was followed laboratory bioassay guideline

No vertebrate animal are involved hence ethical approval is minimal but institutional guideline was observed

### Scientific Summary of methodology used for this study

This Methodology ensured a rigorous evaluation of the larvicidal potential of *Citrus aurantifolia* leave extract by intergrading phytochemical profiling dose- response bioassay and robust statistical analysis. The combined use of LD50 determination and ANOVA ensure both toxicity quantification and statistical validation of efficacy trends foster the reliability of findings

## 3. Results

**Table 1:** Summary of detected Phytochemicals

| Photochemical Groups | Presence |
|----------------------|----------|
| Alkaloids            | +        |
| Flavonoids           | +        |
| Tannins              | +        |
| Saponins             | +        |
| Phenol               | +        |
| Glycoside            | -        |
| Terpenoids           | +        |

### Interpretation of phytochemicals result

The quantitative phytochemical screening of *Citrus aurantifolia* leave extract revealed the presence of several biological active compounds including Alkaloids, Flavonoid, Tannins, saponins, Phenol, and terpenoids while Glycoside was not detected. The presence of Flavonoids, Phenols, and Tannin suggests strong antioxidant and insecticidal potential as these compounds are known to interfere with insect metabolic and neurological functions

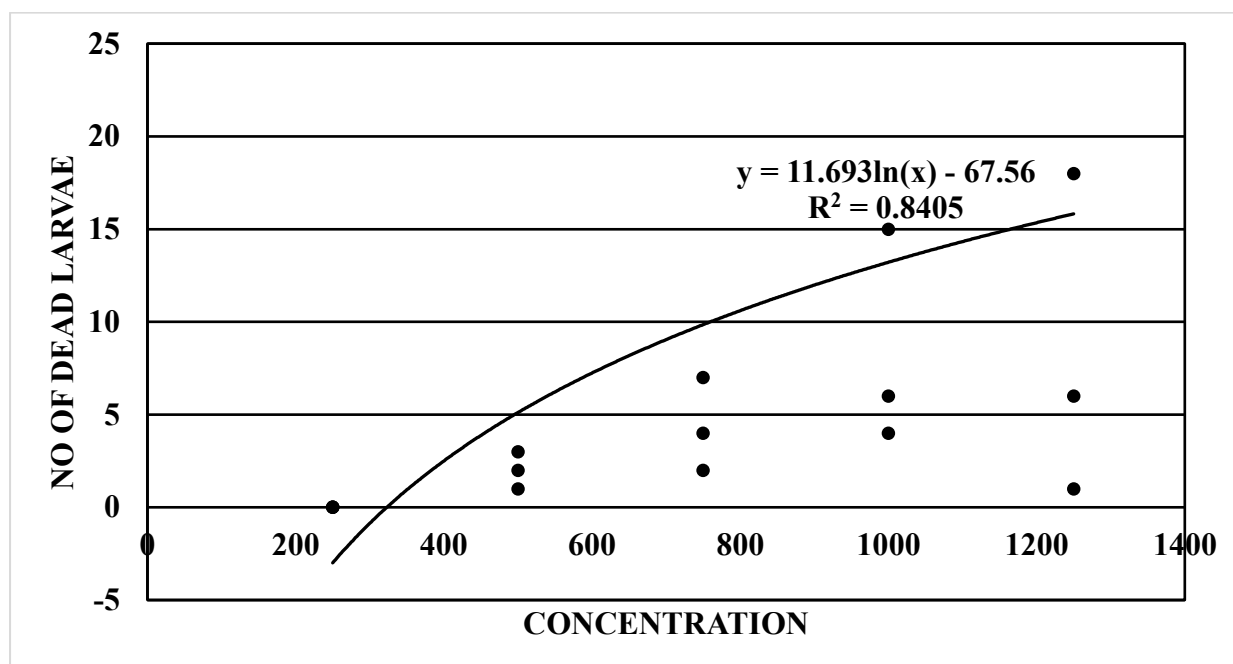
### *Citrus aurantifolia* leave Extract effect on *Musca domestica* larvae

**Table 2:** Larvae mortality rate at different concentrations of leave extract of *Citrus aurantifolia* with time rate of exposure

| Citrus aurantifolia leave extract Concentration (ppm) | No of larvae of Musca domestica | Mortality rate % at 24hrs | Mortality rate % at 48hrs | Mortality rate % at 72hrs | No(%) of alive larvae |
|-------------------------------------------------------|---------------------------------|---------------------------|---------------------------|---------------------------|-----------------------|
| 250 ppm                                               | 25                              | 0(0.00%)                  | 0(0.00%)                  | 0(0.00%)                  | 25(100%)              |
| 500 ppm                                               | 25                              | 1(4%)                     | 2(8%)                     | 3(12%)                    | 19(76%)               |
| 750 ppm                                               | 25                              | 7(36%)                    | 4(16%)                    | 2(8%)                     | 12(48%)               |
| 1000 ppm                                              | 25                              | 15(60%)                   | 6(24%)                    | 4(16%)                    | 0(0.00%)              |
| 1250 ppm                                              | 25                              | 18(72%)                   | 6(24%)                    | 1(4%)                     | 0(0.00%)              |
| MEAN                                                  | 25.00                           | 8.2(32.8%)                | 3.6(14.4%)                | 2(8%)                     | 11.2(44.8%)           |
| CONTROL 0.00mg/L                                      | 25.00                           | 0.00                      | 0.00                      | 0.00                      | 25.00(100%)           |

**3: Concentration response larvicidal bioassay of Citrus aurantifolia leave extract against larvae of Musca domestica**

| Citrus aurantifolia bark | Time  | %mortality | LC50(ppm) | LC95(ppm) |
|--------------------------|-------|------------|-----------|-----------|
| Aqueous extract          | 24hrs | 8.2(32.8%) | 896       | 1729      |
|                          | 48hrs | 3.6(14.4%) | 1812      | 2250      |
|                          | 72hrs | 2(8%)      | 5250      | 7875      |



**Figure 1:** Mortality rates of larvae against the concentration (ppm)of aqueous extract of citrus aurantifolia leave and time period

#### 4. Discussion

##### Overview of findings

This study evaluated the larvicidal activity of Citrus aurantifolia leave extract against Musca domestica larvae across different concentrations and exposure times, The results are discussed in relation to mortality trends, dose-dependent response relationship, LD50 values.

**Table 1: Phytochemical Composition and Bioactivity Implications**

- The qualitative phytochemical screening indicated the presence of key bioactive compounds such as alkanoids, Flavonoids, tannins, saponins, terpenoid and phenolic compounds these secondary metabolites are widely associated with insecticidal and larvicidal effect
- The observed Larvicidal activity can be attributed to these compounds through several mechanism
- Alkanoids may interfere with nervous system signaling in larvse
- Flavonoids and Phenols can distrust cellular metabolism and induce oxidative stress
- Ternnims may bind to protein and digestive enzyme reducing nutrient absorption
- Terpenoids are known to affect insect hormonal regulation and cuticle integrity

These findings support the hypothesis that Citrus aurantifolia bark contains biological active compound capable of inducing larval mortality

**Table 2: Effect of Concentration on Larval Mortality and exposure time**

A clear dose –dependent increase in larval mortality was observed across all exposure periods. Higher concentrations consistently produced greater mortality rates compared to lower concentration and control. This trend suggests that the extract exhibits strong concentration-dependent toxicity increasing concentration likely increase the availability of active phytochemicals interaction with larval physiological systems. The control group showed no mortality, which confirmed that mortality was due to the extract rather than environmental factors.

Furthermore, on the effect of exposure time rate on mortality increased progressively from 24 to 72 hours across all concentrations this signified a time-dependent laticidal effect of the extract, where prolonged exposure enhances toxicity. The possible explanations include: gradual absorption of bioactive compounds through the larval cuticle Accumulation of toxic metabolites within the larval tissues over time. and delayed physiological disruption leading to eventual death.

The interaction effect of time revealed a significant interaction between concentration and exposure time ( $p < 0.05$ ), indicating that the effect of concentration depends on the duration of exposure. This suggests that higher concentration is more effective when exposure time is extended. Lower concentrations may required longer exposure to achieve significant mortality, the combined effect of both factors enhance overall larvicidal efficacy

**Table 3 and Figure 1**,  $LC_{50}$  and  $LC_{95}$  interpretation, the probit analysis produced  $LC_{50}$  and  $LC_{95}$  values that reflect the toxicity level of the extract. The  $LC_{50}$  value indicates the concentration required to kill 50% of the larvae. A lower  $LC_{50}$  value demonstrates higher potency of the extract. The  $LC_{95}$  value provides insight into near complete population control efficiency. The regression line between log concentration and probit mortality showed a strong positive correlation, confirming a reliable dose response relationship

## 5. Comparison with Previous Studies

The larvicidal activity observed in the study is consistent with previous research on plant based insecticides. Many citrus species have demonstrated insecticidal properties due to their rich phytochemical composition, particularly terpenoids and flavonoids. When compared to similar botanical extract, Citrus aurantifolia leave extract shows a promising potential as a natural larvicide, although variation in the potency may deepen on method of extraction, environmental factors and solvent type.

## 6. Summary of Discussion

Overall, the results demonstrated Citrus aurantifolia leave extract exhibited a significant larvicidal activity that was characterized by strong dose-dependent and time dependent effect. With statistically significant mortality difference and a reliable  $LC_{50}$  and  $LC_{95}$  toxicity indicator, bioactivity also supported by phytochemical constituents, these findings, support the potential use of the extract as a natural bio- insecticide vector control strategies.

**Conclusion:** This study evaluated the larvicidal activity of Citrus aurantifolia leave extract with the adoption of laboratory-based experimental design; the findings demonstrated that the extract has a significant larvicidal potential, with mortality rates increasing in a dose-dependent and time dependent manner across all treatment groups. Phytochemical screening also revealed the presence of important bioactive compounds including alkaloids, flavonoids, saponnin, tannins, phenolic compounds and terpenoids. These secondary metabolites are likely responsible for the observed larvicidal activity through mechanisms such as enzyme inhibition, metabolic disruption, and interference with larval physiological processes. Also, probit analysis confirmed the effectiveness of the extract through  $LC_{50}$  and  $LC_{95}$  values, indicating measurable toxicity against the larvae.

It can be concluded from the study that Citrus aurantifolia leave extract is a well promising natural larvicidal agent that could serve as an alternative to synthetic insecticide in control of Musca domestica population.

## Recommendations

Following the findings of this study, the following recommendations are made:

### Further Isolation of Active Compounds

There is need for more research to focus on isolation and characterization of the specific bioactive compounds responsible for larvicidal effect of the *Citrus aurantifolia* leave extract.

### Toxicological Evaluation on Non- Target Organisms

Additional studies should assess the safety of the extract on non-target organisms, beneficial insects, and humans, to ensure environmental safety.

### Field Trials for Practical Application

The larvicidal effectiveness of the extract should be carried out under field conditions to determine its real- world applicability in vector control program.

### Formulation Development

There is need to develop the extract into stable formulations such as sprays, granules, or emulsifiable concentrate to improve ease of application and shelf life.

### Integration into Integrated Pest Management (IPM)

The leave extract should be considered as part of integrated pest management strategies to reduce more reliance on synthetic insecticides and minimize environment pollution.

### Does Standardization Studies

There is need for further studies to establish standardized effective dosages for large scale application to ensure consistent larvicidal performance

### References

- Abiodun, O. A., Adewale, O. O., and Ogunbanwo, S. T. (2024). Larvicidal activity of *Citrus aurantifolia* leaf extract against *Musca domestica* larvae. *Journal of Vector Control and Parasitology*, 12(1), 45–53.
- Adeyemi, Y., Al-Hussein, F. I., Bayoumi, A. E., Alzabib, A. A., and Al-Sarar, A. S. (2022). Organophosphate Insecticides Resistance in Field Populations of House Flies, *Musca domestica* L.: Levels of Resistance and Acetylcholinesterase Activity. *Insects*, 13(2), 192.
- Adebayo-Tayo, B. C., Akinsete, T. O., and Odeniyi, O. A. (2016). Phytochemical composition and comparative evaluation of antimicrobial activities of the juice extract of *C. aurantifolia* and its silver nanoparticles. *Nigerian Journal of Pharmaceutical Research*, 12(1), 59–64.
- Ahmad, I., and Saeed, M. (2018). *Citrus aurantifolia* (Christm.) Swingle: A review of its phytochemistry, pharmacology and therapeutic applications. *Journal of Pharmacy and Pharmacology*, 70(8), 1051–1064.
- Banjo, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2011). Biological effects of essential oils – A review. *Journal of Food and Chemical Toxicology*, 46(2), 446–475.
- Bahrndorff, S., de Jonge, N., Skovgård, H., and Nielsen, J. L. (2017). Bacterial communities associated with houseflies (*Musca domestica* L.) sampled within and between farms. *PLoS ONE*, 12(1), 0169753.
- Barreiro, C., Albano, H., Silva, J., and Teixeira, P. (2013). Role of flies as vectors of foodborne pathogens in rural areas. *ISRN Microbiology*, 2013, 718780.
- Enejoh, O. S., Ogun, I.O., Bala, M.S., Oruene, I.S., Suleiman, M.M and Ambali, S.F. (2015). Ethnobotanical importance of *Citrus aurantifolia* (Christm.) Swingle. *The Pharmainnovation Journal*, 4, 1-6.
- Indriyani, S., Hidayat, R. A., and Putri, E. I. (2023). Chemical composition and biological activities of *Citrus aurantifolia* essential oil: A review. *Journal of Essential Oil Research*, 35(3), 257–266.
- Indriyani, N. N., Anshori, J. A., Permadi, N., Nurjanah, S., and Julaha, E. (2023). Bioactive Components and Their Activities from Different Parts of *Citrus aurantifolia* (Christm.) Swingle for Food Development. *Foods (Basel, Switzerland)*, 12(10), 2036.
- Izah, S. C., Richard, G., and Odubo, T. C. (2023). *Citrus aurantifolia*: Phytochemical constituents, food preservative potentials, and pharmacological values. In *Herbal Medicine Phytochemistry: Applications and Trends* Springer International Publishing. 1–26.





## **A comparative Study on the Canal Transportation of Two Heat Treated Rotary NiTi Instruments; Orodeka Plex-V and AF F-One Files in Sever Curved Simulated Canals (A comparative *in vitro* Study)**

**Saad A. Ramadhan** MSc., Ph.D., Conservative Dentistry, University of Misan, Iraq

**Hamid Kasim** MSc., Ph.D., Sawa University, Samawa, Iraq

Corresponding Author: *Dr. Hamid Kasim*, e-mail: *hamidkasim100@yahoo.com*.

### **Abstract**

**Aim:** to evaluate the shaping ability Orodeka Plex-V and Fanta AF F-One rotary nickle-titanium systems in simulated curved canals.

**Materials and Methods:** twenty artificial curved resin canals were divided into two groups according to the instruments used for canal preparation. All canals were instrumented to master apical file of 25/04. Outer and inner transportation were evaluated. Measurements were carried out at five different levels. Pre-and postoperative images of the canals were taken at 40X magnification. An assessment of the canal shape was determined using Photoshop CS2 software.

**Results:** Plex-V caused greater transportation of the outer walls of the canals than F-One files, with a statistically significant difference at all five levels examined. Similar results were observed on the inner canal walls, without statistical significance, except at the apical level, where the difference was significant. In general, the transportation on the outer walls was greater than on the inner walls along the canals.

**Conclusions:** Within the limitations of the study, F-One instruments maintained the original curvature significantly better than Plex-V.

**Keywords:** Transportation, Fanta AF F-One, Orodeka Plex-V.

### **1. Introduction**

Root canal treatment involves several steps; canal shaping is one of the most important (1). A fundamental aspect of biomechanical preparation is shaping the canal into a tapering form without altering the original shape (2). This phase is crucial for successful canal preparation, especially in the apical third, as uneven cutting of the canal walls may lead to ledge formation or even perforation. Therefore, instruments must be used to cut the dentin evenly from all sides (3). Glossary of Endodontic Terms defined the transportation as “an excessive removal of the outer wall of the apical half of the canal due to the instrument's tendency to return to its original straight shape during instrumentation, which may end with the ledge formation and possibly perforation” (4). Teeth with narrow and curved canals are more prone to these aberrations (5). Hence, degree of transportation of root canal can be used to measure the performance of various endodontic instruments and techniques used for canal preparation (6).

The introduction of NiTi to the world of endodontics, significant advancements occurred in root canal instruments, positively impacting the time required to complete the treatment, enhancing dentist comfort, and reducing the error rate (7). Since these tools were introduced to the market, updates have been ongoing in terms of engineering design and heat treatment (8).

One of the new NiTi rotary systems that was introduced to the field of root canal treatment is the Orodeka Plex-V files. These instruments are manufactured using a special thermal process called CM wire, which aims to increase

flexibility and high resistance to cyclic fatigue. These files feature a triangular cross-section to minimize the friction with canal walls and a non-cutting tip used for canal searching and negotiation (8).

Recently, Fanta introduced a new type of files termed AF F-One system using a special heat treatment called AF Wire. A notable feature of this design is the flat surface along the cutting edge of the file, which makes the file more flexible and reduces friction with the canal walls, thus increasing the file's resistance to breakage and providing a larger space for evacuation debris coronally. In addition, the cross-section is S-shaped, which increases the cutting performance of the file (8).

According to the investigation oversight on scientific websites, there is no research comparing these modern endodontic rotary systems in terms of their ability to prepare canals without deviating from the original paths of the canals. Therefore, this research was conducted in this experiment to find out which of these files is more capable of preparing the severe curved simulated canals while preserving the original shape of the canals in terms of transportation.

## **2. Materials and Methods**

### **2.1 Artificial Root Canals**

Twenty artificial curved root canals (Dentsply Maillefer, Ballaigues, Switzerland) were used in this study. These canals were identical to the geometry of ISO K-file #10. The straight part was 11 mm long, while the curved part was 5 mm long, making the total length of the canal 16 mm. The angle of curvature was 45° according to Schneider (9), and radius with 5.5mm along with Pruett et al., (10).

### **2.2. Canals Photography**

Before starting the instrumentation, and to increase the contrast of the images, each artificial canal was filled with writing ink (Hero, China) using a 27-gauge syringe (Seoul, Korea). Each sample was magnified 40 times by a stereomicroscope (Keyence VH8000, Osaka, OSK, Japan), and the image was taken under this magnification by a digital camera (Cyber-shot, Sony, Japan). The camera was mounted on the microscope lens in a way that allowed the model to be repositioned in the exact same location to take before-and-after photos of sample preparation. After the photography, the simulated canals were washed of ink residue with tap water.

### **2.3. Instrumentation of Simulated Root Canals**

The samples were randomly divided into two groups (n=10) based on the type of rotary system used in the preparation. Canals patency was verified by ISO K file #10 (Dentsply, Maillefer, Ballaigues, Switzerland). The canals were prepared by a root canal specialist experienced with these rotary systems. The rotary files were operated with X-Smart Plus iQ electric motor (Dentsply, maillefer, Ballaigues, Switzerland).

#### **Group 1:**

In this group, the Orodeka Plex-V system (Shandong Province, China) was used by X-Smart motor, according to the manufacturer's recommendations for this system in terms of speed and torque as follow:

Orifice Opener (15/08), WL:5mm, speed:300rpm, torque: 2.5N.

Glide Path (15/03), WL:15mm, speed:300rpm, torque:1.5N.

Shaping file (yellow ring, 20/04), WL:15mm, speed:500rpm, torque:2.5N.

Shaping file (red ring, 25/04), WL:15mm, speed:500 rpm, torque: 2.5N.

#### **Group 2:**

AF F-One (Fanta, Shanghai, China) files were used in preparing the models in this group using X-Smart Plus electronic motor, according to the program designed for this system as follow:

Orifice Opener (17/12), WL: 5mm, speed:500 rpm, torque: 2.5 N.

Path finder (16/04), WL: 15 mm, speed: 500 rpm, torque: 2 N.

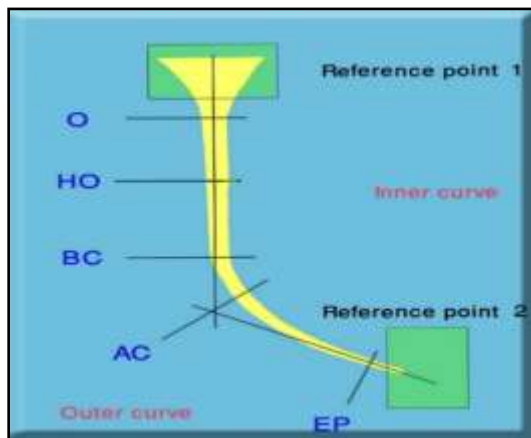
Shaping file (yellow ring, 20/04), WL: 15 mm, speed: 500 rpm, torque: 2.5 N.

Finishing file (red ring, 25/04), WL: 15 mm, speed: 500 rpm, torque: 2.5 N.

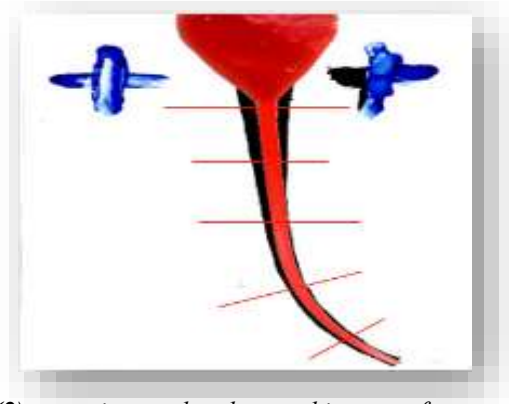
The movement of the files during preparation was slow in and out pecking motion. Distilled water (Baghdad, Iraq) was used as the irrigating fluid using endodontic irrigating syringes (Ultradent Products, Inc., South Jordan, UT, USA) with NaviTip 21 mm (yellow) 30 gauge. The canal was rinsed with 2 ml of distilled water before preparation, and after each file, the canal was rinsed with another 2 ml, resulting in a total of 10 ml of irrigating fluid per canal. Once the file reaches the length of the canal and rotates freely, it is withdrawn. This process takes no more than 5 seconds. The file was used to prepare only 5 canals. After each use, the file was inspected to ensure it was free of cracks and distortion. If these defects were found, the file would be replaced immediately.

## 2.4. Assessment of Canal Transportation

After completing the canal preparation process, ink is injected into the canal, and the canal was imaged in the same way it was imaged before preparation. Digital images of the canals before and after preparation were superimposed and measured. Here, canal transportation was determined by comparing the digital image before and after preparation and was done by measuring the change from the original canal boundaries from the inner and outer sides of the canal at 5 reference points using Adobe Photoshop CS2 software program (fig.1). The measurements were at right angles to the canals (fig.2).



**Fig. (1):** The five levels of measurement.



**Fig. (2):** superimposed and treated images of unprepared and prepared canal with computerized program Adobe Photoshop (C).

Level 1 (O): canal orifice.

Level 2 (HO): the point mid-way from the beginning of the curvature to the orifice.

Level 3 (BC): the point where the canal departs from the long axis of its coronal portion (beginning of the curvature).

Level 4 (AC): the point where the long axes of the coronal and the apical portions of the canal intersect (apex of the curve).

Level 5 (EP): the end point of preparation.

## 3. Results

### 3.1. Outer Transportation.

The mean values and the standard deviations of outer canal transportation after instrumentation at the five measuring levels are given in Table 1.

**Table 1:** Mean and standard deviation of outer transportation (mm) for the two groups.

| System  |      | O          | HO         | BC         | AC         | EP         |
|---------|------|------------|------------|------------|------------|------------|
| Plex-V  | Mean | 7.08       | 6.69       | 5.80       | 3.94       | 2.37       |
|         | SD ± | 0.47796    | 0.57629    | 0.71181    | 0.51467    | 0.27101    |
| F-One   | Mean | 6.52       | 5.24       | 4.27       | 2.90       | 1.64       |
|         | SD ± | 0.53914    | 1.13940    | 1.34582    | 0.86923    | 0.38064    |
| t-test  |      | 2.458      | 3.591      | 3.178      | 3.256      | 4.940      |
| P-value |      | 0.004<br>S | 0.002<br>S | 0.005<br>S | 0.004<br>S | 0.000<br>S |

Based on the table above, it can be observed that Plex-V removed more material from the outer surface than F-One at all levels. Statistical analysis by using the student t-test showed significant differences between the two systems at all levels.

### 3.2. Inner Transportation

The mean values and the standard deviations of inner transportation after instrumentation at the five different levels examined are listed in Table 2.

**Table 2:** Mean and standard deviation of inner transportation (mm) for the two groups.

| System  |      | O           | HO          | BC          | AC          | EP         |
|---------|------|-------------|-------------|-------------|-------------|------------|
| Plex-V  | Mean | 5.780       | 5.080       | 3.640       | 2.390       | 1.590      |
|         | SD ± | 0.70679     | 1.00973     | 0.57194     | 0.53009     | 0.37253    |
| F-One   | Mean | 6.180       | 4.740       | 3.920       | 2.250       | 1.200      |
|         | SD ± | 0.65963     | 1.08341     | 1.18584     | 0.51045     | 0.24944    |
| t-test  |      | 1.308       | 0.726       | 0.673       | 0.602       | 2.751      |
| P-value |      | 0.207<br>NS | 0.477<br>NS | 0.510<br>NS | 0.555<br>NS | 0.013<br>S |

At the orifice of the canals F-One showed the higher means of inner transportation (6.180) than Plex-V (5.780). Next to the second measuring level, Plex-V got the maximum inner transportation (5.080) and F-One showed the minimum mean (4.740). On the third level the results were as follows: F-One (3.920), Plex-V (3.640). On the fourth plane the view was completely inversed as the higher means of inner transportation was with Plex-V (2.390) than F-One (2.250). By the side of the end level of preparation, Plex-V removed more resin from the inner sides (1.590) followed by F-One (1.200).

By using Student t-Test (Table 2), in inner transportation, showed no significant difference between the two systems at all levels, but not at the apical level where the difference was significant.

## 4. Discussion

Canal preparation aims to create a conical shape—with the smallest diameter at the apex and the largest towards the crown—while strictly maintaining the original shape of the canal. One of the most common problems encountered during canal instrumentation is the straightening of the canal; this classically occurs in curved roots, specifically affecting the outer wall in the apical third (11).

According to the American Association of Endodontists (2016), the term "transportation" is defined as the cutting away of the outer wall of the root canal—specifically in the apical half—caused by the instrument's tendency to straighten within the canal during instrumentation; this leads to complications such as root perforation or ledge formation (4).

The degree of transportation is measured by the distance between the edge of the unprepared canal and the corresponding edge after canal preparation (12). The closer the transportation value is to zero, the better the performance of the instruments in canal preparation (13).

Apical transportation plays a crucial role in ensuring the feat of root canal treatment; the degree of curvature throughout preparation affects the seal of the filling materials, thereby determining the success or failure of the root canal management (14).

Several researchers stated that an apical transportation of 0.15 mm is acceptable; however, if it exceeds 0.30 mm, it compromises the apical seal, allowing bacteria or their byproducts to enter the root canal and leading to endodontic treatment failure. Excessive apical transportation leads to several complications; it may cause zipping and the subsequent elbow formation—which hinders the smooth insertion of accessory points of gutta-percha. This prevents a tight apical seal, ultimately resulting in endodontic treatment failure (14, 15).

Reviewing the results of this study reveals that neither of the systems used was able to produce an exact replica of the canal shapes present prior to preparation; in other words, all the instruments caused the original canals to transport to varying degrees. Under conditions of the study, no system exhibited apical transportation exceeding than 0.3 mm, indicating that preparation with these two systems falls within the ideal standards for root canal preparation.

The research by hand was conducted using transparent resin blocks that simulate natural root canals, ensuring standardized dimensional parameters—such as length, diameter, taper, and curvature—while also facilitating imaging before and after preparation. Artificial canals do not replicate the actual anatomy of root canals, but they allow for comparisons regarding canal preparation using various rotary root canal preparation systems. At the same time, it must be noted that the cutting process in these resin blocks differs from that of natural dentin; cutting these canals with rotary systems causes the resin temperature to rise and leads to the accumulation of resin debris in the instrument flutes, which can ultimately result in file breakage (11).

Thus, the results derived from using these blocks must be interpreted with caution when evaluating the performance of preparation systems (16). In the same context, Adobe Photoshop was utilized to analyze images of the canals taken before and after preparation, aided by ink injected into the artificial canals; the software allowed for image enhancement and colorization, facilitating computer-based measurements (11).

Curved canals are ideally used to evaluate root canal instrumentation, as they reflect instrument efficiency—unlike straight canals (17). Hence, rotary instruments are evaluated using curved artificial canals, regardless of whether they maintain the original curvature of the prepared canals or cause them to transport (11).

It is important to note that when comparing rotary root canal systems, the standardization of master apical files size and taper must be considered; in this study, the instruments used were size 25 with a taper of 04. Furthermore, all samples were prepared by a single operator to eliminate operator-related variables (8).

#### **4. 1. Outer Transportation**

Regarding the outer canal wall, Table (1) clearly shows that, across all five canal levels, group Plex-V instruments removed significantly more resin than group F-One files. Accordingly, the canals prepared using F-One instruments were closer to the original shape, as these instruments removed less of the outer canal wall; consequently, there was less transportation, straightening, and distortion of the canals across the five examined levels. These results can be explained by once recognizing that the mechanical and geometric properties of the files significantly influence their performance during rotation within the canals (18, 19).

Tasdemir et al. (2005) stated that several factors directly influence the degree of canal transportation; foremost among these are the flexibility of the instrument used for preparation (20). As well as the cross section of the instrument (21). In the same context, other studies have stated that the flexibility of instruments may be the main factor that allows the

instruments to plane the canal walls rather than engaging and screwing into them and to cut of dentin evenly along the canal wall (22).

According to the conclusions of a study conducted by Saad and Hamed in 2026, the F-One type files are more flexible than the Plex-V files. This high flexibility is due to the unique engineering design of the F-One files, which feature flat side surface; this reduces stress on the file as it rotates and contacts the outer surface of the canal (8).

The cross-section of the file is an important factor in determining the instrument's flexibility. According to the manufacturer, the cross-section of the F system is S-shaped, whereas it is a triangular shape for Plex-V system (8). Some studies have stated that the lower metal mass of S-shaped cross section file gives them greater flexibility (23, 19).

As stated by Di Nardo (2020), the core of a file determines its flexibility. A file with an S-shaped cross-section is more flexible than a triangular cross-section file due to its lower core. Consequently, file F-one is more flexible compared to file Plex-V (24).

Another factor that could explain the results of this study is the cutting efficiency of the file. The unique geometric design of F-one file—featuring a flat side and an S-shaped cross-section— This means there are fewer spaces along the file, causing them to fill up quickly with debris, thereby reducing its cutting efficiency (8).

#### 4. 2. Inner Transportation

Tables (1, 2) show that the direction of transportation was toward the outer wall of the canals; that is, the amount of material removed was greater on the outer aspect compared to the inner aspect of the root canals. This outward transportation has also been confirmed in other studies (25, 26).

These results are attributed to the forces tending to restore the file to its straight shape within the curved canal; this causes friction and cutting against the outer canal wall—with less force exerted on the inner wall—resulting in greater outward transportation compared to inner one (27).

The results of inner transportations indicate that instruments Plex-V removed a greater amount of resin from the inner canal walls compared to instruments F-One; however, the difference was not statistically significant along the length of the canal, except at the apical level. Concerning the original root curvatures, the results of this study reveal that F-One systems obtained better canal geometry, demonstrated less canal transportation and straightening at the five tested levels of the simulated canals and better maintained the original shape of the curved canals compared with Plex-V instruments. Once again, these results can be explained by the differences between the two systems in terms of flexibility and cutting efficiency (8).

It is worth noting a crucial factor determining the shaping capability of root canal instruments: the heat treatment of the metal from which they are manufactured; however, this topic lies beyond the scope of the current study.

### 5. Conclusions

Despite the limitations of this *in vitro* study, both systems caused some transportation in the root canals along the working length, but these transportations were within acceptable limits and did not cause problems with root canal sealing. Considering that system Plex-V caused a greater deviation than F-One system.

#### Conflict of interest

Authors have nothing to declare.

Funding

Nothing apart from personal funds.

## References

- Peters OA (2004) Current challenges and concepts in the preparation of root canal systems: A Review. *Journal of Endodontics* 30, 559–67.
- Schilder H. Cleaning and shaping the root canal. *Dent Clin North Am.* 1974; 18: 269-96.
- Hulsmann, M. Peters, O.A.; Dummer, P.M. Mechanical preparation of root canals: Shaping goals, techniques and means. *Endod. Top.* 2005, 10, 30–76.
- Glossary of Endodontic Terms. American Association of Endodontists 9th Edn. Chicago, American Association of Endodontists. 2016. Available at <https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms/>.
- Weine FS, Kelly RF, Lio PJ (1975) The effect of preparation procedures on original canal shape and on apical foramen shape. *J Endod* 1, 255-262. doi: 10.1016/ S0099-2399(75)80037-9
- Gluskin AH, Brown DC, Buchanan LS. A reconstructed computerized tomographic comparison of Ni–Ti rotary GT™ files versus traditional instruments in canals shaped by novice operators. *Int Endod J.* 2001; 34(6): 476-84.
- Ferraz CC, Gomes NV, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ (2001) Apical extrusion of debris and irrigants using two hand and three engine-driven instrumentation techniques. *International Endodontic Journal* 34, 354–8.
- Ramadhan SA, Kasim H. A study of fracture resistance and deformation of two heat treated rotary NiTi instruments; Orodeka Plex V files and AF F-One files in sever curved simulated canals (a comparative *in vitro* study). *International Journal of Advanced Research in Medicine.* 2026;8(2):12-16.
- Schneider S. A comparison of canal preparations in straight and curved canals. *Oral Surgery* 1971, 32 (2). 271-5.
- Pruett J, Clement D, Garnes D. Cyclic fatigue testing of nickel-titanium endodontic instruments. *Journal of Endodontics* 1997, 23, 77-85.
- Hamid Kasim and A. K. J. Al-Azzawi. The shaping effects of three rotary Nickel-Titanium systems in simulated curved canals. *J Bagh College Dentistry Vol. 24(3),* 2012
- Song Y, Bian Z, Fan B, Fan M, Gutmann J, Peng B. A comparison of instrument-centering ability within the root canal for three contemporary instrumentation techniques. *International Endodontic Journal* 2004, 37(4), 265-271.
- Gambill et al. (1996): “Comparison of nickel-titanium and stainless-steel hand instruments in the preparation of simulated curved root canals.” *Journal of Endodontics*.
- Wu MK, Fan B, Wesselink PR (2000) Leakage along apical root fillings in curved root canals. Part I: effects of apical transportation on seal of root fillings. *J Endod* 26, 210-216.
- Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. *J Endod* 2004; 30: 559–67.
- Bitter K, Gruner D, Wolf O, Schwendicke F. Artificial versus natural teeth for preclinical endodontic training: A randomized controlled trial. *J Endod* 2016; 42:1212-7.
- Berutti E, Chianussi G, Paolino D, Scotti N, Cantatore G, Castellucci A, et al. Canal shaping with Wave one primary reciprocating files and ProTaper system: A comparative study. *J Endod* 2012; 38:505-9.
- Martins JN, Martins RF, Braz Fernandes FM, Silva EJ. What meaningful information are the instruments mechanical testing giving us? A comprehensive review. *J Endod.* 2022; 48:985-1004.
- Silva EJ, Martins JN, Ajuz N, Vieira VT, Pinto R, Marques D, et al. Multimethod analysis of a novel multi-coloured heat-treated nickel-titanium rotary system: Design, metallurgy, mechanical properties, and shaping ability. *J Endod.* 2024; 50:1622-1633.
- Tasdemir T, Aydemir H, Inan U, Ünal O. Canal preparation with Hero 642 rotary NiTi instruments compared with Stainless steel hand K-instrument assessed using computed tomography. *International Endodontic Journal* 2005,38 (2),402-8.
- Yoshimine Y, Ono M, Akamine A. The shaping effects of three nickel-titanium rotary instruments in simulated S-shaped canals. *J Endod* 2005; 31:373-5.
- Tepel J, Schafer E. Endodontic hand instruments: cutting efficiency, instrumentation of curved canals, bending and torsional properties. *Endod Dent Traumatol* 1997,13,201-210
- Bürklein S, Zupanc L, Donnermeyer D, Tegtmeier K, Schäfer E. Effect of core mass and alloy on cyclic fatigue resistance of different nickel-titanium endodontic instruments in matching artificial canals. *Materials (Basel).* 2021; 14:5734.

- Di Nardo D, Miccoli G, Mazzone A, Seracchiani M, Gambarini G, Testarelli L. Centering ability of a new nickel-titanium rotary instruments with a peculiar flat-side design: An in vitro study. *J Contemp Dent Pract.* 2020; 21:539-542.
- Schafer E, Schlingemann R. Efficiency of rotary nickel titanium K3 instruments compared with stainless steel hand K-Flexofile. Part 2. Cleaning effectiveness and shaping ability in severely curved root canals of extracted teeth. *International Endodontic Journal* 2003, 36, 208-217.
- Guelzow A, Stamm O, Martus P, Kielbassa A. Comparative study of six rotary nickel-titanium systems and hand instrumentation for root canal preparation. *International Endodontic Journal* 2005, 38 (10), 743-752.
- Schafer E. Relationship between designs features of endodontic instruments and their properties part I, cutting efficiency. *Journal of Endodontics* 1999, 25 (1), 52-55.



## A Review of Fungal Infection on Plant

SARAH J. JALIL<sup>1</sup>,

ZAINAB G. SADEQ<sup>2</sup>

<sup>1</sup> Iraq Natural History Research Centre and Museum, University of Baghdad, Iraq

[Sarah.g@nhm.uobaghdad.edu.iq](mailto:Sarah.g@nhm.uobaghdad.edu.iq) ORCID ID: [0009-0009-8903-8278](https://orcid.org/0009-0009-8903-8278)

<sup>2</sup> Iraq Natural History Research Centre and Museum, University of Baghdad, Iraq

[Zainab.g@nhm.uobaghdad.edu.iq](mailto:Zainab.g@nhm.uobaghdad.edu.iq) ORCID ID: <https://orcid.org/0009-0004-2632-6575>

Corresponding Author Email: [sarah.g@nhm.uobaghdad.edu.iq](mailto:sarah.g@nhm.uobaghdad.edu.iq)

**Abstract.** The production and product quality of many plants are restricted by plant diseases. The interplay between pathogens, host plants, and ecological factors influences their development. The authors of this review summarize the infection processes, diagnostic methods, and strategies for managing diseases. Efforts to contain disease severity and resistance development have focused on biological control chemical management fungicide resistance and a range of other integrated measures. By correctly diagnosing the disease and integrating biological, cultural and chemical measures, disease can be sustainably managed.

**Keywords:** Fungal diseases; plant pathogens; fungal infection; disease diagnosis; biological control; fungicide resistance; integrated disease management.

### 1. Introduction

Fungal infections are still one of the most important biological limitations of agricultural production and limitation of yield quality and stability. Plant pathogenic fungi can harm various agricultural and horticultural crops and infect the roots, stems, leaves, flowers, fruits and seeds of plants. Besides causing visible disease symptoms, they can have adverse effects on photosynthesis, water and nutrient transport, reproductive development, and the commercial quality of harvested products. As also cited by Khan and Singh, harmful fungal diseases pose as a serious concern for agriculture production and food security.

A complex interaction of susceptible host virulent pathogen and favourable environmental conditions will help in the development of fungal disease.

The effectiveness with which pathogens survive, produce spores, and cause infection and diseases is considerably affected by the temperature, moisture, rainfall, soil condition, crop density and irrigation and agricultural practices. Such interactions are gaining paramount importance due to the new climate conditions, which may alter pathogen distribution, host susceptibility, and disease severity (Singh *et al.*, 2023; Méndez *et al.*, 2026).

Infection with a disease begins when a viable propagule arrives at a surface of the plant, which is susceptible. Infection occurs when a pathogen penetrates into the host due to a natural opening or wound. Infecting a host involves overcoming their defenses or manipulating the host. The interaction will be the result of fungal virulence mechanisms and the ability of the plants to induce structural biochemical and molecular defense mechanisms. (Khan, 2023; Aslam *et al.*, 2024)

A critical diagnosis is essential for disease management. While part one of the note does not cover this sufficiently, the conventional diagnosis based on symptomatology, microscopy and pathogen isolation is still relevant, more so in laboratories that do not have high-throughput technologies to a larger extent. Despite showing similar symptoms, various pathogens or non-infectious stresses can be at work, making the symptom-based diagnosis alone inadequate in many cases. Researchers have developed molecular techniques that allow detecting pathogens more quickly and sensitively than ever before. These methods include PCR-based assays, isothermal amplification, biosensors and sequencing approaches (Sharma *et al.*, 2024; Cotter *et al.*, 2024).

Chemical fungicides are still important for crop protection. Due to the persistent dependence on specific mode of action fungicides, fungicide-resistant populations have come up. Target-site mutations, enhanced efflux, metabolic alterations or other adaptive regulators may all give rise to resistant strains. As a result, modern disease regulation includes a significant aspect of resistance management (Corkley *et al* 2022; Islam *et al* 2024).

Due to the limitations of using chemicals in a more intensive manner, there is an increased emphasis on biological control, host resistance, cultural practices, natural products and other compatible strategies. Microbial competitors including various *Trichoderma*, *Bacillus* and *Pseudomonas* species, and *Paenibacillus* species can kill pathogens using several ways. They compete for space and produce hydrolytic enzymes that can induce plant defence responses. Their mycoparasitic activity is due to antibiosis. (Yao *et al.* 2023; Galli *et al.* 2024; Dobrzyński and Nازیębło 2024)

We can thus consider the implementation of integrated strategies that would prevent any fungal disease from appearing and affecting plants. Together, the results of the surveys enable researchers to assess R&D spending and scope. They also provide insight into new varieties needed in the future for results that grow better (Li *et al.*, 2025).

## 2. Fungal Pathogens and Their Importance in Crop Production

These fungi obtain their nourishment from the host plant and utilize different strategies to overcome the host plant. These organisms can live in a variety of ways. They can be biotrophic, which means that they keep the host tissue alive. On the other hand, they can be necrotrophic, which means that they kill the host cells and live on them. A pathogen that may invest in the first phase of the EARM-spectrum (disease strategy spectrum) and mutilate the host tissue later may be called a hemibiotroph (Maulenbay and Rsaliyev, 2024).

Some examples of fungi that are damaging to plants are sooty molds, smuts, and rusts. Other examples include genera of the fungi *Alternaria*, *Botrytis*, and *Fusarium*. There are significant variations among these pathogens regarding their hosts, infection process, means of survival, and endoparasite's environment requirements (Maulenbay and Rsaliyev, 2024; Aslam *et al.*, 2024).

As capable of causing disease doesn't indicate the worth of any fungal pathogen as a whole. The epidemiological significance of an agent refers to its ability to survive, and between cropping seasons (i.e. grainless periods), to produce and disperse inoculum, to colonize susceptible plant tissues, and to adapt to variation in the environment. Plants absorb nutrients from the soil and, therefore, weeds compete for these resources. Herbicide works either by preventing the weed from absorbing nutrients and water or by killing the weed directly.

Post-harvest fungal diseases increase the loss due to diseases. Pathogens such as *Botrytis*, *Alternaria*, and *Colletotrichum* serve as illustrations of rotting goods. It is essential that fungal disease control programs cover post-harvest protection effort as well as field production effort (Li *et al.* 2025).

## 3. Infection Process and Disease Development

### 3.1 Spore Germination and Host Recognition

A fungal infection typically begins when spores or other infectious agents come into contact with a host's surface. Environmental conditions, especially temperature and moisture, are important factors for germination in addition to

chemical and nutritional signals. The plant surface contracts after germination of fungal structure. Certain pathogens develop unique structures such as appressoria, which help them to enter host cells and / or break the host organisms' tissues (Khan, 2023).

### 3.2 Penetration and Colonization

The skin of a plant is the first line of defence against pests and pathogens and can be described as its first physical barrier. Fungal penetration is reduced by cuticle cell wall and other antifungal compounds. Colonization is limited due to defense mechanisms. A successful pathogen can penetrate the tissues of a plant and disrupt its growth or even kill the plant. When a pathogen penetrates the host tissues, the fungal hyphae will either remain localized or disseminate through the host tissues. The extent to which the above takes place differs from one pathogen to another (Aslam *et al.*, 2024).

### 3.3 Plant Defense Responses

Plants possess different kinds of defense against fungi. The identification of pathogen-related molecules could activate signaling that leads to the production of reactive oxygen species, antimicrobial agents, defence proteins and structural barriers. Structural modifications that occur as a result of pathogen invasion in plants are appressing and cell-wall reinforcement. Hormones such as salicylic acid, jasmonic acid, ethylene, and abscisic acid regulate the plant reaction (Yao *et al.*, 2023; Aslam *et al.*, 2024).

## 4. Symptoms and Consequences of Fungal Diseases

Symptom Presentation of Fungal Diseases Depending upon the pathogen and host infected, the symptoms caused by fungal diseases may vary. The symptoms of the plant diseases include leaf spots and blights, chlorosis and yellowing, necrosis, wilting, stem and branch cankers, damping-off, root and crown rot, vascular discoloration, fruit and seed rot, early defoliation and reduced growth and reproductive performance (Maulenbay and Rsaliyev, 2024).

Leaf diseases which damage functional leaf tissue can reduce photosynthetic capacity, whereas root and vascular tissue damage impairs water and nutrient acquisition. Diseases can reduce yields, affect quality, raise costs of production and reduce post-harvest shelf life with serious economic consequences.

Some fungal pathogens can also produce mycotoxins that cause food and feed safety problems (Singh *et al.*, 2023; Li *et al.*, 2025).

## 5. Environmental and Agricultural Factors Affecting Disease Development

The environmental factors and crop-management practices influence disease development.

Fungi's temperature alters how seed germinate and disease infects. All pathogens have a temperature range of optimum infection and any deviation from this temperature range may alter the disease development. (Singh *et al.*, 2023)

Many leaf pathogens require moisture to thrive. Extended periods of leaf wetness can facilitate spore germination and penetration. High relative humidity may set the stage for sporulation.

Rain can disperse pathogenic through splash dispersal and run off, while overhead irrigation can increase wetness duration of leaves possibly increasing the risk of disease in susceptible crops. Densely packed plants can make a great environment for fungal growth, due to humid conditions (Khan, 2023).

By minimizing the consistent presence of susceptible hosts, crop rotation has the ability to interrupt pathogen life cycles. Appropriate management of infected residues may reduce pathogen survival between years growing (Khan, 2023).

Changing climate conditions have been complicating disease epidemiology through changes in temperature precipitation water availability pathogen distribution and host physiology. A systematic review of 106 studies found that 61% of the studies showed an increase in disease severity under the climate-related conditions investigated. However, the responses varied among different crops and pathogens (Méndez *et al.* 2026).

## 6. Diagnosis of Fungal Plant Diseases

An accurate diagnosis forms the basis of rational disease management.

### 6.1 Symptom-Based Diagnosis

Spotting a Disease Is the First Sign of Illness The lesion structure and symptoms and appearance of organ involvement and visible fungal element can signal useful preliminary information. Symptoms can be misleading, since they cannot help to distinguish pathogens, or infectious and non-infectious disorder (Sharma *et al.*, 2024).

### 6.2 Microscopy and Culture

Observation under microscope can show fungal hyphae. Culturing the pathogen in proper media allows us to identify them further. These methods are useful but take time and expert knowledge (Sharma *et al.*, 2024).

### 6.3 Molecular Diagnosis

The detection of pathogens has benefited from the improved sensitivity and specificity made possible by PCR, quantitative PCR, loop-mediated isothermal amplification, recombinase polymerase amplification, sequencing, and biosensor-based technologies. A review of diagnostics earlier has identified novel molecular methods that can potentially be very useful for detecting fungal and oomycete plant pathogens before disease establishment (Sharma *et al.*, 2024; Cotter *et al.*, 2024).

### 6.4 Imaging and Remote Sensing

More and more researchers are looking into optical hyperspectral thermal and other type of s e n s i n g for early detection of diseases. These methods allow for non-destructive information that can monitor large crop areas. Nonetheless, the necessity for assessment under changing field conditions will always be there as measured in controlled conditions may not be the same in farming time (Pereira *et al.*, 2025; George *et al.*, 2025).

## 7. Chemical Control and Fungicide Resistance

Fungicides are important for protecting crops from disease. The effectiveness of fungicides relies on factors like chemical composition, mode of action, timing, and dose sensitivity (Islam *et al.*, 2024).

Using several fungicides with similar action modes will impose selection pressure on the pathogen population. Strains that develop during treatment can propagate and spread resistance. Resistance to efflux target-site mutations can involve altered metabolism and other mechanism. As a result, it is apt for current and future-resistant management, to enhance the diversification of modes of action, limit unnecessary applications, offer sound dose, and integrate with non-chemical means (Corkley *et al.*, 2022; Naqvi *et al.*, 2024).

## 8. Biological Control

Biological control refers to the strategy of employing beneficial microorganisms in inhibiting plant pathogens. The method is increasingly used in pest control, as there are concerns of chemical residues, environmental pollution, resistance development and sustainability (of the method) (Galli *et al.*, 2024; Madlhophe *et al.*, 2025).

Microbes employed for biocontrol can assist with competition for nutrients and niches, antibiosis, mycoparasitism, production of hydrolytic enzymes, production of antimicrobial metabolites, competition for iron and other elements, as well as stimulation of plant defence mechanisms (Yao *et al.*, 2023; Dobrzyński and Nازیębło, 2024).

### 8.1 *Trichoderma* spp.

*Trichoderma* species have been thoroughly studied and shown to be effective fungal biocontrol agents. Yao *et al.*, 2023, reported that this activity can also be mycoparasitism, competition antibiosis, enzyme production and induction of systemic resistance in plants. Recent studies on formulations based on *Trichoderma* are being used against soil-borne and foliar and post-harvest diseases. Despite being efficacious, field performance may differ with strains, environmental conditions, formulation stability and application method (Li *et al.*, 2025; Pérez-Pizá *et al.*, 2025).

### 8.2 Bacterial Biocontrol Agents

The fungal pathogens have been proved to be controlled biologically by *Bacillus*, *Pseudomonas* and *Paenibacillus* species. Mechanisms may involve the production secretion of enzymes activation of host defenses biofilm formation and more. The genus *Paenibacillus* is of special interest as a potential biocontrol agent other than *P. polymyxa* (Dobrzyński and Nازیębło, 2024).

### 8.3 Arbuscular Mycorrhizal Fungi

Mycorrhizal fungi can prevent plant diseases and help plants obtain nutrients and water. They may change plant nutrition, hormonal signaling, root structure, defence responses, and so on. Scientists have recently found out that they can help manage disease and promote vigour (Galli *et al.*, 2024).

## 9. Botanical and Natural Products

Another potential group of substitutes for synthetic fungicides are plant. The activity of phenolics, flavonoids, terpenoids, alkaloids, and essential-oil components in inhibiting the growth of fungi can be attributed to their action on membrane integrity, enzyme activity, and spore germination (Calefi *et al.*, 2025). Recent reviews indicate that plant-based products can be useful against many pathogens, especially in organic systems. But this is a serious hindrance because of the variability of chemical composition stability formulation phytotoxicity and field efficacy. For real-life use in agriculture, promising compounds will need to be formulated in standard way and field validated (Calefi *et al.*, 2025; Monteiro *et al.*, 2026).

## 10. Emerging and Advanced Approaches

Advancements in plant pathology have created a whole new range of disease-management choices. Researchers are evaluating approaches like genomic editing, molecular diagnostics, and nanotechnology to enhance disease management. Despite the regulatory, ecological, economic, and technical constraints which still exist, Ogwu and Izah (2025) and Monteiro *et al.* (2026) concluded that these technologies have the potential of improving pathogen specificity and minimizing environmental exposure. These methods are the additions to the already-established disease-management principles (Keykhasaber *et al.*, 2026).

## 11. Integrated Disease Management

The different methods for integrated disease management are compatible and among them reduce diseases' intensity. The aim is to avoid heavy dependence on any one method. A practical program can employ resistant or tolerant cultivars, pathogen-free planting material, incorporate crop rotation, ensure proper irrigation and drainage, include handling sanitation and residues, monitor the crop, make an early and accurate diagnosis, practice biological control,

use validated botanicals, use fungicide judiciously and rotate the mode of action of fungicide (Khan, 2023; Galli *et al.*, 2024).

The main advantage of integration is that various components act via various means. This technique can help in improving disease suppression and decreasing the selection pressure for fungicide resistance. As per the recent reviews, it has endorsed integrated frameworks which combine cultural practices, host resistance, bio-control, use of natural products, and targeted application of chemicals. (Galli *et al.* 2024 Monteiro *et al.* 2026).

## 12. Current Challenges and Future Perspectives

Fungal diseases face many challenges that hinder their effective management. Populations of pathogens are genetically varied and adaptable. Fungicide resistance threatens the long-term effectiveness of chemical control. Variable performance shown in the field despite positive laboratory results experienced by a range of biological control agents (Corkley *et al.*, 2022; Pérez-Pizá *et al.*, 2025).

The uncertainty added by climate change stems from alterations in the distribution of pathogens, environmental suitability, host physiology and disease outcomes. The effects are not uniform across the pathosystems indicating that we require disease-specific forecasts making them more specialised than generalised assumptions (Singh *et al.*, 2023; Méndez *et al.*, 2026).

As a result of this, future research focus needs to be on field validation, better formulation of biological agents, standardization of diagnostics, resistance monitoring and combination of complementary disease-management strategies (Keykhasaber *et al.*, 2026; Monteiro *et al.* 2026).

**Table 1:** Major fungal pathogens, associated diseases, symptoms, and main affected plant organs.

| Fungal pathogen/group      | Representative disease | Major symptoms                              | Main affected organs                |
|----------------------------|------------------------|---------------------------------------------|-------------------------------------|
| <i>Fusarium spp.</i>       | Fusarium wilt/root rot | Wilting, vascular discoloration, root decay | Roots, stems                        |
| <i>Alternaria spp.</i>     | Leaf spot/blight       | Necrotic spots, concentric lesions          | Leaves, fruits                      |
| <i>Botrytis cinerea</i>    | Gray mold              | Soft rot, gray sporulation                  | Flowers, fruits                     |
| <i>Colletotrichum spp.</i> | Anthrachnose           | Sunken necrotic lesions                     | Leaves, stems, fruits               |
| <i>Rhizoctonia spp.</i>    | Root/crown rot         | Damping-off, root and stem lesions          | Roots, stems                        |
| <i>Sclerotinia spp.</i>    | White mold             | Soft rot, white mycelium                    | Stems, fruits                       |
| <i>Verticillium spp.</i>   | Vascular wilt          | Wilting, vascular browning                  | Roots, vascular tissues             |
| <i>Magnaporthe spp.</i>    | Blast disease          | Lesions and tissue necrosis                 | Leaves, stems, reproductive tissues |

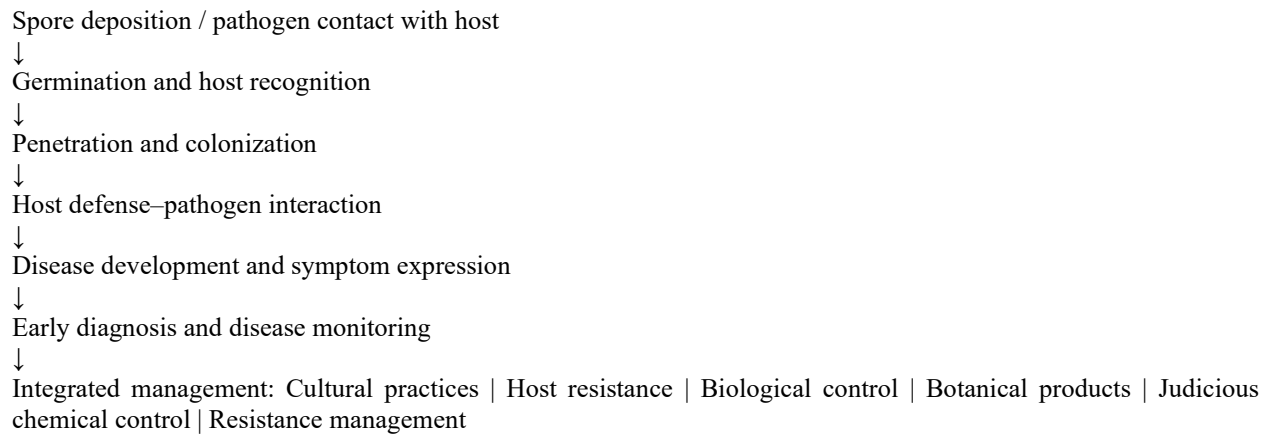
**Table 2:** Comparison of major fungal disease-management strategies.

| Strategy              | Main mechanism                                              | Major advantage                    | Major limitation                            | Sustainability   |
|-----------------------|-------------------------------------------------------------|------------------------------------|---------------------------------------------|------------------|
| Chemical control      | Direct inhibition of pathogen growth                        | Rapid and often effective          | Resistance and environmental concerns       | Moderate         |
| Host resistance       | Restricts infection or disease development                  | Long-term and relatively low input | Pathogen adaptation may overcome resistance | High             |
| Crop rotation         | Interrupts pathogen life cycle                              | Low cost and preventive            | Less effective against broad-host pathogens | High             |
| Biological control    | Competition, antibiosis, mycoparasitism, induced resistance | Environmentally compatible         | Variable field performance                  | High             |
| Botanical products    | Antifungal natural compounds                                | Potentially biodegradable          | Stability and standardization problems      | Potentially high |
| Integrated management | Combines complementary methods                              | More robust disease suppression    | Requires monitoring and planning            | Very high        |

**Figure 1:** Conceptual framework for fungal disease development and integrated management.

Pathogen survival and dissemination

↓



### 13. Conclusion

Fungal diseases are a huge challenge for sustainable crop production and food security. The development of pathogens happens as a result of many factors including pathogen biology. Accurate diagnosis and early intervention are essential for effective management of diseases (Khan, 2023; Singh *et al.*, 2023).

Even with the availability of chemical fungicides, the rising resistance and increasing environmental concerns necessitate the incorporation of host resistance, cultural practices, biological control, natural products and use of chemical fungicides options. Future studies need to increase the reliability of biological and diagnostic technologies under field conditions and explore complementary strategies for effective disease suppression that mitigates environmental impacts (Corkley *et al.*, 2022; Galli *et al.*, 2024; Monteiro *et al.*, 2026).

### References

- Khan, R. A. A. (2023). Advances in integrated disease management (IDM) for soil-borne plant pathogens: innovative approaches and underlying action mechanism at molecular level. *Frontiers in Plant Science*, 14, 1256979. <https://doi.org/10.3389/fpls.2023.1256979>.
- Yao, X., Guo, H., Zhang, K., Zhao, M., Ruan, J., & Chen, J. (2023). Trichoderma and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology*, 14, 1160551. <https://doi.org/10.3389/fmicb.2023.1160551>.
- Madhophe, S., Ogugua, U. V., Makhubu, F. N., & Figlan, S. (2025). Use of biological control agents for managing fungal pathogens in Solanaceae crops: progress and future perspectives—a review. *Discover Applied Sciences*, 7, 83. <https://doi.org/10.1007/s42452-025-06500-9>.
- Galli, M., Feldmann, F., Vogler, U. K., et al. (2024). Can biocontrol be the game-changer in integrated pest management? A review of definitions, methods and strategies. *Journal of Plant Diseases and Protection*, 131, 265–291. <https://doi.org/10.1007/s41348-024-00878-1>.
- Corkley, I., Fraaije, B., & Hawkins, N. (2022). Fungicide resistance: Progress in understanding mechanism, monitoring, and management. *Phytopathology*, 112.
- Sharma, G., Dwibedi, V., Seth, C. S., et al. (2024). Direct and indirect technical guide for the early detection and management of fungal plant diseases. *Current Research in Microbial Sciences*, 7, 100276. <https://doi.org/10.1016/j.crmicr.2024.100276>.
- Cotter, A., Dracatos, P., Beddoe, T., & Johnson, K. (2024). Isothermal detection methods for fungal pathogens in closed environment agriculture. *Journal of Fungi*, 10, 851. <https://doi.org/10.3390/jof10120851>.
- Maulenbay, A., & Rsaliyev, A. (2024). Fungal disease tolerance with a focus on wheat: A review. *Journal of Fungi*, 10, 482. <https://doi.org/10.3390/jof10070482>.
- Aslam, H. M. U., Chikh-Ali, M., Zhou, X.-G., et al. (2024). Epigenetic modulation of fungal pathogens: a focus on *Magnaporthe oryzae*. *Frontiers in Microbiology*, 15, 1463987. <https://doi.org/10.3389/fmicb.2024.1463987>.

- Naqvi, S. A. H., Farhan, M., Ahmad, M., et al. (2024). Deciphering fungicide resistance in Phytophthora: mechanisms, prevalence, and sustainable management approaches. *World Journal of Microbiology and Biotechnology*, 40, 302. <https://doi.org/10.1007/s11274-024-04108-6>.
- Dobrzyński, J., & Naziębło, A. (2024). *Paenibacillus* as a biocontrol agent for fungal phytopathogens: Is *P. polymyxa* the only one worth attention? *Microbial Ecology*, 87, 134. <https://doi.org/10.1007/s00248-024-02450-8>.
- Galli, M., Sede, A., Heinlein, M., et al. (2025). A biocontrol perspective on mycoviruses in fungal pathogen management. *Journal of Plant Diseases and Protection*, 132, 43. <https://doi.org/10.1007/s41348-024-01006-9>.
- Li, X., Liao, Q., Zeng, S., Wang, Y., & Liu, J. (2025). The use of *Trichoderma* species for the biocontrol of postharvest fungal decay in fruits and vegetables: Challenges and opportunities. *Postharvest Biology and Technology*, 219, 113236. <https://doi.org/10.1016/j.postharvbio.2024.113236>.
- Pérez-Pizá, M. C., et al. (2025). Quick and effective evaluation methods for biocontrol agents and biostimulants against phytopathogenic fungi relevant for various cropping systems. *Plant Pathology*. <https://doi.org/10.1111/ppa.14064>.
- Ogwu, M. C., & Izah, S. C. (2025). Nanotechnology for fungal pathogen control in crops: innovations, public health impacts, and disease prevention. *Frontiers in Fungal Biology*, 6, 1653214. <https://doi.org/10.3389/ffunb.2025.1653214>.
- Keykhasaber, M., Tzelepis, G., & Rafiei, V. (2026). Converging technologies for next-generation plant protection: an integrated framework for fungal disease management. *Frontiers in Agronomy*, 8, 1760693. <https://doi.org/10.3389/fagro.2026.1760693>.
- Monteiro, F., et al. (2026). Non-chemical control of fungal pathogens in crops: a one-health perspective on strategies, mechanisms, and future directions. *Frontiers in Plant Science*. <https://doi.org/10.3389/fpls.2025.1746521>.
- George, R., Thuseethan, S., Ragel, R. G., et al. (2025). Past, present and future of deep plant leaf disease recognition: A survey. *Computers and Electronics in Agriculture*, 234, 110128. [10.1016/j.compag.2025.110128](https://doi.org/10.1016/j.compag.2025.110128).
- Méndez, I., et al. (2026). Does climate change increase disease severity caused by crop pathogens? A systematic review. *Plant Disease*, 110, 2556–2571. <https://doi.org/10.1094/PDIS-03-25-0573-SR>.
- Islam, T., Danishuddin, Tabassum Tamanna, N., Matin, M. N., Barai, H. R., & Haque, M. A. (2024). Resistance mechanisms of plant pathogenic fungi to fungicide, environmental impacts of fungicides, and sustainable solutions. *Plants*, 13, 2737. <https://doi.org/10.3390/plants13192737>.
- Calefi, G. G., Silva, N. B. S., Alhatlani, B. Y., & Abdallah, E. M. (2025). Harnessing nature's arsenal: sustainable plant-based strategies for phytopathogen control. *Frontiers in Microbiology*, 16, 1588462. <https://doi.org/10.3389/fmicb.2025.1588462>.
- Pereira, M. R., Tosin, R., dos Santos, F. N., Tavares, F., & Cunha, M. (2025). Digital assessment of plant diseases: A critical review and analysis of optical sensing technologies for early plant disease diagnosis. *Computers and Electronics in Agriculture*, 236, 110443. [10.1016/j.compag.2025.110443](https://doi.org/10.1016/j.compag.2025.110443).
- Singh, B. K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J. E., Liu, H., & Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology*, 21, 640–656. <https://www.nature.com/articles/s41579-023-00900-7>.



## Knowledge and Beliefs Regarding Harmful Traditional Health Practices among University Staff: A Cross-Sectional Study

KAREEM RISHAG SAJIT<sup>1</sup>, KHOLOUD ABDUL RAZZAQ MANAHI<sup>2</sup>, SAJJAD S. ISSA<sup>3</sup>,  
ALI MALIK TIRYAG<sup>4\*</sup>, MAJED A. MOHAMMAD<sup>5</sup>, FIRAS A. JASSIM<sup>6</sup>

1 College of Nursing, University of Baghdad, Baghdad, Iraq

2 Pediatric Department, Al-Basrah Teaching Hospital, Ministry of Health, Basrah, Iraq

3 Community Health Nursing Department, College of Nursing, University of Basrah, Basrah, Iraq

4 Fundamentals of Nursing, College of Nursing, University of Basrah, Basrah, Iraq

5 College of Nursing, University of Basrah, Basrah, Iraq

6 Community Health Nursing Department, College of Nursing, University of Basrah, Basrah, Iraq

\*Corresponding Author: [ali.malik@uobasrah.edu.iq](mailto:ali.malik@uobasrah.edu.iq)

### Abstract

**Background:** Certain customs have their roots in cultures, religions, or the counseling of new mothers by family members.

**Methods:** A descriptive cross-sectional study was conducted from December 1st, 2025 to March 30th, 2026. The study included a convenience sample of 200 male and female staff members from different colleges at the University of Basrah, Bab Al-Zubair campus. Data were collected using a closed-ended questionnaire consisting of two parts: socio-demographic characteristics and questions related to the staff's opinions about several traditional health practices. The Statistical Package for Social Sciences (SPSS) version 26 was used to analyze the data using both descriptive and inferential statistics.

**Results:** The results showed that most participants were university graduates (58.5%), while 23% had postgraduate degrees. The Participants demonstrated variable levels of knowledge, with persistence of several misconceptions. However, some incorrect beliefs were still present among the participants, such as using ash or flour to stop wound bleeding, hanging beads for neonatal jaundice, and using kohl to protect infants' eyes. Significant correlations were found between knowledge scores and some demographic variables, including age, gender, and marital status.

**Conclusion:** The study concluded that despite relatively high educational levels among participants, some harmful traditional health practices are still believed and practiced. Increasing awareness and providing accurate health education are essential to reduce the spread of these misconceptions.

### 1. Introduction

Due to poverty and poor levels of education, harmful traditional behaviors are carried out in many civilizations, particularly in developing nations. The link between the environment and human views toward nature is typically the source of traditional behaviors. Certain customs have their roots in nations, religions, or family members counseling new mothers (1). Since prehistoric times, people have utilized natural products, such as plants, animals, and marine organisms, to treat and alleviate illnesses (2).

For early people, using natural things as medicines surely posed a significant problem. When looking for food, that is highly likely. Poisonous plants were frequently eaten by early humans, who may have died as a result of vomiting, diarrhea, stupor, or other toxic effects. However, they were able to expand their understanding of natural remedies and edible ingredients in this way (3). Humans then discovered how to create new medications. Natural items are used in traditional remedies, which are quite significant. Natural goods are used in traditional medicine and folk medicine, which have been used for hundreds or even thousands of years worldwide. They have consistently thrived in official medicine, which continues to be a storehouse of human knowledge (4).

Arabs are credited with creating and establishing the principles and guidelines of traditional medicine, particularly those who specialized in traditional and folk Arab medicine (5). Traditional medicine includes procedures and manual exercises used alone or in combination to cure, diagnose, prevent, or maintain happiness, as well as plant, animal, and mineral remedies, spiritual therapies, magic, and superstition. It also refers to all of the knowledge, skills, and approaches that are employed to maintain health, prevent, diagnose, improve, or treat physical, mental, and other ailments and are taken from indigenous theories, beliefs, and experiences of other cultures, whether or not they can be explained (6).

A yellowish or, less commonly, greenish coloring of the skin and sclera caused by elevated bilirubin levels is called jaundice, or icterus. Adult cases of jaundice are usually indicative of underlying conditions including aberrant heme metabolism, liver malfunction, or obstruction of the biliary tract (7, 8). While jaundice is uncommon in adults, it is frequent in babies, affecting an estimated 80% of them during the first week of life. The most typical signs of jaundice include dark urine, pale feces, and itching (9). Excessive disintegration of red blood cells may be the cause of elevated unconjugated bilirubin. The underlying cause of jaundice usually determines the course of treatment (10, 11).

The process during which a baby's first teeth—the deciduous teeth—emerge through their gums, usually in pairs, is known as teething. The infant experiences discomfort and anguish as the mandibular central incisors, the first primary teeth to erupt, typically appear between the ages of six and ten months (12). The eruption of all 20 teeth may take several years. The body releases hormones that lead to the death and separation of some gum cells, allowing the teeth to erupt. Teething does not appear to be a major cause of vomiting, diarrhea, or fever (13,14).

Every newborn has a varied threshold for pain. Usually starting three to five days before the tooth erupts, the sensations go away as soon as the tooth penetrates the skin (15, 16). Some people think that teething causes other symptoms, such as diarrhea, but there is no evidence to support this (17).

Measles is an airborne disease that spreads quickly from person to person through the coughs and sneezes of infected people. Symptoms usually start 10–12 days after interaction with an infected person and continue for 7–10 days. Common initial symptoms include fever, often more than 40 °C (104 °F), cough, runny nose, and itchy eyes (18). Koplik spots are little white patches that might develop inside the mouth two or three days after the onset of symptoms (19). A red, flat rash typically develops on the face three to five days after symptoms start, then spreads to the rest of the body (20).

Pneumonia (6%), middle ear infection (7%), and diarrhea (8% of cases) are common consequences (21). Measles-induced immunosuppression contributes to these (22). Seizures, blindness, or brain inflammation are less prevalent (23, 24). While there are historical reports from the early 20th century suggesting that red light therapy could "abort" or speed up the recovery of measles, there is no modern scientific evidence or clinical guidelines recommending "red dressing" (red clothing) to treat or relieve measles symptoms (25, 26).

A collection of middle ear inflammatory conditions is known as otitis media. Acute otitis media (AOM), a fast-onset illness that typically manifests as ear pain, is one of the two primary forms (27, 28). Otitis media with effusion (OME) is the other primary form (29). Middle ear inflammation that causes a tympanic membrane perforation and ear discharge is known as chronic suppurative otitis media (CSOM) (30, 31).

Inflammation of the conjunctiva, the thin, transparent membrane covering the inner eyelid and the white surface of the eye, is referred to as conjunctivitis, or pink eye (32, 33). While bacterial causes are more common in children, viruses are the most common infectious causes in adults (34, 35).

An injury to skin or other tissues brought on by heat, electricity, chemicals, friction, or ionizing radiation (like sunburn, which is produced by UV radiation) is referred to as a burn (36). Burns mostly happen at home or at work (36, 37). Superficial or first-degree burns are those that only damage the outermost layers of skin. The pain usually lasts around three days, and they appear red without blisters. A partial-thickness or second-degree burn occurs when the damage penetrates part of the underlying skin layer. Blisters are common and frequently cause excruciating agony. Scarring may result, and healing may take up to eight weeks (38). All of the skin's layers are damaged in a full-thickness or third-degree burn. The burned area is often stiff and painless. Usually, healing doesn't happen on its own (39).

## 2. Research Methodology

**Study's Design:** At the University of Basrah Bab Al-Zubair campus, a descriptive cross-sectional study approach was used, involving a sample of 200 participants (male and female). The study started from December 1st, 2025, up to 30th March, 2026, in order to know the University of Basrah staff's opinions on some wrong health traditional practice at Bab Al-Zubair campus.

**Setting of the Study:** The current study was conducted at the Bab Al-Zubair campus of the University of Basrah.

**The Sample of the Study:** A convenience sample from the university staff, which consisted of 200 participants at Basrah University.

**Study's Instrument:** Data were collected using a closed-ended questionnaire. The questionnaire is divided into two sections. The first portion includes six questions about the population's sociodemographic characteristics, such as age, gender, college department, education level, marital status, and place of residence. The population's opinions toward several health customs are the subject of 15 questions in the second section of the survey. For the study, a standardized Likert scale with three points—agree, neutral, and disagree—was employed. Good (2.34-3), middling (1.67-2.33), and poor (1-1.66). 200 samples were given the pre-made questionnaire, which they read and completed. The researchers then collected the completed forms. Every form was examined and given a percentage score based on the correct usual response.

**Statistical Analysis:** Analysis was conducted using SPSS 26, so we measured: frequency, Percentage (%), Spearman correlation, and means.

**Ethical Approval:** In addition to receiving official approval from the Council of the College of Nursing at the University of Basrah, the study also received ethical approval from the committee overseeing the college's ethical research and was permitted to proceed. The researcher explained the purpose of the study to each participant. Oral consent was obtained from each participant before data collection.

## 3. Results

**Table 1:** Distribution of the study sample according to educational levels

| Educational levels          | Frequencies | Percentages |
|-----------------------------|-------------|-------------|
| Primary school graduation   | 7           | 3.5         |
| Secondary school graduation | 16          | 8           |
| University graduation       | 117         | 58.5        |
| Institute education         | 14          | 7           |
| Master degree               | 35          | 17.5        |
| Doctorate                   | 11          | 5.5         |
| <b>Total</b>                | <b>200</b>  | <b>100</b>  |

The table shows the distribution of the study sample according to educational levels. We found that 3.5% had primary school education, 8% had secondary school education, 7% had institution education, 58.5% had university education, 17.5 % were having Master graduation, and 5.5% had doctorate graduation.

**Table 2:** Distribution of the study sample according to workplace location

| Workplace                             | Frequencies | Percentages |
|---------------------------------------|-------------|-------------|
| College of administration and economy | 48          | 24          |
| College of education for girls        | 34          | 17          |
| College of Nursing                    | 34          | 17          |
| College of Arts                       | 32          | 16          |
| College of Fine Arts                  | 19          | 9.5         |
| College of Law                        | 10          | 5           |
| Others                                | 23          | 11.5        |
| <b>Total</b>                          | <b>200</b>  | <b>100</b>  |

The table showed the distribution of the study sample according to workplace; 24% of the sample were from the College of Administration and Economy, 17% of the sample were from the College of Education for Girls, 17% were from the College of Nursing, 16% were from the College of Art, 9.5% were from Fine Arts, 5% were from the College of Law, and 11.5% were from other places in the Bab Al-Zubair campus at the University of Basrah.

**Table 3:** Distribution of the study sample according to Age intervals

| Age intervals in years | Frequencies | Percentages |
|------------------------|-------------|-------------|
| 20-29                  | 38          | 19          |
| 30-39                  | 68          | 34          |
| 40-49                  | 57          | 28.5        |
| 50-59                  | 37          | 18.5        |
| <b>Total</b>           | <b>200</b>  | <b>100</b>  |

The table showed that 19% of the sample were within the age interval 20-29 years, 34% of the sample were within the age interval 30-39 years, 28.5% of the sample were within the age interval 40-49 years, and 18.5% of the sample were within the age interval 50-59 years.

**Table 4:** Distribution of the study sample according to Gender

| Gender       | Frequencies | Percentages |
|--------------|-------------|-------------|
| Males        | 89          | 44.5        |
| Females      | 111         | 55.5        |
| <b>Total</b> | <b>200</b>  | <b>100</b>  |

The table shows the distribution of the study samples according to gender; 44.5% were males and 55.5% were females.

**Table 5:** Distribution of the study sample according to Residencies

| Residencies  | Frequencies | Percentages |
|--------------|-------------|-------------|
| Urban        | 150         | 75          |
| Rural        | 50          | 25          |
| <b>Total</b> | <b>200</b>  | <b>100</b>  |

The table shows the distribution of the study samples according to residency; 75% were urban, and 25% were rural.

**Table 6:** Distribution of the study sample according to Marital Status

| Marital status | Frequencies | Percentages |
|----------------|-------------|-------------|
| Single         | 77          | 38.5        |
| Married        | 123         | 61.5        |
| <b>Total</b>   | <b>200</b>  | <b>100</b>  |

The table shows the distribution of the study samples according to marital status; 38.5% were single, and 61.5% were married.

**Table 7:** Distribution of the study sample according to Scores

| Scores             | Frequencies | Percentages |
|--------------------|-------------|-------------|
| Less than 50 marks | 6           | 3           |
| 50-59 marks        | 31          | 15.5        |
| 60-69 marks        | 53          | 26.5        |
| 70-79 marks        | 45          | 22.5        |
| 80-89 marks        | 32          | 16          |
| 90 and above       | 33          | 16.5        |
| <b>Total</b>       | <b>200</b>  | <b>100</b>  |

The table showed the distribution of the study sample according to scores they get after corresponding the typical answers with staff answers to measure their levels of knowledge using the classical way in classifying the scores (marks), 3% were having less than 50 scores (fail to answer the questionnaire items) 15.5% were having 50-59 score (low level of knowledge), 26.5% were having 60-69 score (moderate level of knowledge), 22.5% were having 70-79 scores (good level of knowledge), 16% were having 80-89 scores (very good level of knowledge) and 16.5 % were having 90 and above (excellent level of knowledge).

**Table 8:** Distribution of the Study Sample according to questionnaire answers

| No | Item                                                                                                     | AG<br>(1) | Neut.<br>(2) | DA<br>(3) | Means |
|----|----------------------------------------------------------------------------------------------------------|-----------|--------------|-----------|-------|
| 1  | Ear dissection for a patient with Jaundice is an acceptable way of treating babies and adults            | 44        | 60           | 96        | 2.26  |
| 2  | Hanging Garlic pieces on the body of neonates is beneficial in decreasing neonatal Jaundice              | 64        | 46           | 90        | 2.13  |
| 3  | Hanging yellow jaundice beadle on the body of neonate had a role in decreasing the level of jaundice     | 153       | 31           | 16        | 1.31  |
| 4  | Teething process in infants can cause some symptoms like diarrhea, vomiting, fever, and loss of appetite | 50        | 56           | 94        | 2.22  |
| 5  | Red dressing for measles patients can relieve the disease symptoms                                       | 71        | 65           | 64        | 1.96  |
| 6  | Introduction of onion pieces on ear for managing ear diseases                                            | 80        | 53           | 67        | 1.93  |
| 7  | Using a pack of tea to treat eye infection                                                               | 31        | 49           | 120       | 2.44  |
| 8  | Skin cauterization for management of headache                                                            | 22        | 21           | 157       | 2.67  |
| 9  | Putting animal feces on umbilical cord stump in neonate to enhance healing process                       | 23        | 30           | 147       | 2.62  |
| 10 | Using animal urine in the treatment of skin diseases                                                     | 60        | 53           | 87        | 2.13  |
| 11 | Using tomato paste for managing burns at home                                                            | 50        | 52           | 98        | 2.24  |
| 12 | Using ash or flour to stop wound bleeding                                                                | 122       | 54           | 24        | 1.51  |
| 13 | Baby swaddling is useful for future body alignment                                                       | 118       | 49           | 33        | 1.57  |
| 14 | Using rice water in feeding infants                                                                      | 87        | 41           | 72        | 1.92  |
| 15 | Using Kohl for protecting infants' eyes from infections                                                  | 115       | 45           | 40        | 1.62  |

AG= agree, Neut.= neutral, DA = Disagree

The table showed the distribution of the study sample according to the questionnaire answers. Regarding the answer to question (1-Ear dissection for a patient with Jaundice is an acceptable way for treating babies and adults), 22% agreed with such a wrong tradition, 30% had a neutral answer, while 48% only disagreed. Question (2-Hanging Garlic pieces on the body of neonates is beneficial in decreasing neonatal Jaundice): 32% agreed with such a wrong tradition, 23% had a neutral answer, while 45% only disagreed. Regarding question (3-Hanging yellow jaundice beadle on the body of neonate had a role in decreasing level of jaundice), 76.5% agreed with such a wrong tradition, 15.5% had a neutral answer, while 8% only disagreed.

Question (4- Teething process in infants can cause some symptoms like diarrhea, vomiting, fever, and loss of appetite), 25% agreed with such a wrong tradition, 28% had a neutral answer, while 47% only disagreed. Question (5-Red dressing for measles patients can relieve the disease symptoms): 35.5% agreed with such a wrong tradition, 32.5% had a neutral answer, while 32% only disagreed. Question (6-Introduction of onion pieces on ear for managing ear diseases): 40% agreed with such a wrong tradition, 26.5% had a neutral answer, while 33.5% only disagreed. Question (7-Using a pack of tea to treat eye infection): 15.5% agreed with such a wrong tradition, 24.5% had a neutral answer, while 60% only disagreed.

Question (8-Skin cauterization for management of headache): 11% agreed with such a wrong tradition, 10.5% had a neutral answer, while 78.5% only disagreed. Question (9-Putting animal feces on umbilical cord stump in neonate to

enhance healing process): 11.5% agreed with such a wrong tradition, 15% had a neutral answer, while 73.5% only disagreed. Question (10-Using animal urine in the treatment of skin diseases): 30% agreed with such a wrong tradition, 26.5% had a neutral answer, while 43.5% only disagreed.

Question (11-Using tomato paste for managing burns at home): 25% agreed with such a wrong tradition, 26% had a neutral answer, while 49% only disagreed. Question (12-Using ash or flour to stop wound bleeding): 61% agreed with such a wrong tradition, 27% had a neutral answer, while 12% only disagreed. Question (13-Baby swaddling is useful for future body alignment) 59% were agree with such a wrong tradition, 24.5% had a neutral answer, while 16.5% only disagreed. (14-Using rice water in feeding infants) Question 43.5% agreed with such a wrong tradition, 20.5% had a neutral answer, while 36% only disagreed. Question (15-Using Kohl for protecting infants' eyes from infections): 57.5% agreed with such a wrong tradition, 22.5% had a neutral answer, while 20% only disagreed.

Table 9: The Correlations of the scores with some variables

|                      | Scores | Age     | Gender  | Level  | Residence | Marital status |
|----------------------|--------|---------|---------|--------|-----------|----------------|
| Spearman Correlation | 1      | -0.181* | 0.222** | -0.105 | -0.026    | 0.526**        |
| Sig. (2-tailed)      |        | 0.010   | 0.002   | 0.137  | 0.713     | 0.000          |
| N                    | 201    | 201     | 194     | 201    | 201       | 201            |

The table showed the correlation of scores with demographic variables, using Spearman correlation. There was a significant correlation between scores and age at the 0.05 level, and there was high significant correlation between scores and both gender and marital status at the 0.01 level.

Table 10: Distribution of the study sample according to knowledge scores (No. 15 question)

| Score     | Classes  | Frequencies | Percentages |
|-----------|----------|-------------|-------------|
| 1-1.66    | Poor     | 4           | 26.6        |
| 1.67-2.33 | Moderate | 7           | 46.7        |
| 2.34-3    | Good     | 4           | 26.7        |

The table showed the distribution of the study sample according to knowledge scores; 26.6% had poor knowledge, 46.7 % had moderate knowledge, and 26.6 had good knowledge, so 73.3 % were having high level of knowledge.

#### 4. Discussion

The present study aimed to explore the opinions of University of Basrah staff regarding some common traditional health practices that may negatively affect health. Although the majority of the participants had relatively high educational levels, the findings showed that several incorrect health beliefs are still present.

According to the results, most of the participants were university graduates or held postgraduate degrees. Despite this, a noticeable proportion of the respondents agreed with some traditional practices that are not supported by scientific evidence. This suggests that educational level alone may not be sufficient to eliminate beliefs that are deeply rooted in cultural traditions.

The results of the knowledge scores indicated that most participants had moderate to good levels of knowledge about these practices. However, a smaller proportion of the sample showed low knowledge levels, which indicates that some misconceptions still exist among university staff.

The responses to the questionnaire also showed that certain traditional practices are still widely accepted. For example, a high percentage of participants believed in practices such as hanging yellow beads on neonates to reduce jaundice, using ash or flour to stop bleeding, and using kohl to protect infants' eyes. These practices are often inherited through generations and may be considered normal in some communities despite the lack of scientific evidence supporting them.

On the other hand, some practices such as skin cauterization for headache and placing animal feces on the umbilical cord stump were rejected by the majority of participants. This may indicate that awareness about some harmful practices has improved. The correlation analysis revealed significant relationships between knowledge scores and some demographic variables. Age showed a significant association with knowledge level, which may suggest that experience and exposure to information influence health awareness. Gender and marital status also showed significant relationships with the knowledge scores.

## 5. Conclusions

More than half of the sample had university graduation, two-thirds of the sample were from urban areas, more than half were married, and more than half of the sample were within the ages between 30-49 years. About half of the sample answered the questionnaire with 60 and 79 evaluation marks, as with standard answers. Some wrong traditional health practices are still present among University of Basrah staff. Most participants demonstrated moderate to good knowledge about these practices. A noticeable proportion still believes in some harmful practices of the population. Demographic factors such as age, gender, and marital status were significantly associated with knowledge level.

## References

- Ammar A. Features of traditional treatment in the Tafna region in western Algeria [Internet]. 2014 [cited 2025 Oct 24]. Available from: <https://www.alukah.net/culture/0/67951>
- Shi QW, Li LG, Huo CH, Zhang ML, Wang YF. Study on natural medicinal chemistry and new drug development. *Chin Tradit Herb Drugs*. 2010; 41:1583-1589.
- Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect*. 2001;109(Suppl 1):69-75.
- Alves RR, Rosa IL. Biodiversity, traditional medicine and public health: where do they meet? *J Ethnobiol Ethnomed*. 2007; 3:14. doi:10.1186/1746-4269-3-14.
- Maqboub I. Ritual folk treatment in Morocco. *J Pop Cult*. 2016;9(34):104-121.
- World Health Organization. Traditional medicine: report by the Secretariat. Executive Board, 134th session, provisional agenda item 9.1. Geneva: World Health Organization; 2013 Dec 13.
- National Library of Medicine. Jaundice [Internet]. MedlinePlus; 2025 Jun 25 [cited 2025 Oct 24]. Available from: <https://medlineplus.gov/jaundice.html>
- Al-Tubaikh JA. Internal Medicine. Cham: Springer; 2017. doi:10.1007/978-3-319-39747-4.
- Kaplan M, Hammerman C. Hereditary contribution to neonatal hyperbilirubinemia. In: *Fetal and Neonatal Physiology*. 5th ed. Philadelphia: Elsevier; 2017. p. 933-942.e3. doi:10.1016/B978-0-323-35214-7.00097-4.
- Roger J. Oxford Textbook of Primary Medical Care. Oxford: Oxford University Press; 2004. p. 758.
- Winger J, Michelfelder A. Diagnostic approach to the patient with jaundice. *Prim Care*. 2011;38(3):469-482. doi: 10.1016/j.pop.2011.05.004. PMID:21872092.
- Lunt RC, Law DB. A review of the chronology of eruption of deciduous teeth. *J Am Dent Assoc*. 1974;89(4):872-879. doi: 10.14219/jada.archive.1974.0484. PMID:4609369.
- National Health Service. Teething [Internet]. NHS; [cited 2025 Oct 24]. Available from: <https://www.nhs.uk/baby/babys-development/teething/>
- Jaber L, Cohen IJ, Mor A. Fever associated with teething. *Arch Dis Child*. 1992;67(2):233-234. doi:10.1136/adc.67.2.233. PMID:1543387. PMCID.
- WebMD. Teething: overview [Internet]. WebMD; [cited 2010 Jan 25]. Available from: <https://www.webmd.com/oral-health/tc/teething-topic-overview>
- Macknin ML, Piedmonte M, Jacobs J, Skibinski C. Symptoms associated with infant teething: a prospective study. *Pediatrics*. 2000;105(4):747-752. doi:10.1542/peds.105.4.747. PMID:10742315.
- National Health Service. Baby teething symptoms [Internet]. NHS; [cited 2025 Oct 24]. Available from: <https://www.nhs.uk/baby/babys-development/teething/baby-teething-symptoms/>
- Tesini BL. Measles [Internet]. Merck Manual Professional Edition. 2023 Jul [cited 2025 Apr 10]. Available from: <https://www.merckmanuals.com/professional/pediatrics/common-viral-infections-in-infants-and-children/measles>
- Centers for Disease Control and Prevention. Measles (rubeola): signs and symptoms [Internet]. 2014 Nov 3 [cited 2015 Feb 5]. Available from: <https://www.cdc.gov/measles/about/signs-symptoms.html>

- Centers for Disease Control and Prevention. Chapter 13: Measles. In: Pink Book: epidemiology and prevention of vaccine-preventable diseases [Internet]. 2024 Apr 24 [cited 2025 Apr 9]. Available from: <https://www.cdc.gov/pinkbook/hcp/table-of-contents/chapter-13-measles.html>
- Rota PA, Moss WJ, Takeda M, de Swart RL, Thompson KM, Goodson JL. Measles. Nat Rev Dis Primers. 2016;2:16049. doi:10.1038/nrdp.2016.49. PMID:27411684.
- World Health Organization. Measles [Internet]. Geneva: World Health Organization; 2024 Nov 14 [cited 2025 Apr 10]. Available from: <https://www.who.int/en/news-room/fact-sheets/detail/measles>
- Guerra FM, Bolotin S, Lim G, Heffernan J, Deeks SL, Li Y, et al. The basic reproduction number (R0) of measles: a systematic review. Lancet Infect Dis. 2017;17(12). doi:10.1016/S1473-3099(17)30307-9. PMID:28757186.
- Delamater PL, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the basic reproduction number (R0). Emerg Infect Dis. 2019;25(1):1-4. doi:10.3201/eid2501.171901. PMID:30560777. PMCID.
- Selina SP, Chen MD. Measles [Internet]. Medscape; 2019 Jun 6 [cited 2025 Apr 10]. Available from: <https://emedicine.medscape.com/article/966220-overview>
- World Health Organization. Measles [Internet]. Geneva: World Health Organization; [cited 2025 Apr 30]. Available from: <https://www.who.int/news-room/fact-sheets/detail/measles>
- Lieberthal AS, Carroll AE, Chonmaitree T, Ganiats TG, Hoberman A, Jackson MA, et al. The diagnosis and management of acute otitis media. Pediatrics. 2013;131(3). doi:10.1542/peds.2012-3488. PMID:23439909.
- Centers for Disease Control and Prevention. Ear infections [Internet]. Atlanta (GA): CDC; 2013 Sep 30 [cited 2015 Feb 14]. Available from: <https://www.cdc.gov/getsmart/antibiotic-use/URI/ear-infection.html>
- Minovi A, Dazert S. Diseases of the middle ear in childhood. GMS Curr Top Otorhinolaryngol Head Neck Surg. 2014;13. doi:10.3205/cto000114. PMID:25587371. PMCID.
- Emmett SD, Kokesh J, Kaylie D. Chronic ear disease. Med Clin North Am. 2018;102(6):1063-1079. doi:10.1016/j.mcna.2018.06.008. PMID:30342609.
- Ruben RJ, Schwartz R. Necessity versus sufficiency: the role of input in language acquisition. Int J Pediatr Otorhinolaryngol. 1999;47(2):137-140. doi:10.1016/S0165-5876(98)00132-3. PMID:10206361.
- National Eye Institute. Pink eye [Internet]. Bethesda (MD): National Eye Institute; 2025 Nov [cited 2026 Jan 16]. Available from: <https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/pink-eye>
- Azari AA, Barney NP. Conjunctivitis: a systematic review of diagnosis and treatment. JAMA. 2013;310(16):1721-1729. doi:10.1001/jama.2013.280318. PMID:24150468. PMCID.
- Richards A, Guzman-Cottrill JA. Conjunctivitis. Pediatr Rev. 2010;31(5):196-208. doi:10.1542/pir.31-5-196. PMID:20435711.
- Durand ML, Barshak MB, Sobrin L. Eye infections. N Engl J Med. 2023;389(25):2363-2375. doi:10.1056/NEJMra2216081. PMID:38118024.
- Tintinalli JE. Emergency Medicine: A Comprehensive Study Guide. New York: McGraw-Hill; 2010. p. 1374-1386.
- World Health Organization. Burns [Internet]. Geneva: World Health Organization; 2016 Sep [cited 2017 Aug 1]. Available from: <https://www.who.int/mediacentre/factsheets/fs365/en/>
- World Health Organization. Burns: fact sheet no. 365 [Internet]. Geneva: World Health Organization; 2014 Apr [cited 2016 Mar 3]. Available from: <https://www.who.int/mediacentre/factsheets/fs365/en/>
- Granger J. An evidence-based approach to pediatric burns. Pediatr Emerg Med Pract. 2009;6(1).
- Nasrawi AJM, Hussien SRM. Is garlic necklace effective in the treatment of neonatal jaundice? Iran J Neonatol. 2020;11(2). doi:10.22038/ijn.2020.41675.1688.



## Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Staphylococcus aureus* (VRSA) among admitted patients at Baqubah Teaching Hospital in Diyala

MAWJ MOHAMMED ABBOOD<sup>1</sup>,  
ENAS AMMAR MOHAMMED<sup>3</sup>,

HANAN RAHEEM HASOONI<sup>2\*</sup>,  
WISSAM F. HASSAN<sup>4</sup>

1 Baqubah Teaching Hospital, Diyala, Iraq. [mawjmohammedta23@gmail.com](mailto:mawjmohammedta23@gmail.com).

2 Department of Internal Medicine, College of Medicine, University of Diyala, Diyala, Iraq. [hanan.hassouni@uodiyala.edu.iq](mailto:hanan.hassouni@uodiyala.edu.iq).

3 Department of Internal Medicine, College of Medicine, University of Diyala, Diyala, Iraq. [enas.a@uodiyala.edu.iq](mailto:enas.a@uodiyala.edu.iq).

4 Department of Internal Medicine, College of Medicine, University of Diyala, Diyala, Iraq. [wissam@uodiyala.edu.iq](mailto:wissam@uodiyala.edu.iq).

Corresponding Author: Hanan Raheem Hassouni, [hanan.hassouni@uodiyala.edu.iq](mailto:hanan.hassouni@uodiyala.edu.iq).

### Abstract

**Background:** Antibiotic resistance in *Staphylococcus aureus* has emerged as a critical global public health threat, driven primarily by the rising prevalence of methicillin-resistant strains (MRSA) and their diminishing susceptibility to conventional antimicrobials.

**Objective:** To determine the prevalence of methicillin-resistant (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) and to evaluate their antimicrobial susceptibility patterns across various clinical isolates.

**Patients and Methods:** A cross-sectional study was conducted at Baqubah Teaching Hospital in Diyala, Iraq, from March 2025 to April 2026. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include 110 *S. aureus* isolates were consecutively Antimicrobial susceptibility testing was performed using standard methods. Data were analyzed using SPSS version 24, and Chi-square tests were applied to assess the associations between resistance patterns and specimen sources. P-values less than 0.05 were considered statistically significant.

**Result:** The isolated *S. aureus* strains exhibited substantial resistance to several commonly used antibiotics. The most prominent resistance rates were observed against benzylpenicillin (95.45%) and oxacillin (80.91%), indicating a high phenotypic prevalence of methicillin-resistant *S. aureus* (MRSA). Moderate resistance profiles were recorded for moxifloxacin (65.45%), erythromycin (66.36%), gentamicin (51.82%), levofloxacin (51.82%), tobramycin (50.91%), and rifampicin (50.00%). Conversely, the isolates retained relatively higher susceptibility to teicoplanin (73.64%), trimethoprim/sulfamethoxazole (62.73%), vancomycin (56.36%), and tetracycline (56.36%). Notably, the distribution of antimicrobial resistance varied significantly based on the specimen source; isolates recovered from urine samples demonstrated the highest resistance rates, followed by wound and vaginal swabs, whereas ear swabs exhibited the lowest. Statistical analysis confirmed significant variations in susceptibility patterns ( $P \leq 0.01$ ), underscoring a non-random distribution of resistance among the tested pathogens.

**Conclusion:** In conclusion, this study demonstrates a high prevalence of multidrug-resistant *S. aureus*, including a substantial proportion of methicillin-resistant (MRSA) strains, at Baqubah Teaching Hospital. The significant variations in susceptibility patterns across different antibiotics and anatomical infection sites emphasize the necessity of tailored empirical therapy. These alarming resistance rates warrant the immediate implementation of strict antimicrobial stewardship programs and regular local surveillance to curb the dissemination of these refractory pathogens.

**Keywords:** *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VRSA), Antibiotic resistance, Antimicrobial susceptibility.

## 1. Introduction

*S. aureus* is a gram-positive bacterium that colonizes the skin and nasal passages of humans and animals, acting as both a commensal organism and a potent pathogen capable of causing a wide range of diseases [1]. The high adaptability of *S. aureus*, coupled with its ability to form biofilms on medical devices and persist in hospital environments, further complicates eradication efforts and contributes to recurrent infections [2]. The emergence of methicillin resistance in *S. aureus* strains is primarily mediated by the acquisition of the *mecA* gene, which encodes a penicillin-binding protein (PBP2a) with reduced affinity for beta-lactam antibiotics, rendering standard methicillin and related beta-lactam therapies ineffective [3]. Consequently, MRSA infections pose substantial clinical and economic burdens due to prolonged hospital stays, increased morbidity and mortality rates, and higher healthcare costs associated with the use of advanced antimicrobial agents [4]. In parallel, the emergence of vancomycin-resistant *S. aureus* (VRSA) has raised additional concerns regarding therapeutic options, as vancomycin has long been considered the antibiotic of last resort for severe MRSA infections. Vancomycin resistance in *S. aureus* is typically mediated by the acquisition of *vanA* gene clusters from vancomycin-resistant enterococci (VRE), resulting in altered peptidoglycan synthesis that reduces vancomycin binding and efficacy [5,6]. Although VRSA remains relatively rare compared to MRSA, documented cases have been reported in multiple countries, underscoring the potential for rapid dissemination in healthcare settings and the urgent need for robust surveillance systems. VRSA infections often arise in patients with chronic medical conditions, prior exposure to vancomycin, or prolonged hospitalization, highlighting the interplay between antimicrobial pressure and bacterial evolution [7]. Infection control measures, including hand hygiene, environmental cleaning, contact precautions, and screening of high-risk patients, have demonstrated variable efficacy in limiting the spread of resistant strains [8]. In this context, antimicrobial stewardship programs play a pivotal role in mitigating selective pressure by optimizing the use of antibiotics, reducing inappropriate prescriptions, and promoting adherence to evidence-based guidelines. Moreover, public health education and awareness campaigns targeting healthcare workers, patients, and the general population are essential to curtail the dissemination of resistant *S. aureus* strains and reduce the burden of infections [9]. This study aimed to determine the prevalence of methicillin- and vancomycin-resistant *S. aureus* (MRSA and VRSA) and to evaluate the antibiotic susceptibility patterns of these isolates obtained from various clinical specimens.

## 2. Patients and Methods

### 2.1 Study Design and Setting

This cross-sectional analytical study was conducted to evaluate the prevalence and antimicrobial resistance patterns of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) at Baqubah Teaching Hospital in Diyala Governorate, Iraq. The study was carried from March 2025 to April 2026 in Baqubah Teaching Hospital was selected as the study site due to its high patient turnover and diverse clinical presentations, and because it features a fully equipped microbiology laboratory staffed by trained personnel capable of performing standardized bacterial isolation and phenotypic characterization. A total of 385 samples collected from various clinical specimens, including urine, wound, vaginal, and ear swabs. Include only 110 *S. aureus*.

### 2.2 Study Population and Sampling Strategy

Non-probability consecutive sampling was employed, recruiting all patients who presented with suspected bacterial infections and met the selection criteria until the target sample size was achieved.

**Inclusion Criteria:** Patients of all age groups and both sexes with microbiologically confirmed *S. aureus* infections.

**Exclusion Criteria:** Patients who had received antibiotic therapy within 72 hours prior to sample collection, individuals with mixed bacterial infections, immunocompromised status with systemic infections, or those with incomplete medical and clinical records.

## 2.3 Ethical Considerations

The study protocol was reviewed and officially approved by the Research Ethics Committee of the College of Medicine, University of Diyala. Prior to specimen collection, informed written consent was obtained from all adult participants or from the legal guardians of minors. Participants were briefed on the study objectives, procedures, and their right to withdraw at any stage without compromising their medical care. Data confidentiality was strictly maintained by converting all personal data into anonymized alphanumeric codes. All procedures aligned with the ethical standards established in the Declaration of Helsinki.

## 2.4 Data Collection and Specimen Processing

Demographic information and clinical data were documented using a structured data collection sheet. Clinical specimens were gathered under strict aseptic conditions by trained healthcare professionals following standardized institutional protocols. All specimens were immediately placed in appropriate transport media and transferred to the microbiology laboratory to ensure bacterial viability and minimize pre-analytical errors.

## 2.5 Bacterial Isolation and Phenotypic Identification

Specimens were inoculated onto selective and differential media, including Mannitol Salt Agar and Blood Agar plates, and incubated aerobically at 37°C for 24–48 hours [10]. Golden-yellow colonies exhibiting  $\beta$ -hemolysis on blood agar and mannitol fermentation on salt agar were provisionally identified as *S. aureus* [11]. Microscopic confirmation was performed using Gram staining to observe Gram-positive cocci in clusters. Biochemical confirmation was achieved via positive catalase and coagulase (both slide and tube methods) tests. Verified isolates were preserved in nutrient broth supplemented with 15% glycerol and stored at –20°C for subsequent testing [12].

## 2.6 Antimicrobial Susceptibility Testing (AST)

In vitro phenotypic susceptibility testing was performed utilizing the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, strictly adhering to the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Bacterial inocula were adjusted to a 0.5 McFarland turbidity standard ( $1.5 \times 10^8$  CFU/mL) to ensure uniform lawn growth. The panel of antibiotic disks tested included: Benzylpenicillin, Oxacillin, Gentamicin, Tobramycin, Levofloxacin, Moxifloxacin, Erythromycin, Clindamycin, Teicoplanin, Vancomycin, Tetracycline, Tigecycline, Nitrofurantoin, Fusidic acid, Rifampicin, and Trimethoprim/Sulfamethoxazole. Following incubation at 37°C for 24 hours, zones of inhibition were measured in millimeters. Isolates were categorized as Susceptible (S), Intermediate (I), or Resistant (R) based on the specific CLSI interpretive breakpoints. Multidrug resistance (MDR) was defined as acquired resistance to at least one agent in three or more antimicrobial categories.

## 2.7 Quality Control

To guarantee the accuracy, reliability, and reproducibility of the laboratory procedures, *S. aureus* ATCC 25923 was utilized as a reference quality control strain for all phenotypic and susceptibility assays. All prepared culture media and chemical reagents underwent routine sterility and performance checks prior to use.

## 2.8 Statistical Analysis

Data management and statistical evaluations were performed using SPSS software (version 24.0, IBM Corp., Armonk, NY, USA). Categorical variables (frequencies and percentages of susceptibility profiles across different specimen sources) were summarized in descriptive tables. The Chi-square ( $\chi^2$ ) test (or Fisher's exact test where applicable) was utilized to determine the statistical significance of associations between antibiotic resistance patterns and clinical specimen sources. Continuous variables were expressed as mean  $\pm$  standard deviation. A *p*-value less than 0.05 was considered statistically significant for all analyses.

### 3. Results

#### 3.1. Antibiotic Susceptibility and Resistance Profiling

The antimicrobial susceptibility profiles of the *S. aureus* isolates (n=110) revealed high resistance rates toward several conventional antibiotics. The highest resistance rate was observed against Benzylpenicillin (95.45%, n=105), then Oxacillin (80.91%, n=89). Moderate resistance patterns were observed for Levofloxacin (51.82%), Gentamicin (51.82%), and Tobramycin (50.91%). Furthermore, resistance to Moxifloxacin and Erythromycin remained relatively high at 65.45% and 66.36%, respectively. Conversely, comparatively lower resistance rates—and thus better sensitivity profiles—were exhibited toward Nitrofurantoin (46.36%), Tigecycline (45.45%), Fusidic acid (45.45%), Clindamycin (44.55%), Vancomycin (43.64%), and Tetracycline (43.64%). Remarkably, Teicoplanin emerged as the most effective agent, demonstrating the lowest resistance rate (26.36%) and the highest susceptibility rate (73.64%). Trimethoprim/Sulfamethoxazole also maintained favorable clinical utility with a sensitivity rate of 62.73%. Statistical evaluation demonstrated a significant association between the specific antibiotic type and the resulting susceptibility patterns (Chi-square = 9.71, P=0.001), as showed in table (1).

**Table (1):** Antibiotic Susceptibility Profile of *S. aureus* Isolates

| Antibiotics                   | Resistance No. | %     | Sensitive No. | %     | P. value & Chi-square                  |
|-------------------------------|----------------|-------|---------------|-------|----------------------------------------|
| Benzylpenicillin              | 105            | 95.45 | 5             | 4.55  | P. value = 0.001*<br>Chi-square = 9.71 |
| Oxacillin                     | 89             | 80.91 | 21            | 19.09 |                                        |
| Gentamicin                    | 57             | 51.82 | 53            | 48.18 |                                        |
| Tobramycin                    | 56             | 50.91 | 54            | 49.09 |                                        |
| Levofloxacin                  | 57             | 51.82 | 53            | 48.18 |                                        |
| Moxifloxacin                  | 72             | 65.45 | 38            | 34.55 |                                        |
| Erythromycin                  | 73             | 66.36 | 37            | 33.64 |                                        |
| Clindamycin                   | 49             | 44.55 | 61            | 55.45 |                                        |
| Teicoplanin                   | 29             | 26.36 | 81            | 73.64 |                                        |
| Vancomycin                    | 48             | 43.64 | 62            | 56.36 |                                        |
| Tetracycline                  | 48             | 43.64 | 62            | 56.36 |                                        |
| Tigecycline                   | 50             | 45.45 | 60            | 54.55 |                                        |
| Nitrofurantoin                | 51             | 46.36 | 59            | 53.64 |                                        |
| Fusidic acid                  | 50             | 45.45 | 60            | 54.55 |                                        |
| Rifampicin                    | 55             | 50.00 | 50            | 45.45 |                                        |
| Trimethoprim/Sulfamethoxazole | 41             | 37.27 | 69            | 62.73 |                                        |

\*Statistically significant (p < 0.05).

#### 3.2. Distribution of Resistance Profiles Across Clinical Specimen Sources

The distribution of *S. aureus* isolates varied significantly based on the anatomical site of the clinical sample. Overall, urine samples were the primary source of *S. aureus* strains, yielding 53 isolates (48.2%), followed by wound swabs (38 isolates; 34.5%) and ear swabs (17 isolates; 15.5%), whereas vaginal swabs yielded only two isolates (1.8%). Statistical analysis revealed a significant association between the clinical sample source and the number of *S. aureus* positive isolates (P. value = < 0.0001), indicating that the clinical source of the isolate is a critical factor, as shown in Table (2).

**Table (2):** Distribution of *S. aureus* isolates according to clinical specimen source

| Specimen source | Positive for <i>S. aureus</i> NO. | (%)     | P. value |
|-----------------|-----------------------------------|---------|----------|
| Ear swab        | 17                                | (15.5%) |          |

|              |     |         |          |
|--------------|-----|---------|----------|
| Wounds       | 38  | (34.5%) | < 0.0001 |
| Vaginal swab | 2   | (1.8%)  |          |
| Urine        | 53  | (48.2%) |          |
| Total        | 110 | (100%)  |          |

\*Statistically significant ( $p < 0.05$ ).

#### 4. Discussion

The emergence of antibiotic-resistant *S. aureus* poses a significant challenge to public health worldwide. The increasing prevalence of methicillin-resistant and multidrug-resistant (MDR) strains complicates treatment strategies, particularly in hospital and community settings. Understanding the patterns of antimicrobial susceptibility and resistance is essential to guide rational antibiotic use and improve clinical outcomes. The analysis of antimicrobial susceptibility in this study revealed that  $\beta$ -lactam antibiotics, particularly Benzylpenicillin and Oxacillin, were largely ineffective against the isolates, indicating a high prevalence of methicillin-resistant *S. aureus* (MRSA) strains. These findings align with a previous study reporting high beta-lactam resistance among nasal *S. aureus* isolates [14], attributing this resistance to  $\beta$ -lactamase production or the presence of the *mecA* gene. In contrast, another study observed greater susceptibility to fluoroquinolones in isolates from medical students—a variation likely reflecting differences in prior antibiotic exposure and the traditional epidemiological distinction between community-acquired (CA-MRSA) and hospital-acquired (HA-MRSA) methicillin-resistant *S. aureus* strains [15]. This comparison emphasizes how population characteristics and local antibiotic prescribing patterns heavily influence contemporary resistance trends. Furthermore, the statistical evaluation of resistance patterns revealed significant variation among the tested antibiotics. This variation aligns with a 2021 study that documented significant differences in antimicrobial activity between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) strains, underscoring the need for susceptibility-guided therapy rather than empirical protocols [16].

These differences also reflect the complex biological mechanisms driving resistance—including genetic diversity, horizontal gene transfer, and divergent selective pressures. This aligns with molecular insights from previous studies demonstrating that community-associated and hospital-associated strains harbor distinct resistance determinants; this directly contributes to variations in treatment outcomes and confirms that the clinical source and strain type dictate susceptibility patterns [17].

Analysis based on sample source revealed a critical epidemiological pattern: urine samples accounted for the majority of resistant bacterial isolates, whereas ear and vaginal swabs showed the lowest levels of resistance (reflected in a smaller number of isolates) suggesting that the specific anatomical site, local microbial environment, and environment-specific antibiotic pressures accelerate the development of resistance [18]. In contrast, Babić et al. (2016) [19] indicated that beta-lactam resistance operates largely independently of sample type. This discrepancy highlights the complex interplay between systemic clonal expansion and local antibiotic selective pressure, underscoring the urgent need for source-specific surveillance to improve infection control and the rational use of antibiotics.

#### 5. Conclusion

This study demonstrates a widespread prevalence of antibiotic resistance among clinical *S. aureus* isolates in Baqubah. The observed resistance to beta-lactams—specifically benzylpenicillin and oxacillin—highlights a significant burden of methicillin-resistant *S. aureus* (MRSA) in the region. Furthermore, the resistance rates observed against fluoroquinolones and macrolides underscore the emergence of multidrug-resistant (MDR) strains. In contrast, vancomycin, teicoplanin, trimethoprim/sulfamethoxazole, and tetracycline showed high efficacy, making them more effective therapeutic alternatives. Notably, the distribution of isolates was significantly influenced by the clinical source; urine samples yielded the highest proportion of isolates, followed by wound and ear swabs, while vaginal swabs recorded the lowest. Collectively, these findings emphasize the critical need for rigorous antibiotic surveillance, rational use programs, and enhanced infection control protocols to curb the spread of resistant *S. aureus* strains in clinical settings.

## References

- van den Akker EL, Nouwen JL, Melles DC, van Rossum EF, Koper JW, Uitterlinden AG, Hofman A, Verbrugh HA, Pols HA, Lamberts SW, van Belkum A. *Staphylococcus aureus* nasal carriage is associated with glucocorticoid receptor gene polymorphisms. *J Infect Dis*. 2016;194:814–818.
- Krebes J, Al-Ghusein H, Feasey N, Breathnach A, Lindsay JA. Are nasal carriers of *Staphylococcus aureus* more likely to become colonized or infected with methicillin-resistant *Staphylococcus aureus* on admission to a hospital? *J Clin Microbiol*. 2021;49(1):430–432.
- Weidenmaier C, Kokai-Kun JF, Kulauzovic E, Kohler T, Thumm G, Stoll H, Götz F, Peschel A. Differential roles of sortase-anchored surface proteins and wall teichoic acid in *Staphylococcus aureus* nasal colonization. *Int J Med Microbiol*. 2018; 298:505–513.
- Stapleton MR, Horsburgh MJ, Hayhurst EJ, Wright L, Jonsson IM, Tarkowski A, Kokai-Kun JF, Mond JJ, Foster SJ. Characterization of IsaA and SceD, two putative lytic transglycosylases of *Staphylococcus aureus*. *J Bacteriol*. 2017;189:7316–7325.
- Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, Agata T, Mizunoe Y. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature*. 2020;465:346–349.
- Park B, Nizet V, Liu GY. Role of *Staphylococcus aureus* catalase in niche competition against *Streptococcus pneumoniae*. *J Bacteriol*. 2008;190:2275–2282.
- Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of *Staphylococcus aureus*? *Trends Microbiol*. 2021; 9:605–610.
- Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, Nouwen JL. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis*. 2025; 5:751–762.
- Nouwen JL, Ott A, VandenBergh MFQ, Boelens HAM, Hofman A, van Belkum A, Verbrugh HA. Predicting the *Staphylococcus aureus* nasal carrier state: derivation and validation of a —culture rule. *Clin Infect Dis*. 2024; 39:806–811.
- Chakraborty, S. P., KarMahapatra, S., Bal, M., & Roy, S. (2011). Isolation and identification of vancomycin resistant *Staphylococcus aureus* from post operative pus sample. *Al Ameen J Med Sci*, 4(2), 152-168.
- Abdullah, M. M., & Mahdi, W. M. (2025). Isolation and identification of bacteria from Respiratory tract and Phenotypic and molecular investigation of Hla gene bacteria in *Staphylococcus aureus* isolated. *Samarra Journal of Pure and Applied Science*, 7(3), 152-162.
- Hassooni, H. R., Al Ezee, A. S., Al-Azawi, E. F., & Alaa, M. O. A. (2022). Detection of virulence of *Staphylococcus aureus* isolated from wounds.
- James, S. L. I. I., Amy, J. M., April, M. B., Alexandra, L. B., Shelley, C., & Sharon, K. C. (2025). CLSI M100 performance standards for antimicrobial susceptibility testing. *Wayne Pa Clin Lab Stand Inst*.
- Onanuga A, Temedie TC. Nasal carriage of multi-drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. *Afr Health Sci*. 2021;11(2):176–81.
- Adesida SA, Abioye OA, Bamiro BS, Brai BIC, Smith SI, Amisu KO, et al. Associated risk factors and pulsed field gel electrophoresis of nasal isolates of *Staphylococcus aureus* from medical students in a tertiary hospital in Lagos, Nigeria. *Braz J Infect Dis*. 2017;11(1):63–9.
- Al-Geobory HA. Comparative study between Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin sensitive *Staphylococcus aureus* (MSSA), and detect the antimicrobial effects of some plant extracts on them. *MSc Thesis*. Baghdad University, College of Science; 2021.
- Fey PD, Said-Salim B, Hinrich SH, Boxrud DJ, Davis CC, Kreiwirth BN, Schlievert PM. Comparative molecular analysis of community or hospital-acquired Methicillin-Resistant *Staphylococcus aureus*. *J Antimicrob Chemother*. 2023; 78:196–203.
- Sonavane ADA, Mathur M. Screening for Vancomycin intermediate resistant *Staphylococcus aureus* among clinical isolates of MRSA. *Indian J Med Microbiol*. 2017; 25:79–80.
- Babic M, Hujer A, Bonomo RA. What's new in antibiotic resistance? Focus on beta-lactamases. *Drug Resist Updat*. 2016; 9:142–56.



## Assessment of Awareness toward Healthcare and Lifestyle Modifications among Patients with Coronary Artery Disease in Cardiac Care Units

AMER J. KADUSH

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

[amer.kadoosh@mu.edu.iq](mailto:amer.kadoosh@mu.edu.iq),

<https://orcid.org/0000-0003-4690-4446>

### Abstract

**Background:** Coronary artery disease is a high burden cardiovascular disease and patients need basic awareness on the relevant health behaviours. Adequate awareness in patients improves self-care, enables better use of health care services and encourages effective long-term management of diseases.

**Objective:** To assess the level of awareness regarding health and lifestyle modifications among patients suffering coronary artery disease (CAD) admitted in a Cardiac Care Unit, and to correlate the awareness level between patient demographics and clinical characteristics.

**Methods:** A descriptive cross-sectional study in Al Hussein Teaching Hospital, Al Samawa City, Al-Muthanna Governorate, Iraq from April 1-June 30 2026. A carefully curated sample of 50 adult patients with documented coronary artery disease was collected. Data were collected using a structured questionnaire developed by the investigator. The questionnaire included two parts; socio-demographic and clinical characteristics and a 10-questions awareness test on a domain of health care and lifestyle modifications. Content validity was assessed by a panel of 15 experts in the fields of research and clinical practice. The test-retest reliability was good ( $r = 0.83$ ). Data were analyzed with the SPSS version 26 using both descriptive and inferential statistical methods. A p-value less than 0.05 was considered statistically significant.

**Results:** The study results showed that there was a limited level of awareness in the top patients regarding healthcare and lifestyle changes in heart disease. Awareness was analysed in relation to several socio-demographic and clinical characteristics. The assessment identified the characteristics associated with patient awareness and highlighted areas for additional patient education and nursing support.

**Conclusion:** Firstly, evaluating the perception of healthcare and lifestyle changes among patients with coronary artery disease is vital for understanding education needs. Education within the cardiac setting, lifestyle advice, support for self-monitoring and self-care should be further developed as this improves patients understanding of their condition and promotes effective long-term management.

**Keywords:** Awareness; Coronary Artery Disease; Lifestyle Modifications.

### 1. Introduction

At its core, cardiovascular illness arises from arterial disease, where both adaptive and maladaptive remodelling of the arterial wall are central mechanisms upon which these diseases depend and therefore are targeted by therapies. It is an active structural-restructuring process entailing cellular proliferation, apoptosis and migration in/throughout or from the tissue as well as signs of degradation and remodeling of the extracellular matrix (ECM) components with minimal secreted soluble factors within the arterial wall. Normally, this dynamic mechanism is essential for maintaining blood pressure homeostasis; however, in pathological conditions, these adaptive modifications become maladaptive vascular remodelling (Lyle & Taylor 2019).

Age-related changes to the cardiovascular system, which is relatively characteristic for any physiological element. Changes such as decreased vascular awareness, left ventricular hypertrophy (LVH), and increased stiffness (increased

arterial awareness) contribute to a greater hemodynamic workload in elderly patients. Consequently, patients suffering from heart diseases which require cardiac procedures especially cardiac catheterisation are more prone toward anxiety. This indicates that effects may become exacerbated including further heart strain, impairment haemodynamic stability and functioning. Apparently, a nonpharmacological method such as hand massage must be used to relieve nonsurgical anxiety before the procedure (Capriotti and Frizzell 2016 in Hinkle, Cheever & Overbaugh 2022)

The circulatory system is indispensable in transporting oxygen and nutrients from blood to organs and tissues, as well as in extracting toxins and carbon dioxide (CO<sub>2</sub>) from peripheral tissues of healthy persons (Commisso et al., 2018). Nurses play the roles of educating patient, counseling, and monitoring adherence to medication. Ruswati 2024 noted that their treatments especially focused on adherence problems, such as the adverse effects of prescription or lifestyle influences, and aimed to better health literacy to empower patients (Ruswati 2024).

Patient nonawareness is frequently multifactorial and may involve personal factors including fear of side effects (actual or anticipated), perception that guidelines underplay the diet effect, real or perceived ineffectiveness of treatment, forgetfulness, complex dosing schedules or polypharmacy. In addition, factors such as physician inertia (defined as reluctance to start or increase therapy for patients with high blood pressure) and poor patient-physician communication may contribute to an increased number of unmet therapeutic goals (Hamrahian et al., 2022). Through 2019, cardiovascular diseases ranked first in the causes of deaths worldwide and were associated with an estimated 18.6 million deaths [1]. According to the report, cardiovascular disease accounted for more than 868,000 deaths in the United States during 2017. (Concistre, 2023).

Traditionally in developed countries, criminal heart disease (IHD), as well as other cardiovascular diseases (CVDs), has been the leading cause of death; indeed "heart disease in all its manifestations has headed the mortality tables of the u.s. Centers for Disease Control and Prevention since 1921. (Lemos and Omland, 2018). Ischaemic heart disease (IHD) now constitutes the leading cause of morbidity and mortality in many countries worldwide [1, 2]. Coronary artery disease, also known as ischaemic heart disease, is the leading type of heart disease that affects humans. Another name for this ailment as coronary heart disease (Morton and Thurman, 2023).

Coronary heart disease (CHD) is a major contributor to the world's mortality, killing more people annually than any other cause of death combined (World Health Organization (WHO), 2017). Secondary prevention, on the other hand, focuses on those with manifestations of cardiovascular disease and is expressed in advances to change risk factors due to dietary interventions (Saleh et al., 2021) and smoking cessation (Ralston et al., 2018). Those without an understanding of the relationship among diseases and risk factors may be inclined to refrain from acting in accordance with behavioural modification or disease treatment guidelines. On the other hand, those who understand this concept usually strive to change their lifestyle in order to prevent further cardiac events (Arnett et al., 2019).

Coronary artery disease is defined by the accumulation of exudate (mostly cholesterol) within the endothelial walls that nourish coronary vessels, causing vascular illness or loss. These substances build up and fills, then squeezes the vessels out of the arteries. Atherosclerosis (Del Buono et al., 2021) is the main cause of this ailment. Prompted by the goal to give consumers greater control over their health, the World Health Organization introduced its Global Action Plan for the Prevention and Control of Cardiovascular Diseases, among other noncommunicable diseases. Advocate through patient record data and care pathways; through health behaviour education, assistance and resources; build a patient management team (Mechanic et al., 2021).

Despite the well-known importance of lifestyle modification in coronary artery disease management and guideline-developed recommendations, there has been limited focus on the awareness of patients regarding these guidelines in cardiac care settings. Thus, the investigator conducted this study to examine the awareness of healthcare or lifestyle changes in patients with coronary artery disease admitted in cardiac care units and if that awareness is associated with certain socio-demographic and clinical characteristics.

## 2. Methodology

### 2.1 Research Framework

In Cardiac Care Units, patients with coronary artery disease were evaluated for awareness and the effectiveness of health care changes using a cross-sectional study design. The cross-sectional design was appropriate to assess patient awareness and its relationship with certain socio-demographic and clinical characteristics within a defined period of time.

## **2.2 Research Environment**

For this study, we conducted a research prototype in Al-Hussein Teaching Hospital -Al-Samawa City, Al-Muthanna Governorate, Iraq. Medical clinic that serves people with various medical problems, including cardiovascular disease, providing healthcare and nursing assistance. Cardiac Care Unit. The Cardiac Care Unit provides special cardiac monitoring, medical treatment with nursing support and patient education for individuals suffering from cardiovascular disease.

The specific location is ideal given that it will have direct access to patients who are receiving cardiac treatment and health advice for coronary artery disease.

## **2.3 Research Duration**

The conducted research is from the 2nd quarter of 2026, between Apr-1 and Jun-30, 2026.

## **2.4 Research Cohort**

The study cohort included adults with a diagnosis of coronary artery disease (CAD) following the treatment at Al-Hussein Teaching Hospital in Al-Muthanna Governorate, Iraq.

## **2.5 Sample Size and Sampling Method**

Sample of the study: The research cohort included 50 adult patients with confirmed coronary artery disease. Participants were recruited using a purposeful non-probability sampling approach by selecting individuals who met the defined eligibility criteria. Sample size calculator using Raosoft Sample Size Calculator Module based on expected population size, chosen confidence level and margin of error as well as response distribution.

## **2.6 Standards for Sample Selection**

### **Eligibility Requirements**

Individuals were incorporated if they:

- People aged 18 and over.
- Diagnosed with coronary artery disease.
- Patients were being treated in the Cardiac Care Unit.
- The patients were receiving some form of medical treatment and health state advice for coronary artery disease.
- Understood the survey and solved it exactly.
- Voluntarily agreed to take part in the research.

### **Criteria for Exclusion**

Exclusion Criteria

Participants were omitted if they:

- Data gathering process was not able to communicate the information properly
  - Cognitive weaknesses that made it difficult for them to understand or answer to the questionnaire.
  - They were clinically unstable and unable to participate at the time of data collection.
- Declined to participate in this research.

Surveys returned with large omissions making it impossible to calculate the awareness score.

## **2.7 Research Tool**

The study instrument was a systematic questionnaire designed by the researcher and supervised by the research supervisor, following a review of relevant literature on coronary artery disease, healthcare guidelines, disease management, lifestyle modifications, medication administration, follow-up treatment and self-care.

The survey included of two main sections.

### **Section 1: Socio-Demographic and Clinical Characteristics**

This aspect included some socio-demographic and clinical features, including age, gender, level of education, income per month and the period of coronary artery disease.

### **The second section: Awareness of professional care and life changes**

This part consisted of 10 awareness -based questions evaluating the patient's awareness about healthcare and life style changes that are relevant to coronary artery disease. The ten points together expressed one single, large area: awareness of Health and Lifestyle Changes. Topics among them were drug purchase, health surveillance, dietary change, exercise practice, no smoking request, controlling weight gain and its loss, lowering distress level/diminishing stress-related condition taking care of themselves; risk perception and detection of alarms requiring medical treatment.

### **Alternatives for Responses and Evaluation Framework**

- Each was answered with a simple Yes, No.
- For each correct answer the score was 1 and for incorrect answers and "Don't Know" gave a score of 0.

The total awareness score was between 0–10, with higher scores representing an increased level of awareness.

Awareness level was classified into: • Low: 0–3 • Moderate: 4–6 • High: 7–10

### **Validity of the Tool**

A panel of 15 academic and clinical experts in nursing, cardiovascular health and patient education and other health disciplines evaluated the content validity of the questionnaire. The scale items were rated by specialists in terms of applicability, clarity, consistency with study objectives, and comprehensiveness. As per the recommendations of experts, appropriate changes were made in selected items to increase their clarity, relevance and applicability.

### **Preliminary Investigation**

A pilot study of 10 subjects demonstrating characteristics similar to the research population had been performed. The pilot study was conducted prior to the actual data collection to test the clarity, comprehensibility, feasibility and estimated time for completing the questionnaire. The original volunteers were excluded from the final study population.

### **Reliability of the Tool**

The internal consistency and stability of the 10-item awareness questionnaire were assessed to check its reliability.

Test-retest reliability was analyzed by having the questionnaire administered to the same subjects on two different occasions, and the relationship between both assessments was evaluated using Pearson's correlation coefficient.

Validity was assessed against a comparable self-report measure and identified high concurrent validity ( $r = 0.87$ ). The 10-item awareness questionnaire was tested for reliability measured by Cronbach's alpha coefficient. Finally, the observed Cronbach's alpha must be calculated from the research data acquired and reported in the final publication.

### **Data Collection**

Data were collected from April 1 to June 30, 2026.

Patients were identified who met the inclusion and exclusion criteria. The investigator explained the purpose and procedures of the research to each participant.

The questionnaire was administered once signed informed consent was obtained. If they were able to complete the questionnaire alone, this was allowed. The questionnaire was conducted as structured interviews with standardized wording for participants who required assistance.

The survey took about 15 to 20 minutes to complete.

### **Analysis of Data**

The collected data were coded, entered and analysed using the Statistical Package for the Social Sciences (SPSS) version 26.

### **Statistical Analysis Techniques: Descriptive and Inferential**

This analysis is descriptive in nature; it contains the occurrence and proportion for categorical variables. For continuous variables and awareness metrics, mean + s.d. Frequency, coverage and mean for awareness metrics. Statistical analyses were performed as appropriate:

An independent samples t-test to compare the means of two different groups on some awareness rating.

Awareness Scores: One-way ANOVA to compare mean awareness scores across three or more independent groups

Use Pearson's correlation coefficient to investigate relation between awareness scores and continuous variables like age, duration of coronary artery disease.

Chi-square analysis to explore associations between category awareness levels and selected socio-demographic/clinical characteristics where applicable.

All p-values less than 0.05 were defined as significant.

### **Moral Implications**

The necessary official approvals were obtained prior to commencing data collection.

The Council of the College of Nursing at the University of Al-Muthanna granted consent, while internal ethical approval from the Al-Muthanna Directorate of Health was also given to conduct research in Al-Hussein Teaching Hospital.

The study protocol was sanctioned by the ethical committee of the University of Al-Muthanna.

The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

### **Limitation of the Research**

Limitations of this research were its method, singular institution, and relied on participant feedback. Such elements may limit the generalizability of the findings to patients with coronary artery disease in other clinical settings.

### 3. Results

The results of the data analysis are presented in tables organized directly according to the objectives of the study as follows:

**Table (1): Disposition of Study Subjects by Socio-Demographic Characteristics**

| Socio-Demographic characteristics |                 | Frequency  | Percentage |
|-----------------------------------|-----------------|------------|------------|
| Age                               | <= 29           | 6          | 12         |
|                                   | 30-53           | 12         | 24         |
|                                   | 54-76           | 21         | 42         |
|                                   | 77-90           | 11         | 22         |
|                                   | Mean= 65.84     | SD= 16.568 |            |
| Sex                               | Male            | 19         | 38         |
|                                   | Female          | 31         | 62         |
| Educational Level                 | Primary         | 30         | 60         |
|                                   | Secondary       | 11         | 22         |
|                                   | Tertiary        | 6          | 12         |
|                                   | College         | 3          | 6          |
| Monthly Income                    | Enough          | 12         | 24         |
|                                   | Somewhat enough | 18         | 36         |
|                                   | Not enough      | 20         | 40         |

According to the table (1), most of patients were aged between 54 years up to 76 years with average age was  $65.84 \pm 16.568$  years, which is the mean [42%]. The sample consisted of 62% females, with 60% having primary education, and an inadequate monthly income reported by 40%.

**Table (2): Distribution of Patients according to Awareness Questions**

| NO | Questions                                                                                                                                                       | Yes |    | NO |    | Mean | Assessment |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|----|----|----|------|------------|
|    |                                                                                                                                                                 | F   | %  | F  | %  |      |            |
| 1  | Did you know that you must stick to the recommended schedule for heart medications to prevent future cardiac arrests?                                           | 26  | 52 | 24 | 48 | 1.54 | Good       |
| 2  | Discontinuing cardiac medicine without the help of your cardiologist cold turkey could lead to major health problems, did you know?                             | 30  | 60 | 20 | 40 | 1.42 | Poor       |
| 3  | Did you have any idea that regular heart follow-up appointments are important, even when you feel completely healthy and asymptomatic?                          | 14  | 28 | 35 | 70 | 1.30 | Poor       |
| 4  | Did you know that before you're going to stop any medicine, it is a must to tell your healthcare staff particularly if having some new or unusual side effects? | 7   | 14 | 42 | 84 | 1.13 | Poor       |
| 5  | This can be monitoring your blood pressure, and monitoring pulse rate at home.                                                                                  | 4   | 8  | 45 | 90 | 1.08 | Poor       |
| 6  | Did know that reducing routine usage of sodium (salt) and harmful fats assists in lessening blood pressure and protecting coronary arteries from blocking.      | 27  | 54 | 22 | 44 | 1.53 | Good       |
| 7  | Are you aware that smoking and passive cigarette smoke also have an immediate influence on the narrowing and blockage of coronary arteries?                     | 5   | 10 | 45 | 90 | 1.02 | Poor       |
| 8  | Did you know moderate exercise as prescribed by your doctor strengthens the heart muscle?                                                                       | 31  | 62 | 18 | 36 | 1.63 | Good       |
| 9  | Do you know that habitual mental stress and anxiety may negatively impact both your heartbeat as well as blood pressure?                                        | 16  | 32 | 34 | 68 | 1.32 | Poor       |
| 10 | Did you know that maintaining a healthy weight reduces the overall pressure on your heart?                                                                      | 27  | 54 | 23 | 46 | 1.55 | Poor       |

F-frequency, % - percentage, Assessment: Good> 1.5, Poor less than or equal 1.5

According to table (2), it shows that patients' general awareness regarding health care and lifestyle changes was insufficient as most of the items under awareness categories were marked as poor.

**Table (3): Overall awareness levels regarding Health care and Life style modifications in Patients of CAD**

| Variables         | Classification | F  | %   |
|-------------------|----------------|----|-----|
| Overall awareness | Poor           | 42 | 88  |
|                   | Good           | 8  | 16  |
|                   | Total          | 50 | 100 |

As shown in Table (3), most of the patients (88%) had insufficient overall awareness: on the contrary, only 16% of them had good awareness regarding both healthcare and lifestyle changes.

**Table (4): Association between Patients Awareness and Socio-Demographic Variables**

| Socio-Demographical Characteristics | Significance                                                                |
|-------------------------------------|-----------------------------------------------------------------------------|
| Age                                 | Chi-square=2.213 <sup>a</sup><br>df=3<br>P. value=0.563<br>Non-significant  |
| Sex                                 | Chi-square= 0.021 <sup>a</sup><br>df=1<br>P. value=0.900<br>Non-significant |
| Educational level                   | Chi-square= 1.410 <sup>a</sup><br>df=3<br>P. value=0.842<br>Non-significant |
| Monthly Income                      | Chi-square=1.542 <sup>a</sup><br>df=2<br>P. value=0.394<br>Non-significant  |

HS: Highly Significant at P<0.01; S: Significant at P<0.05; NS: Non-Significant at P>0.05; significant.

Results show no significant association between awareness and age, gender, education level and monthly income (P > 0.05).

#### 4. Discussion of Results

##### Results Analysis of the Demographics Related Outcomes:

##### Patient's Age

A total of 42% were classified in the age range between 54–76 years, with an average age of  $65.84 \pm 16.568$  years.. As indicated by Tengiz Verulava and Giorgi Mikiashvili (2021), more than 50% of the patients were aged  $\geq 50$  years, but only 27% was younger than 39 years old.

This is attributed to the increased risk of coronary artery disease due to aging, an outcome related to a lifetime accumulation of cardiovascular risk factors and/or age-related vascular changes.

##### Sex

Results indicated females were a larger portion of the study at 62% compared to males=38%.

Sex analysis by Liza et al. (2023) observed that 67.5% of the participants were female, indicating that a large proportion of the study sample consisted of this sex.

This result is in conflict with the results of Samer Alwey et al 2018, who reported a female proportion of 40% and male proportion of our paper.

These could be linked to differences in etiopathogenesis and clinical expression of cardiovascular disease in men versus women, especially among the elderly.

### **Educational Attainment**

Results revealed simple training for many participants (60%), secondary schooling was retained by 22%. Kelli et al, support this conclusion. in patients with coronary artery disease, (2019) reported a relationship between lower educational achievement and poorer cardiovascular outcomes and increased rates of adverse events. It might be attributed to differences in human levels of health literacy and the ability to define, comprehend (eg. using medical terminologies) and perform recommended behaviour changes related to disease management.

### **Monthly Income**

Regarding monthly incomes, most patients (40%) rated their income as insufficient. This conclusion matches the findings of Ahmad Homoud et al. (2025). Around eighty-two point eight % earned above seven thousand SAR and a bulk assured from 81.4% on their responses. A reason behind this may be the financial stress associated with long-term treatment, medications, medical care and several health prices.

### **Analysis Finding Awareness levels related to the Healthcare and Lifestyle**

Health consciousness is an important factor in the effective management of coronary artery disease. The findings showed that the majority of patients had general ignorance most likely due to a lack of health education and inadequate understanding of appropriate recommendations for healthcare and lifestyle changes.

Tengiz and Giorgi (2021) Research result indicated that some areas in understanding have been improved. This finding contradicts the findings of Ahmad Homoud et al. (2025). The outcome presented moderate awareness of patients 66.5%.

The researchers argue that the lower awareness levels stem from how long patients have been sick and the medical mechanisms of the treatment, but they add that it is important to examine patients' mindsets as these may potentially affect adherence to the therapy.

### **Study of the association of general Patient Awareness with Demographic Variables**

It shows that the Analysis of association between awareness and socio-demography helps to find out factors associated with health consciousness among patients. Results showed that awareness was not significantly associated with the patients' socio-demographic characteristics ( $P > 0.05$ ). This may indicate that the improvement of patients' awareness was not solely due to demographic characteristics such as age and sex, but also by other factors including quality and consistency of health education.

The results contradict the conclusions of Badr and Ahmed (2014) regarding patient awareness and age,  $P = 0.020$ , however are consistent with respect to gender ( $P = 0.165$ ) and monthly income ( $P = 0.217$ ).

Kaur and Aggarwal (2022) backed this study and showed that age  $P$ . Value=16.92, sex  $P$ . value 7.82, educational status  $P$ . value 16.92 and occupations  $P$ . value=16.92 is equal to the awareness levels of coronary artery disease which was not statistically significant compared to socio-demographic variables but were found to be significant at 0.05 level of confidence.

The limited level of awareness may be due to inadequate health education, limited access to organized and continuous advice, and insufficient support for adherence to recommended management and lifestyle change in patients at high risk for coronary artery disease.

## 5. Conclusion

The results showed that people with coronary artery disease had an inadequate general awareness of health, especially on topics related to lifestyle change. Patients did not perform significantly better in expected domains; however, major gaps were present in many aspects of cardiac care and healthy living habits. No significant relationship was established between the overall awareness and socio-demographic characteristics of the study subjects. These findings highlight the need for continuous and structured health education to improve patient awareness and self-management of coronary artery disease.

## 6. Recommendations

- Implement structured and continuous health education programmes targeting coronary artery disease patients on aspects of medications adherence, heart surveillance, as well as lifestyle modifications.
- Improve the role of nurses in teaching clients through appropriate diet, activities such as exercise, quitting smoking, reducing stress and home monitoring of BP/pulse rate.
- Provide customized and simple educational materials grounded on patients' information needs for their understanding of coronary artery disease and self-management.

## References

- Hinkle, J.L., Cheever, K.H. & Overbug, K. (2022). Brunner & Suddarths Text of Medical Surgical Nursing. China. Wolters kluwer. (15 edition). Pp (2392-2380). Retrieved from: <https://www.tandfonline.com/doi/full/10.2147/PPA.S368784#d1e282>
- Comisso, I., Lucchini, A., Bambi, S., Giusti, G. D., & Manici, M. (2018). Nursing in critical care setting: an overview from basic to sensitive outcomes. Retrieved from: [https://books.google.iq/books?hl=ar&lr=&id=y\\_VeDwAAQBAJ&oi=fnd&pg](https://books.google.iq/books?hl=ar&lr=&id=y_VeDwAAQBAJ&oi=fnd&pg)
- Lyle, A. N., & Taylor, W. R. (2019). The pathophysiological basis of vascular disease. Laboratory Investigation, 99(3), 284-289. Retrieved from: <https://www.nature.com/articles/s41374-019-0192-2>
- Ruswati, R. (2024). The Role of Nurses in Enhancing Medication Adherence and Patient Outcomes in Hypertension Management. *International Journal of Nursing and Midwifery Research*, 2(3), 78-87. Retrieved from: <https://journals.iarn.or.id/index.php/ners/article/view/286>
- Hamrahian, S. M., Maarouf, O. H., & Fülöp, T. (2022). A critical review of medication adherence in hypertension: barriers and facilitators clinicians should consider. *Patient preference and adherence*, 2749-2757.
- Concistrè, G. (Ed.). (2023). *Ischemic heart disease*. From Diagnosis to Treatment. Springer Nature. Retrieved from: <https://link.springer.com/book/10.1007/978-3-031-25879-4>.
- Michaud, K. Basso, C. d'Amati, G. et al. (2020). *Diagnosis of Myo-cardial Infarction at Autopsy: AECVP Reappraisal in the Light of the Current Clinicalclassification*. Virchows Archiv 476 (2) 179–194. Retrieved from: [https://www.researchgate.net/publication/335824777\\_Diagnosis\\_of\\_myocardial\\_infarction\\_at\\_autopsy\\_AECVP\\_reappraisal\\_in\\_the\\_light\\_of\\_the\\_current\\_clinical\\_classification](https://www.researchgate.net/publication/335824777_Diagnosis_of_myocardial_infarction_at_autopsy_AECVP_reappraisal_in_the_light_of_the_current_clinical_classification).
- De Lemos, J., A. & Omland, T. (2018). *Chronic coronary artery disease: A companion to braunwald's heart disease*. Elsevier Inc. Retrieved from: <https://www.sciencedirect.com/book/9780323428804/chronic-Coronary-Artery-Disease>.
- Michaud, K. Basso, C. d'Amati, G. et al. (2020). *Diagnosis of Myo-cardial Infarction at Autopsy: AECVP Reappraisal in the Light of the Current Clinicalclassification*. Virchows Archiv 476 (2) 179–194. Retrieved from: [https://www.researchgate.net/publication/335824777\\_Diagnosis\\_of\\_myocardial\\_infarction\\_at\\_autopsy\\_AECVP\\_reappraisal\\_in\\_the\\_light\\_of\\_the\\_current\\_clinical\\_classification](https://www.researchgate.net/publication/335824777_Diagnosis_of_myocardial_infarction_at_autopsy_AECVP_reappraisal_in_the_light_of_the_current_clinical_classification).
- Morton, P., G. & Thurman, P. (2018). *Critical care nursing: A holistic Approach*. (11<sup>th</sup> Edition). Lippincott Williams & Wilkins. Retrieved from: <https://www.amazon.com/Critical-Care-Nursing-Holistic-Approach/dp/1496315626>.

- World Health Organization. (2017). *Global Action Plan for the Prevention and Control of non Communicable Diseases 2017-2020.Summary*. World Health Organization . Retrieved from: <https://iris.who.int/bitstream/handle/10665/259232/WHO-NMH-NVI-17.9-eng.pdf>.
- Ralston, S., H. Penman, I., D. Strachan, M., W. & Hobson, R. (Eds.). (2018). *Davidson's principles and practice of medicine*. (23<sup>rd</sup> Edition). Elsevier Health Sciences. <https://www.amazon.com/Davidsons-Principles-Practice-Medicine-Ralston/dp/0702070289> .
- Mozaffarian, D. Benjamin, E. Arnett, D. Blaha, M. Cushman, M. et al. (2016). *Heart Disease and Stroke Statistics–2016 Update*. *Circulation*, 135, e146-603. <https://pubmed.ncbi.nlm.nih.gov/26673558/> .
- Del Buono, M., G. Montone, R., A. Camilli, M. Carbone, S. Narula, J. Lavie, C., J. & Crea, F. (2021). *Coronary Microvascular Dysfunction Across the Spectrum of Cardiovascular Diseases: JACC State-of-the-Art review*. *Journal of the American College of Cardiology*, 78(13), 1352-1371. <https://www.jacc.org/doi/10.1016/j.jacc.2021.07.042>.
- Mechanic, O. J. Gavin, M. Grossman, S., A. & Ziegler, K. (2021). *Acute Myocardial Infarction (Nursing)*. In StatPearls [Internet]. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK568759>
- Verulava, T., & Mikiashvili, G. (2021). Knowledge, awareness, attitude and medication compliance in patients with hypertension. *arterial hypertension*, 25(3), 119-126. <https://doi.org/10.5603/AH.a2021.0021>
- Sana, N. E., & Kurniawati, J. (2024). Factors That Influence The Level Of Compliance Take Medication For Hypertension Patients In The Village Tosaren, Pesantren District, Kediri City. *Strada Journal of Pharmacy*, 6(1), 46-54. <https://pdfs.semanticscholar.org/aab1/1e9ebabec0f1d1ae2817e353357926676bbf.pdf>
- Kelli, H. M., et al. (2019). Educational attainment and coronary artery disease outcomes. *Journal of the American Heart Association*, 8(17), e013165. <https://doi.org/10.1161/JAHA.119.013165>
- Ahmad Homoud et al., (2025), Evaluation of hypertension knowledge and its association with medication adherence among hypertensive patients attending primary health centers: a cross-sectional study from eastern Saudi Arabia, Volume 12 <https://doi.org/10.3389/fpubh.2024.1378561>
- Verulava, T., & Mikiashvili, G. (2021). Knowledge, awareness, attitude and medication compliance in patients with hypertension. *arterial hypertension*, 25(3), 119-126. <https://doi.org/10.5603/AH.a2021.0021>
- Ahmad Homoud et al., (2025), Evaluation of hypertension knowledge and its association with medication adherence among hypertensive patients attending primary health centers: a cross-sectional study from eastern Saudi Arabia, Volume 12 <https://doi.org/10.3389/fpubh.2024.1378561>
- Ahmed, B. I., Abd EL-Razi, B. G., & Mohammed, M. A. E. A. (2014). Factors Affecting Compliance of Patients with Essential Hypertension toward Therapeutic Regimen. *Zagazig Nursing Journal*, 10(1), 66-85. [https://znj.journals.ekb.eg/article\\_39185\\_45e5d396649b52b6cbbddd130ff50172.pdf](https://znj.journals.ekb.eg/article_39185_45e5d396649b52b6cbbddd130ff50172.pdf)
- Brar, J. K., Aggarwal, S., & Kaur, G. (2022). A Descriptive Study to Assess the Level of Knowledge Regarding Risk Factors and It's Prevention of Coronary Artery Disease Among Patients Attending Medicine Outpatient Department in Dasmesh Hospital, Faridkot, Punjab. *Asian Journal of Nursing Education and Research*, 12(1), 42-46. <https://www.researchgate.net/profile/Jasinder-Kaur-2/publication/359181491>



## Association between DNA Damage and Glycemic Control in Iraqi Patients with Type 2 Diabetes Mellitus

SAJA SALEM ABDUL HASSAN RASAN  
The Islamic University, Al-Diwaniyah, Iraq  
Email: [sajasalem888888@gmail.com](mailto:sajasalem888888@gmail.com).

### Abstract.

**Background:** Type 2 diabetes (T2DM) is defined as the chronic disorder associated with metabolic issues, having hyperglycemia and insulin resistance as the main drivers. It appears that oxidative stress and damage to DNA may contribute significantly to the emergence of diabetes and its complications. The present study aims to analyze the interrelation between the markers of DNA damage and glycemic control in Iraqi individuals suffering from T2DM.

**Objectives:** The aim of the research is to reveal the existence of any interrelationship between DNA damage (8-hydroxy-2'-deoxyguanosine [8-OHdG] and parameters obtained from comet assay) and glycemic control (HbA1c levels) among the individuals suffering from T2DM in Iraq.

**Methods:** This research is based on the data collected about 180 diabetes patients and 60 healthy individuals. The measurements of fasting blood glucose (FBG), HbA1c, 8-OHdG, and DNA damage were taken. The assessment of DNA damage was performed using comet assay and statistical procedure.

**Results:** Diabetes patients demonstrated a higher level of 8-OHdG in comparison with healthy individuals ( $p < 0.001$ ) and show an increase in DNA damage. DNA damage has exhibited a positive correlation with HbA1c ( $r = 0.742$ ,  $p < 0.001$ ) and FBG ( $r = 0.698$ ,  $p < 0.001$ ). Multiple regression showed that an independent predictor of DNA damage was HbA1c ( $\beta = 0.521$ ,  $p < 0.001$ ).

**Conclusions:** The findings indicate that there exists a close association between glycemic control and DNA damage in patients in Iraq. Low glycemic control influences the increase in oxidative and DNA damage stressing the necessity of intensive glycemic control in diabetes treatment.

**Keywords:** Type 2 diabetes mellitus, DNA damage, glycemic control, 8-OHdG, comet assay, oxidative stress, HbA1c, Iraq.

### 1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the biggest public health crises of our time, with around 537 million adults suffering from it worldwide in 2021, and this number may reach 783 million by 2045 [1]. The rates of T2DM in the Middle East and North Africa, including Iraq, show significant growth over the last 20 years due to rapid urbanization, changes in lifestyle, decreased activity levels, and increase in high-caloric food consumption [2]. Iraq is undergoing a diabetes crisis, with current estimates showing about 4 million to 5 million people suffering from T2DM, although the exact figures are unclear due to a lack of proper surveillance systems [3]. T2DM is a metabolic disease marked by chronic elevation of blood sugar, which may be caused by a problem with insulin secretion, insulin action, or both, giving rise to serious vascular and non-vascular complications [4].

The pathophysiology of T2DM involves an intricate combination of factors such as insulin resistance, pancreatic beta-cell dysfunction, diseases of liver metabolism of glucose, and chronic low-grade inflammation [5]. The researched traditional risk factors include obesity, lack of physical activity, and genetics, yet more studies indicate the role of oxidative stress in T2DM and its complications [6]. Oxidative stress is a state in which there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the body. In terms of diabetes,

chronic hyperglycemia results in too much production of ROS through various mechanisms such as auto-oxidation of glucose, increased mitochondrial respiration, and activation of NADPH oxidase [8]. Excessive accumulation of ROS in diabetic patients can certainly lead to damage of cellular macromolecules such as proteins, lipids, and DNA, and hence more development of diabetic complications [9].

DNA damage is one of the important consequences of oxidative stress, since damaged DNA is sensitive to oxidative modification due to the effect of ROS on DNA [10].

About 70% of spontaneous DNA lesions is caused by oxidative damage whereby the most common and stable oxidatively damaged DNA lesion at the same time is 8-hydroxy-2'-deoxyguanosine (8-OHdG) [11]. The damage occurs when ROS attack guanine DNA base. This results in mutagenic lesion which must be repaired in order to avoid GC to AT transversions [12]. High levels of urinary and serum 8-OHdG are recorded in multiple conditions and can serve as a marker of oxidative stress and DNA damage [13]. Several works show elevated amounts of 8-OHdG in diabetic patients in relation to healthy controls and this increase is related to uncontrolled glycemia and diabetes complications [14, 15]. This method involves measuring and besides 8-OHdG, single-cell gel electrophoresis, or the comet test may be considered a good method of assessment of DNA damage in cells [16]. The comet assay is a technique that allows the quantitative measurement of DNA damage and repair capability in individual cells, giving a better representation of genotype [17]. There has been an increased application of this technique in research to study metabolic disorders impacting genome stability [18]. The link between glycaemic control and damage to DNA has been previously shown in various studies and populations [19, 20]. Many studies have shown that poor glycaemic control, defined by high levels of hemoglobin A1c (HbA1c), contributes to an increase in DNA damages and oxidative stress [21]. HbA1c is considered a 'gold standard' method of assessing long term glycaemic control with its proven correlation to the degree of vascular damage and complications due to diabetes [22].

The literature investigating the association between glycaemic control and DNA damage in Middle Eastern countries, in particular Iraq, is lacking. Iraq is dealing with many specific issues regarding treatment of diabetes, such as low availability of medical facilities, inadequate glycaemic monitoring centers, high incidence of complications due to diabetes, and lack of educational programs aimed at patients [23]. The treatment of diabetes is aggravated by the consequences of prolonged military conflicts reducing access to healthcare services and creating difficulties in the establishment of high-quality medical service [24]. Understanding the mechanisms linking poor glycaemic control to different destructive processes, such as DNA damage, is essential for selection correct strategies leading to positive results in diabetes treatment in Iraq.

There is extensive evidence that chronic hyperglycemia leads to development of diabetic complications; however, the exact mechanisms are yet to be determined. Recently, it was found that the connection between hyperglycemia and occurrence of diabetes complications is linked to the inducing of DNA damage due to oxidative stress [25].

Our study aimed at finding the relationship between DNA markers and glycemic control in Iraqi patients. Our primary goal was to define the link between the DNA damage markers (8-OHdG and comet assay parameters) and glycemic control (HbA1c and FBG) in patients suffering from T2DM in Iraq. The second goal was to find out the relationship between DNA damage and duration of diabetes, and the third goal was to study the impact of different types of glycemic control on DNA damage. We assumed that poor glycemic control will lead to higher levels of DNA damage.

## **2. Methodology**

### **2.1 Study Design and Setting**

This was a cross-sectional comparative study conducted at Aldiwania Teaching Hospital. The study was conducted over a 12-month period from January 2023 to December 2023.

### **2.2 Study Population**

The study population comprised of two groups: (1) patients with T2DM (n=180) and (2) age-matched healthy controls who were free of diabetes (n=60). T2DM patients were recruited from both the outpatient diabetes clinic and inpatient

services at Aldiwania Teaching Hospital. Inclusion criteria for T2DM patients included: being aged 30 years or above, being diagnosed as having T2DM as per the criteria of American Diabetes Association (i.e. FBG 126 mg/dL or more, 2-hour glucose equal to or greater than 200 mg/dL in an oral glucose tolerance test, or HbA1c of  $\geq 6.5\%$ ), illness duration of over one year, and ability to give informed consent. Exclusion criteria comprised: type 1 diabetes, secondary diabetes, acute diabetic complications (diabetic ketoacidosis/hyperosmolar hyperglycemic state), acute infection or sepsis, recent surgery (within prior 3 months), malignant illness, chronic kidney disease stage 4-5, liver cirrhosis, autoimmune disorders and being on immunosuppressive drugs. Healthy controls were recruited from hospital staff and relatives of such patients having no history of diabetes in themselves and their families and having no chronic illness, with FBG  $< 100$  mg/dL and HbA1c  $< 5.7\%$ .

### 2.3 Data Collection

A standardized questionnaire was used by trained researchers to gather demographic and clinical information. Collected information consisted of age, sex, body mass index (BMI), duration of diabetes, medications taken, smoking status, and complications due to diabetes (i.e. retinopathy, nephropathy, neuropathy). Medical checks were carried out to measure blood pressure, height, and weight. Blood was acquired (10 mL) after an overnight fast (8-12 hours) from all participants. Collected blood was split into three parts: 1) 2mL in EDTA tube to be processed immediately for the comet assay analysis, 2) 3mL in serum separator tube for biochemical analyses.

### 2.4 Biochemical Measurements

The hexokinase method was used to test FBG levels through an automated analyzer (Roche Cobas c702 Chemistry Analyzer) while determining HbA1c through HPLC (high-performance liquid chromatography), using a Bio-Rad Variant II analyzer with coefficient of variation matches of less than 2%. Serum creatinine, blood urea nitrogen (BUN), liver function tests, and biochemical analysis were done with standard enzymatic automated analyzers. The 8-OHdG levels in serum were tested through the ELISA (enzyme-linked immunosorbent assay) method. The manufacturer's instructions by Cayman Chemical Company, Ann Arbor, MI, USA specifies a minimum detection limit of 0.15 ng/mL for 8-OHdG construction and the coefficient of variation is 4.6% for the intra-assay and 6.8% for the inter-assay respectively.

### 2.5 Comet Assay (Single-Cell Gel Electrophoresis)

DNA damage was evaluated using the alkaline comet assay techniques as previously described [26]. In brief lymphocytes were prepared from blood using Ficoll-Paque density gradient centrifugation. The cells obtained ( $1 \times 10^5$  cells/mL) were then embedded in the 0.5% low melting-point agarose in microscope slides previously prepared with 1% normal melting-point agarose. On completion of cell lysis in the high salt buffer (pH 10) for 1 hour at 4°C and cell unwinding in alkaline buffer (pH  $> 13$ ) for 20 minutes, results of electrophoresis were recorded at 25 V and 300 mA for 25 minutes. After neutralization and ethanol precipitation slides were stained with ethidium bromide. Fifty cells from each slide (totaling 100 cells from 2 slides) were evaluated using the fluorescence microscopy with the CometScore software set to 400 $\times$  magnification. DNA damages were assessed using the following measurements: (1) tail length (in micrometers) – the distance from the middle of comet blob till the end of comet tail, (2) tail intensity – the content of total DNA in the tail, (3) Olive moment – the overall index simultaneously considering tail length and tail intensity. All measurements of comet assay were done by one person that was blinded in participant's group assignment.

### 2.6 Glycemic Control Categories

Based on the levels of HbA1c, participants were divided into three groups: good glycemic control (HbA1c  $< 7\%$ ), moderate glycemic control (HbA1c 7-8.9%), and poor glycemic control (HbA1c  $\geq 9\%$ ). The groups were determined according to American Diabetes Association recommendations for personalized glycemic targets in diabetes management.

## 2.7 Statistical Analysis

SPSS version 26.0 (IBM Corporation, Chicago, IL) was used to execute all statistical evaluations. All of the parameters were evaluated descriptively, thus we present continuous variables in terms of means  $\pm$  standard deviations (SDs) and the categorical ones in terms of frequencies and percentages. The normality of the data distribution was evaluated by means of Shapiro-Wilk test. Diabetic patients were compared to control using independent samples t test for continuous normally distributed and Mann-Whitney U test for continuous non-normally distributed variables. Chi-square test was applied to compare categorical data. The correlations of DNA damage variables and markers of glycemic control were evaluated using Pearson's and Spearman's coefficients. Multiple linear regression analysis was utilized for evaluation of DNA damage variables (tail length and olive moment) with respect to HbA1c value, FBG value, age, BMI and diabetes duration employing them as predictors. Multiple analysis of variance (MANOVA) has been performed for comparison of DNA damage values.

## 3. Results

### 3.1 Demographic and Clinical Characteristics

The T2DM and control groups were comparable in terms of age, gender, and smoking status since there was no statistically significant difference noted ( $p > 0.05$ ). On the other hand, the groups were different from each other with respect to different clinical parameters. The T2DM subjects had the mean value of BMI to be much higher than the controls (28.9 vs. 23.4 kg/m<sup>2</sup>). Moreover, both systolic and diastolic blood pressures were found to be higher in the T2DM group compared to the controls (SBP: 135.4 vs. 118.6 mmHg; DBP: 82.3 vs. 74.2 mmHg), the differences were shown to be highly statistically significant ( $p < 0.001$ ).

**Table 1:** Demographic and Clinical Characteristics of Study Participants. Data presented as mean  $\pm$  SD for continuous variables and frequencies (%) for categorical variables. SBP, systolic blood pressure; DBP, diastolic blood pressure. \* $P < 0.001$  indicates statistical significance.

| Characteristic            | T2DM Patients (n=180) | Controls (n=60)     | P-value |
|---------------------------|-----------------------|---------------------|---------|
| Age (years)               | 52.3 $\pm$ 9.8        | 51.8 $\pm$ 10.2     | 0.671   |
| Sex (M/F), n (%)          | 96/84 (53.3%/46.7%)   | 32/28 (53.3%/46.7%) | 1.000   |
| BMI (kg/m <sup>2</sup> )  | 28.9 $\pm$ 4.6        | 23.4 $\pm$ 2.8      | <0.001* |
| Diabetes duration (years) | 8.2 $\pm$ 6.4         | —                   | —       |
| Smoking, n (%)            | 54 (30.0%)            | 12 (20.0%)          | 0.112   |
| SBP (mmHg)                | 135.4 $\pm$ 15.2      | 118.6 $\pm$ 8.4     | <0.001* |
| DBP (mmHg)                | 82.3 $\pm$ 10.6       | 74.2 $\pm$ 7.3      | <0.001* |

The values of all considered biochemical indexes in patients with T2DM were notably higher than in the rest of the subjects of the research. Poor glycemic control seen in T2DM patients was demonstrated by rather elevated fasting blood glucose (172.6 vs. 89.4 mg/dL) as well as HbA1c levels (8.4% vs. 5.2%) in comparison with healthy individuals. In addition, T2DM patients presented worse lipid profile having much higher levels of total cholesterol as well as LDL-cholesterol. Most importantly, the urinary marker of oxidative DNA injury 8-hydroxy-2-deoxyguanosine (8-OHdG) was recorded to be four times higher in T2DM patients (4.8 ng/mL) compared to its concentration in healthy subjects (1.2 ng/mL), thus proving the much higher level of systemic oxidative stress in diabetic patients ( $p < 0.001$  for all).

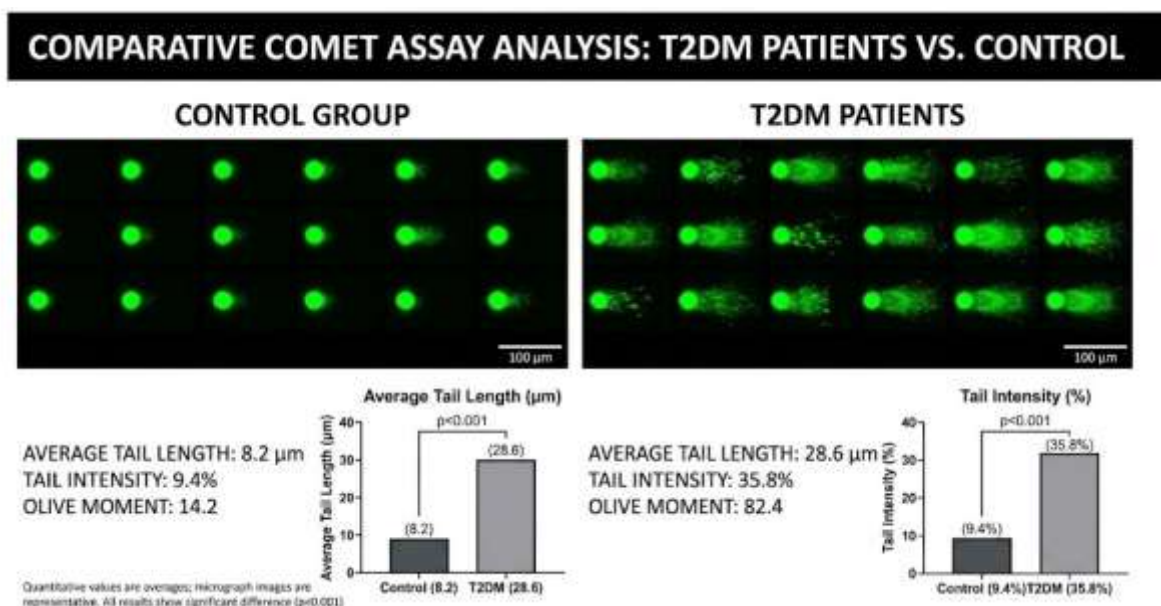
**Table 2:** Biochemical Parameters and 8-OHdG Levels in Study Groups. FBG, fasting blood glucose; 8-OHdG, 8-hydroxy-2'-deoxyguanosine. \* $P < 0.001$  indicates statistical significance.

| Biochemical Parameter     | T2DM Patients (n=180) | Controls (n=60)  | P-value |
|---------------------------|-----------------------|------------------|---------|
| FBG (mg/dL)               | 172.6 $\pm$ 54.2      | 89.4 $\pm$ 8.6   | <0.001* |
| HbA1c (%)                 | 8.4 $\pm$ 1.8         | 5.2 $\pm$ 0.4    | <0.001* |
| Total Cholesterol (mg/dL) | 218.4 $\pm$ 48.6      | 182.3 $\pm$ 32.4 | <0.001* |
| LDL-C (mg/dL)             | 142.8 $\pm$ 38.6      | 108.4 $\pm$ 28.3 | <0.001* |
| 8-OHdG (ng/mL)            | 4.8 $\pm$ 2.1         | 1.2 $\pm$ 0.6    | <0.001* |

Compared to those in the control group, diabetes patients showed generally higher values for comet assay parameters, which directly indicate the presence of DNA breaks. The average tail length was noticed to be 28.6  $\mu\text{m}$  among the diabetes patients, as opposed to the control group's value of 8.2  $\mu\text{m}$ . The tail intensity was three times as high as that recorded in the control group (35.8% vs. 9.4%). Moreover, the Olive moment measure of the T2DM group (82.4) is significantly higher than that of control (14.2) as well.

**Table 3:** Comet Assay Parameters Measuring DNA Damage. Olive moment is a composite measure combining tail length and tail intensity. All differences between groups are statistically significant at  $P < 0.001$ .

| Comet Assay Parameter         | T2DM Patients (n=180) | Controls (n=60) | P-value |
|-------------------------------|-----------------------|-----------------|---------|
| Tail Length ( $\mu\text{m}$ ) | 28.6 $\pm$ 12.4       | 8.2 $\pm$ 3.6   | <0.001* |
| Tail Intensity (%)            | 35.8 $\pm$ 14.2       | 9.4 $\pm$ 4.8   | <0.001* |
| Olive Moment                  | 82.4 $\pm$ 38.6       | 14.2 $\pm$ 7.8  | <0.001* |



**Figure 1:** Comparative comet assay analysis

### 3.2 Association between DNA Damage and Glycemic Control

The examination of Pearson correlation tests showed considerable positive relations between DNA damage indicators and glycemic markers. Tail length had direct relation with HbA1c ( $r = 0.742$ ,  $p < 0.001$ ) and FBG ( $r = 0.698$ ,  $p < 0.001$ ) values. Furthermore, olive moment positively correlated with the values of HbA1c ( $r = 0.718$ ,  $p < 0.001$ ) and FBG ( $r = 0.672$ ,  $p < 0.001$ ). Serum 8-OHdG levels also positively correlated with HbA1c ( $r = 0.704$ ,  $p < 0.001$ ) and FBG ( $r = 0.641$ ,  $p < 0.001$ ) respectively. It was revealed that HbA1c was independently significant for predicting tail length from multiple linear regression analysis ( $\beta = 0.521$ ,  $p < 0.001$ , 95% CI: 2.18-3.64) and olive moment ( $\beta = 0.489$ ,  $p < 0.001$ , 95% CI: 8.96-14.32). There were significant results of differences between DNA damage measurements categories for different three groups when splitting the participants by glycemic control categories ( $p < 0.001$ ). Patients with poor glycemic profile (HbA1c  $\geq 9\%$ ) showed 3.2 times increased tail length ( $34.8 \pm 13.2 \mu\text{m}$ ) in comparison to individuals with good glycemic profile ( $10.8 \pm 5.4 \mu\text{m}$ ).

## 4. Discussion

Research indicates a notable link between measurements of glycemic control and DNA damage among Iraqi patients suffering from T2DM. Results indicate that T2DM patients exhibit a remarkable increase in markers of DNA damage than normal controls and there is a strong and independent relationship between DNA damage and measurements of glycemic control. These results fit into the previously existing research and add fresh evidence to the research done on the same subject in different populations at the same time providing new data concerning the Iraqi population.

Thus, the increase in levels of 8-OHdG in diabetic individuals ( $4.8 \pm 2.1$  ng/mL) compared to controls ( $1.2 \pm 0.6$  ng/mL) which is 4 times higher is supported by the data from many studies that show that prolonged hyperglycemia leads to oxidative stress and DNA damage [27].

Moreover, the explanation of the observed process can be found in many mechanisms such as (1) glucose auto-oxidation which results in increasing the amount of superoxide anions, (2) mitochondrial dysfunction followed by enhanced activity of the respiratory chain and increased production of ROS, (3) activation of NADPH oxidase due to availability of high glucose levels, and (4) disruption of antioxidant system including superoxide dismutase, catalase, and glutathione peroxidase [28]. Thus, the fact that HbA1c is positively correlated with various DNA damage indicators ( $r = 0.742$  for tail length,  $r = 0.718$  for olive moment,  $r = 0.704$  for 8-OHdG) is a great insight that adds to the body of knowledge of the molecular effects of poor glycemic control. The fact that HbA1c still remained an independent predictor of DNA damage, even after adjusting for other confounding variables, indicates that glycemic regulation is one of the important changes in the modification of genomic integrity among diabetic patients. Results obtained from the comet assay revealed an increase in patients' tail length and olive moment, thus indicating higher odds for DNA double-stranded breaks in diabetic patients. Comet assay is superior in terms of sensitivity to 8-OHdG assay in terms of early and transient DNA damage, as it reveals the fact of DNA destruction at the cellular level [29]. The increase in patients' tail length determined by the assay is about 3.5 times the same indicator among healthy controls; thus, it may indicate a high possibility for the occurrence of genomic instability leading to cellular malfunction and complication processes in diabetes. The findings from the stratification analysis demonstrating the difference in the tail length among patients with poor glycemic regulation in diabetes compared to those with good glycemic regulation pose serious clinical significance of this observation. It can be concluded that managing glycemics by means of the strategies aimed at reaching less than HbA1c (%) may significantly decrease the incidence of oxidative stress responsible for DNA damage. These findings are particularly important for the context of Iraq since lots of diabetic patients are presented with poor glycemic control due to limited access to medical facilities and medications, low patient education, and weak monitoring facilities [30].

It should be highlighted that improving glycemic status among diabetic patients may also reduce DNA damage theoretically leading to lesser risks of complications from diabetes in practice. The fact that oxidative stress and DNA damage could act as the mechanism connecting hyperglycemia with diabetes complications is supported by scientific evidence. Oxidative DNA damage is widely known to play a key role in the development of diabetic nephropathy, retinopathy, and cardiovascular disease [31]. Failure to repair or incorrectly repairing DNA damage can result in mutations, instability of chromosomes, and changes in gene expression which could play a role in the progressive nature of diabetes complications [32]. In addition, persistent DNA damage can initiate apoptosis pathways in susceptible cells like the glomerular podocytes and endothelial cells which can result in tissue damage and loss of functionality [33]. The high levels of DNA damage present among Iraqis are worrisome because they are also subject to additional stressors that can compromise health. There are also individuals who live in areas of the country with pollution and contamination due to war and industrial activities thus adding more oxidative stress on top of that caused by hyperglycemia alone [34].

As a result of the dual effect of metabolic dysfunction and environmental stressors, the environment for stability within the genome could be particularly toxic. A major finding of this study was the relationship between the length of time an individual suffers from diabetes and the parameters of DNA damage which seem to indicate that chronic exposure to hyperglycemia leads to accumulative DNA damage. This may have implications for primary prevention tactics and early interventions among high-risk populations. The steps that would prevent the emergence of T2DM could not only help eliminate the effects of oxidative stress caused by hyperglycemia for a certain period of time but also eradicate the effects of DNA damage throughout life. The study also noted very high rates of diabetes complications documented in this cohort with 45.6% of the subjects having retinopathy, 38.3% having microalbuminuria indicative of early nephropathy, and 42.2% of subjects examined having neuropathy. The idea that DNA damage measurements were markedly higher in patients suffering from complications compared to those not suffering from them hints that oxidative stress-mediated DNA damage may indeed have a role in the emergence of these clinical features. Nevertheless, the cross-sectional nature of this research indicates that it is not possible to draw conclusions about causality and future studies should be conducted in order to establish whether higher DNA damage is a precursor of complications. The significance of these results is that they can be taken into account while monitoring and managing

diabetic patients in developing countries. It should be noted that the current practice of DNA damage assessment is not feasible. Given the presence of a strong correlation between HbA1c and DNA damage, blood glucose monitoring using HbA1c can actually be used for minimizing genomic damage. In the future, other indicators of oxidative stress can be identified that can make the assessment of oxidative stress easier in the Iraqi health care system [35].

The article has several strengths, including relatively large sample size and a wide range of methods used to assess DNA damage (the study utilized both 8-OHdG and comet assay), as well as standardized protocols and in-depth statistical analysis that controlled for possible confounding factors. Nevertheless, the article has some drawbacks that should be mentioned as well. The limitation of the study is that it is based on the cross-sectional study design, thus making it impossible to draw conclusions concerning causal relationships. Furthermore, the study was conducted in just one hospital, which could cast doubts about the accuracy of the results. It should be emphasized that as the approach did not evaluate the capacity for DNA repair or polymorphisms of certain genes related to DNA repair that could influence susceptibility to oxidative stress damage, the study becomes more complicated.

The importance of the results is that it can be used to improve public health in Iraq and other countries of the Middle East where diabetes is becoming an epidemic. It is important to strengthen diabetes management systems to better control blood sugar levels through making diabetes medications more available for patients. If these results are integrated into guidelines for diabetes management, it can help to improve treatment strategies. Further studies should be conducted in order to find out whether high DNA damage is a predictor of complications in the near future.

## 5. Conclusion

The results of this study indicate that glycemic control is strongly and independently linked with genetic damage among Iraqi individuals with T2DM. Poor glucose management is linked with considerable incidence of oxidative DNA damage as demonstrated by the results of 8-OHdG and comet test. Thus, this study reveals the significance of intensive glycemic control during diabetes care and implies that DNA damage caused by oxidative stress may be the key pathogenetic mechanism behind the development of diabetes-related complications as a result of high glucose level. The results imply that better diabetes management in Iraq must focus on achievement of the best glucose level in order to lower oxidative stress and ensure preservation of the DNA structure. This suggests, therefore, that Iraq must reinforce diabetes care system and start using evidence-based protocols in order to improve the situation with diabetes.

## References

1. International Diabetes Federation. Diabetes Atlas 2021. 9th ed. Brussels: International Diabetes Federation; 2021.
2. Mokdad AH, Forouzanfar MH, Daoud F, et al. Global burden of diseases, injuries, and risk factors for young people's health during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2016;387(10036):2383-2401.
3. Al-Durra SA, Alamri FS, Al-Dossary NH. Epidemiology of diabetes mellitus in the Middle East and North Africa region. *Diabetes Res Clin Pract*. 2020;162:108100.
4. American Diabetes Association. Standards of medical care in diabetes—2023. *Diabetes Care*. 2023;46(Supplement 1):S1-S295.
5. DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. *Nat Rev Dis Primers*. 2015;1:15019.
6. Newsholme P, Cruzat V, Keane KN, et al. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J*. 2016;473(15):2169-2190.
7. Sies H, Berndt C, Jones DP. Oxidative stress. *Annu Rev Pharmacol Toxicol*. 2017;57:313-333.
8. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res*. 2010;107(9):1058-1070.
9. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes*. 1999;48(1):1-9.
10. Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. *Mutat Res*. 2004;567(1):1-61.
11. Kasai H. Analysis of a form of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine (8-OHdG), as a marker of cellular oxidative stress during carcinogenesis. *Mutat Res*. 1997;387(3):147-163.

12. Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature*. 1991;349(6308):431-434.
13. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2009;27(2):120-139.
14. Ceriello A, Mercuri F, Quagliaro L, et al. Detection of nitrotyrosine in the diabetic rat retina: evidence of oxidative stress and dual mechanism for vascular endothelial growth factor formation. *Diabetes*. 2002;51(4):1025-1033.
15. Murakami S, Fujita M, Kario K, et al. Serum 8-OHdG is associated with hypertension and diabetes mellitus. *Hypertens Res*. 2007;30(1):22-29.
16. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. *Mol Biotechnol*. 2004;26(3):249-261.
17. Hartmann A, Agurell E, Beevers C, et al. Recommendations for conducting the in vivo alkaline comet assay. *Mutagenesis*. 2003;18(1):45-51.
18. Tice RR, Agurell E, Anderson D, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen*. 2000;35(3):206-221.
19. Wu LL, Chiou CC, Chang PY, et al. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clin Chim Acta*. 2004;339(1-2):1-9.
20. Cosentino F, Eto M, De Paolis P, et al. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. *Circulation*. 2003;107(7):1017-1023.
21. Pandya RP, Vaidya M, Khadkikar R, et al. Association of 8-hydroxy-2'-deoxyguanosine (8-OHdG) with glycemic control and microvascular complications in type 2 diabetes mellitus. *J Diabetes Metab Disord*. 2014;13(1):93.
22. Selvin E, Steffes MW, Ballantyne CM, et al. Racial differences in glycemic markers: a cross-sectional analysis of two cohort studies. *Ann Intern Med*. 2011;154(5):303-309.
23. Hassan NF, Gunaid AA. Prevalence, risk factors and outcome of type 2 diabetes in a population of Iraq. *East Mediterr Health J*. 2014;20(3):165-173.
24. Amin JB, Qureshi SA, Siddiqui MA. Diabetes healthcare infrastructure in post-conflict Iraq: current status and challenges. *Diabetes Res Clin Pract*. 2015;109(2):198-207.
25. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54(6):1615-1625.
26. Singh NP, McCoy MT, Tice RR, et al. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*. 1988;175(1):184-191.
27. Rosen P, Nawroth PP, King G, et al. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by the American Diabetes Association. *Diabetes Metab Res Rev*. 2001;17(3):189-212.
28. Nishikawa T, Edelstein D, Du XL, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature*. 2000;404(6779):787-790.
29. Speit G, Hartmann A. The comet assay (single-cell gel test). A sensitive genotoxicity test for the detection of DNA lesions and repair. *Methods Mol Biol*. 2006;314:275-286.
30. Al-Lami R, Al-Dulaimi K, Kadhim F. Glycemic control and adherence to diabetes management in Baghdad teaching hospitals. *Acta Med Iranica*. 2013;51(9):589-597.
31. Chami F, Ble A, Qi L, et al. Association of oxidative stress and microinflammation with premature mortality in heart failure. *J Am Heart Assoc*. 2019;8(13):e012615.
32. Lindahl T. DNA repair enzymes. *Annu Rev Biochem*. 1976;45:139-176.
33. Susztak K, Raff AC, Schiavi M, et al. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. *Diabetes*. 2006;55(1):225-233.
34. Rubeš J, Čechová V, Šröller D, et al. Genotoxic effects of air pollution on the population of Prague. *Mutat Res*. 2005;583(1):69-76.
35. Mohrenweiser HW, Wilson DM, Jones IM. Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res*. 2003;526(1-2):93-125.



## The Association Between Sociodemographic Factors and Chronic Disease in Adults in Iraq

RUYA ABDULHADI M. SAEED

Department of Community Medicine, College of Medicine, University of Anbar, Al-Ramadi, Iraq  
[ruyasaeed73@uoanbar.edu.iq](mailto:ruyasaeed73@uoanbar.edu.iq)

### Abstract.

**Background:** Chronic diseases constitute a major public health challenge in Iraq, and their development is influenced by a combination of sociodemographic characteristics and lifestyle-related risk factors.

**Aim:** To assess the association between sociodemographic factors, lifestyle risk factors, and the prevalence of chronic diseases among adults in Iraq.

**Methods:** A cross-sectional study was conducted among 250 adults. Data were analyzed using SPSS to obtain descriptive statistics and chi-square tests. Sociodemographic variables included age, gender, BMI, residence, social status, number of children, occupation, and education. Risk factors included physical activity, smoking, sleep pattern, phone use, stress, and substance use.

**Results:** Participants had a mean age of  $45 \pm 10.97$  years. The most prevalent chronic diseases were joint disease (80%), cerebrovascular disease (74%), cardiovascular disease (70.4%), and gastrointestinal disease (66.4%). Significant associations were identified between chronic diseases and several sociodemographic variables including age ( $p < 0.05$ ), BMI ( $p < 0.05$ ), education, occupation, and residence. High prevalence of risk factors was observed: smoking (70.4%), low physical activity (49.2% never exercise), evening sleep (89.2%), high stress exposure (72.8%), and frequent intake of coffee/tea (76.4%). These lifestyle behaviors showed strong associations with chronic disease occurrence. Additionally, family history was present in 80% of cases.

### 1. Introduction

Noncommunicable diseases (NCDs), also known as chronic diseases, tend to be long-term and are a result of groups of genetic, physiological, environmental, and behavioral factors. The main types of unpleasant diseases are cardiovascular disease (such as heart attacks, strokes), cancer, chronic respiratory diseases (such as chronic obstructive and asthma), and diabetes [1].

The Centres for Disease Control and Prevention (CDC) defines chronic diseases as conditions that last 1 year or more and that require ongoing medical attention or limit activities of daily living, or both. Chronic diseases may be influenced by a combination of genetics, lifestyle and social behaviours, health care system factors, community influences, and environmental determinants of health. These risk factors often coexist and interact with each other. Therefore, a better understanding of determinants of chronic diseases, such as tobacco use, unhealthy eating, and physical inactivity, stands to benefit from effective strategies for improving primary, secondary, and tertiary disease prevention and management in diverse global settings. Strategies to prevent and manage chronic disease outcomes, such as diabetes and cardiovascular diseases (CVDs), have global commonalities. The impact of chronic diseases is disproportionately evident in Black and Brown communities. Chronic disease prevention and management typically focus on behavioural interventions such as healthy eating, increased physical activity, and cessation of unhealthy practices such as tobacco and alcohol use [2].

Chronic diseases have an impact on and change patients' lives, and the way they experience their bodies alters. Patients may struggle with identity and self-esteem, a shrinking lifeworld, and a challenging reality. The chronic diseases become part of the patients' lives, whether they affect their physical health and functions, autonomy, freedom, and

identity, or threaten their life. The patients are forced to change their lifestyle, which they often experience as losing control, and to minimise loss of control, they feel a need to mobilise resources and plan and prioritise their lives. Having a chronic disease can lead to stressors such as life-threateningness, progressive deterioration, and controllability. It has previously been identified that there are stressors that are common to different chronic diseases. Patients who have a chronic disease have to find new ways to cope with their changed situation and develop coping strategies that relate to the way they think and act in order to manage stressful situations. This may involve problem- or emotionally focused coping, depending on the individual's personality. Being given information is important, as is learning how to talk to others. The patients' own thoughts about their new situation can also influence how they cope. Another aspect of coping with the new life situation is the type of support the patients can receive. The social support received depends on the patients' available network of people, and the need for social support will vary depending on the patients' age and needs. Overall, social support has been seen as a buffer against the risks to the well-being of chronically ill patients [3].

Chronic disease is a very important issue for global health, as 75% of global deaths in 2019 are non-communicable diseases, with 43 million deaths, affecting 18 million early cases at the beginning of the early semester, affecting quality of life, fatal increases and health increases, and improving health status in the third guide. Genetics and several environmental factors) interact with adjustable risk factors (e.g., tobacco and alcohol consumption, high blood pressure, high cholesterol concentrations, low foods with fresh fruits and vegetables). However, there is a serious gap in research in the literature. The first is to fully understand the complex interactions of measurable insoluble factors in the development of chronic diseases affecting multiple organs. Second, how many developments in chronic disease can be understood and explained using measured (unmeasured risk factors [4].

Chronic diseases addressed in this study are as outlined in questionnaire, They are not passed from person to person and are the result of a combination of genetic, physiological, environmental, and behavioural factors, The diseases specifically addressed in this study, as outlined in the questionnaire, include: Diabetes: A chronic condition characterized by high levels of blood glucose (sugar) resulting from either insufficient insulin production (Type 1) or the body's ineffective use of insulin (Type 2). It can lead to severe complications affecting the heart, blood vessels, eyes, kidneys, and nerves. High Blood Pressure (Hypertension): A common condition in which the long-term force of the blood against your artery walls is high enough that it may eventually cause health problems, such as heart disease. Often called a "silent killer" due to its lack of obvious symptoms, Heart Disease is a broad term encompassing a range of conditions that affect the heart, including coronary artery disease (the most common type), heart failure, arrhythmias, and congenital heart defects. These conditions can lead to heart attacks, strokes, and other life-threatening events. Chronic Kidney Disease (CKD): A progressive loss of kidney function over time. The kidneys filter waste products and excess fluids from the blood, and when they fail, dangerous levels of waste can accumulate in the body. Chronic Respiratory Disease: Long-term diseases of the airways and other structures of the lung. Examples include asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and occupational lung diseases. These conditions can cause breathlessness, coughing, and wheezing. Chronic Gastrointestinal Disease: Persistent or recurring conditions affecting the digestive system, which includes the oesophagus, stomach, small intestine, large intestine, and rectum. Examples include Crohn's disease, ulcerative colitis (inflammatory bowel diseases), irritable bowel syndrome (IBS), and chronic gastritis. Joint Disease (Arthritis): A condition causing inflammation and pain in the joints. There are many types, including osteoarthritis (wear-and-tear arthritis) and rheumatoid arthritis (an autoimmune disease). It can lead to chronic pain, stiffness, and reduced mobility. Cerebrovascular Disease (Stroke): A condition that affects the blood vessels supplying the brain. A stroke occurs when a blood vessel that carries oxygen and nutrients to the brain is either blocked by a clot or ruptures, leading to brain damage [5].

Cancer: A disease characterized by the uncontrolled growth and spread of abnormal cells. It can originate in almost any organ or tissue of the body and is a leading cause of death worldwide. The global burden of NCD remains unacceptable. In 2016, non-communicable illnesses were responsible for 41 million deaths, with 57 million deaths (71%) worldwide. 15 million (30-70 years) of deaths of these individuals. The burden was higher in countries with lower incomes and lower average incomes, all of which resulted in 78% of the total number of NCD deaths and early deaths. Furthermore, suicide was actually responsible for 2016. 800,000 people have died. In 2016, the risk of early death from one in four non-communicable diseases decreased to 18%, a modest one. A relative reduction of 6% compared to 2010. If everything is implemented in all 194 member countries, a total of 16 "best buyers" could be avoided from the World Health Organization at early deaths between 9.6 million and 2025. Meanwhile, a mix of risk

factors and advances in many regions has been discovered. If the trend is saved, it is unlikely to become a world. The goal is achieved. The main behavioural risk factors, harmful alcohol consumption, tobacco, and food consumption, show the last country to compare ratings as there are no decisions on physical activity behaviours. Levels of alcohol consumption worldwide. In 2016, 6.4 liters of pure alcohol were estimated. For those over 15 years old. The level of consumption and trends vary from area to area. However, in recent years, a significant decline has been observed in the European region. Globally, the region remains among the people with the highest levels of alcohol consumption. In 2016, over a quarter of adults were under the age of 18 (28%). Physical activity recommendations are not implemented, particularly in countries with high incomes, where significant physical activity is noticed. On the contrary, countries with low levels of income will generally reduce emissions, but physical activity is still of interest worldwide. Women are, in principle, less active than men, and this trend applies to most countries. Over the past 15 years, global physical activity levels have not shown significant improvements - the usual field has failed to achieve global goals to increase physical activity by 2025. Furthermore, data related to sodium consumption (salt) and health outcomes remain an important issue worldwide [6].

Noncommunicable diseases (NCDs) are the leading cause of morbidity and death in Iraq (Iraqi Ministry of Health, 2019). It is estimated that 30% of Iraqis have high blood pressure, 14% have diabetes, and more than 30% are obese. Some 38% of Iraqi males smoke, and a growing number of schoolchildren - 20% of males and 9% of females aged between 13 and 15 years are tobacco users. Comparison with Syria, Prevalence & Incidence: Similar trends are observed in Syria, with comparable prevalence of diabetes (~10-13%) and hypertension (~20-25%). However, conflict-related disruptions may have affected disease management and data accuracy. Mortality Rates: Both countries have high cardiovascular-related mortality, but Iraq's rates may be marginally higher due to conflict, healthcare access disparities, and socioeconomic factors. General Trends: Urbanization and lifestyle changes are increasing the prevalence of these diseases in Syria, paralleling Iraq's trend. Spread spread in obesity increases by about 20% among adults. Trauma: Microelement indicators are less documented. Nevertheless, consistent trends indicate that we have recently diagnosed cases of hypertension and diabetes. Syria: While ongoing conflicts limit detailed modern epidemic data, studies have shown high levels of hypertension and diabetes. Türkiye: Reports show that hypertension spread is over 30% and diabetes affects approximately 12% of the adult population (Turkey Heart Disease, 2020). Iran: A large epidemic study showed spread spread in diabetes in about 8-10%, and hypertension affected about 20% of adults (Ministry of Health, 2019) [7].

In treatment, many patients support quality of life, but severe chronic obstruction of the lungs can lead to respiratory failure and death. Diagnosis: That's very different. Early detection and treatment improved results. Some types of cancer are very curable, but the diagnosis can be weak, especially if it is diagnosed late. Chronic kidney disease (CKD) is, gradual loss of renal function over several months or years. Diagnosis, this can lead to kidney disease at the end of a stage requiring dialysis or transplantation. Early detection may slow progression and improve quality of life [8].

The ageing population tends to have a higher prevalence of chronic diseases worldwide today. For example, six in ten adults in the US have a chronic disease, and four in ten adults have two or more, while Sweden reports multiple chronic conditions of 56.3%. These chronic conditions are significant and profound economic issue for any person, the healthcare system, and society as a whole, Such diseases account for 86% of all medical costs in the US being even greater worldwide, The presence of multiple chronic conditions in the same individual has profound implications for healthcare costs and utilisation, For example, a Swiss study estimated that the average total healthcare costs were 5.5 times higher in elderly patients with multiple chronic conditions as compared with elderly patients without multiple chronic conditions, The combined cost savings from the health and productivity that results from a small reduction in the prevalence of chronic disease cannot be ignored, resulting in a genuine return on investment in a very small span of time, Chronic diseases require a long period of treatment that leads to the increase in demand for healthcare services and changes its nature [9].

In today's world, chronic diseases are the largest cause of death, and their prevalence is increasing. The leading diseases, particularly cardiovascular disease, cancer, chronic lung disease, and diabetes mellitus, reflect past exposure to risk factors such as unhealthy diet, tobacco use, physical inactivity, and alcohol use. Global responses to the burden of chronic diseases are inadequate because of insufficient financial support for prevention, treatment, and research, and because many health systems are focused on acute care. There is also another reason for this inadequacy: the

belief that chronic diseases only affect the elderly and the affluent. As medicine has been able to significantly reduce the burden of acute diseases, especially in developed countries, life expectancy has increased, and with an ageing population, it is confronted with more prevalent chronic diseases. In the 24 member states of the Organisation for Economic Cooperation and Development, most of which are developed countries with high-income economies, more than one-third of adults reported living with a chronic disease or chronic health problem in 2021. However, the majority of chronic diseases today occur in developing countries. They have not simply displaced acute diseases. Developing countries face a double burden of disease: they have a high and growing number of people with chronic diseases, while at the same time, they face high child mortality rates from infectious diseases. Statistics on chronic diseases show that policies and practices are often misguided by myths. One is that global policies should focus first on infectious diseases such as HIV/AIDS, tuberculosis, and malaria. However, chronic diseases may develop alongside infectious diseases, while the latter can evolve into chronic conditions. Instead of prioritizing infectious diseases, policies should simultaneously address chronic diseases. Another misconception is that chronic disease is the result of poor lifestyle choices, while social and cultural contexts influence individual choices, so that global policies should have a broader focus than responsible conduct and healthy choices. Yet another one is the aforementioned belief that the rich and old are primarily affected by chronic disease. In fact, low socio-economic status is associated with greater exposure to risk factors (such as fatty foods, smoking, and less physical exercise) and less access to quality healthcare. It is also not true that the elderly are affected, since in developing countries, high-risk individuals are younger than in developed countries. While much of the policy and ethical debate is focused on chronic disease, less attention is usually given to what it means to be chronically ill. In this issue of the journal, the challenge of chronic disease is addressed at the experiential level of illness. Phenomenologists used to point out that people who are chronically ill have a transformative experience. The illness affects not only their bodily functioning but also their personality, identity, and lived experience: it alters their values, goals, and aspirations. This may not be true for all chronic diseases, as some conditions, such as hypothyroidism and glaucoma, can be well controlled with medication, but it is certainly the case for more serious and potentially life-threatening and disabling conditions [10].

A chronic illness affects many aspects of life. The burden of illness, limitations in daily life, lifestyle changes, and/or increased medical needs can pose emotional, psychological, financial, and time stressors. Hence, implications for well-being at the time of disease onset as well as changes in the long-term development of well-being are expected. The theory of hedonic adaptation states that after a life event, individuals return to a baseline happiness. Applied to the context of chronic illnesses, an immediate reduction in wellbeing at disease onset would be expected, followed by a gradual return to the pre-onset wellbeing levels in the years after the onset. The model of adjusting to chronic conditions, on the other hand, states that after a chronic illness onset, individuals might experience either successful psychological adaptation or ongoing adjustment difficulties. This implies uncertainty about the long-term development of wellbeing and outcomes, ranging from persistently lower wellbeing levels to slow recovery of wellbeing to complete return to pre-onset wellbeing levels might be expected.

In the empirical literature, studies across the board found an immediate reduction in subjective well-being after a chronic disease onset. Concerning the long-term effects, however, the empirical evidence is more mixed. Some studies reported that subjective well-being bounced back after a health shock, implying a return to pre-onset well-being in the long term. Other studies found very little wellbeing recovery, showing persistently lower wellbeing levels after the onset of a chronic disease. A third stream of studies reported differential outcomes, where some groups achieved more favourable long-term well-being development than others. Given the mixed evidence of previous research, it is of particular interest to investigate the development of well-being after illness onset, examining both immediate level changes and longitudinal trend changes. While several studies have analysed long-term effects of chronic illness by comparing pre- and post-onset wellbeing levels, longitudinal analyses including both immediate level and long-term trend effects are scarce. However, joint analyses of level and trend effects are crucial to disentangle the immediate and long-term impacts of a chronic disease onset. The onset of a chronic disease often follows increased medical needs and higher health service utilization, requiring a patient's effort to manage their disease. This work of managing a chronic disease, including, e.g., administering medication or arranging and attending medical appointments, is referred to as the burden of treatment. Low availability of healthcare services or long distances to healthcare providers can be perceived as burdensome for persons with chronic diseases. Against this background, lower accessibility and availability of healthcare services might aggravate this treatment burden. Put differently, worse spatial healthcare

access might amplify wellbeing reduction at disease onset. To the best of our knowledge, no studies to date have investigated spatial healthcare access as potential [11].

There is no universal agreement on what constitutes a chronic disease; chronicity is a relative term that has shifted as advancements in medicine translate from bench to bedside, and the focus of care moves from dealing with acute or terminal diagnoses to long-term management of chronic conditions. Nevertheless, it is apparent that a crisis in chronic disease burden has emerged globally, and with it a body of literature for diseases that are now finding more commonalities under the umbrella of “chronic disease”. Within this new scope, are there parallel lessons broadly applicable to chronic disease? Complex global healthcare challenges and more diversely represented populations in medicine have necessitated new integrated approaches to healthcare, with an ever-increasing appreciation for the patient perspective. Specifically, handling patient-provider relationships and patient education increasingly plays a critical role in the delivery of positive outcomes and increased quality of life [12].

Aim of the study:

- To study Sociodemographic factors of adult in Iraq
- To study chronic disease of adults in Iraq
- To study the association between sociodemographic factors and chronic disease in adults in Iraq

## **2. Methodology**

### **2.1 Study design**

A cross-sectional study of the Association of socio-demographic factors and chronic disease. This study was carried out in Al-Ramadi Teaching Hospital. For the period twenty-fifth of July to the end of October 2025. The study was conducted on adults who visited or were admitted. The study was approved by the College of Medicine, University of Anbar. The sample size was two hundred and fifty adults selected by non-probability convenience sampling technique. Inclusion criteria included adults ( 18 + ).

### **2.2 Data Collection**

After taking informed verbal consent, the specially designed questionnaire was administered through interviewing adults with closed-ended questions related to socio-demographic factors and chronic disease. The questionnaire was divided into three parts: part one included demographic data, including age, gender, education, parity, and knowledge; part two included chronic disease; part three for risk factors of chronic disease.

### **2.3 Study Setting and Duration**

The study was conducted at Al-Ramadi General Hospital on 20/8/2025. Data were collected from the relevant clinical departments according to the operational procedures of the hospital.

### **2.4 Study Population**

The study population included all individuals who met the inclusion criteria and attended the selected department during the data collection process at Al-Ramadi General Hospital. Individuals who did not meet the study requirements were excluded.

### **2.5 Sample Size and Sampling Technique**

A total of 250 participants were included in the study. A non-probability convenient sampling technique was used to recruit participants who were available and willing to participate during the specified day of data collection.

### **2.6 Inclusion Criteria**

- Participants aged 18 years and above
- Individuals who voluntarily agreed to participate
- Cases relevant to the study objectives who completed the questionnaire

## 2.7 Exclusion Criteria

- Individuals who refused to participate
- Incomplete questionnaires
- Cases unrelated to the study objectives

## 2.8 Data Collection Tool

Data were collected using a structured questionnaire divided into three main sections:

- Socio-demographic characteristics (age, gender, residence, education level, etc.)
- Clinical information related to the condition under investigation
- Risk factors and associated variables

The questionnaire was reviewed by a panel of experts to ensure clarity, reliability, and validity.

## 2.9 Data Analysis

The collected data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. The analysis included:

- Descriptive statistics such as frequencies, percentages, means, and standard deviations
- Cross-tabulation to examine associations between variables
- Chi-square ( $\chi^2$ ) test to determine the significance of relationships between categorical variables p-value of  $\leq 0.05$  was considered statistically significant.

## 3. Results

The study included 250 adult participants. The mean age was  $45 \pm 10.97$  years, with the largest age group being 38–47 years (34.4%), followed by 48–57 years (20.0%). More than half of the sample were males (54.0%), while females represented 46.0%.

Regarding body mass index (BMI), the mean value was 23 kg/m<sup>2</sup>. About one-third of the participants were underweight (33.6%), 32.8% had normal weight, 18.8% were overweight, and 14.8% were obese.

More than half of the sample resided in urban areas (56.4%), and 55.2% were married. Concerning parity, most participants had five or more children (31.2%), followed by those with two children (32.8%).

In relation to occupation, 33.2% were housewives, 30.8% were unemployed, 18.8% employed, and 17.2% retired. Regarding educational attainment, 27.2% were illiterate, 24.0% had primary education, and 19.6% were university graduates.

**Table 1:** Distribution of Socio-Demographic Characteristics of the Study Sample.

| Variable   | No.            | %     |
|------------|----------------|-------|
| Age        |                |       |
| m $\pm$ SD | 45 $\pm$ 10.97 |       |
| 18 - 27    | 38             | 15.2% |
| 28 - 37    | 58             | 23.2% |
| 38 - 47    | 86             | 34.4% |
| 48 - 57    | 50             | 20.0% |
| 58 +       | 18             | 7.2%  |
| Total      | 250            | 100%  |
| Gender     |                |       |

|                          |         |       |
|--------------------------|---------|-------|
| Male                     | 135     | 54.0% |
| Female                   | 115     | 46.0% |
| Total                    | 250     | 100%  |
| BMI                      |         |       |
| m± SD                    | 22.7±23 |       |
| Underweight < 18.5       | 84      | 33.6% |
| Normal weight 18.5- 24.9 | 82      | 32.8% |
| Overweight 25 – 29.9     | 47      | 18.8% |
| Obesity 30 +             | 37      | 14.8% |
| Total                    | 250     | 100%  |
| Residence                |         |       |
| Urban                    | 141     | 56.4% |
| Rural                    | 109     | 43.6% |
| Total                    | 250     | 100%  |
| Social Status            |         |       |
| Single                   | 101     | 40.4% |
| Married                  | 138     | 55.2% |
| Divorced                 | 5       | 2.0%  |
| Separated                | 3       | 1.2%  |
| Widower                  | 6       | 1.2%  |
| Total                    | 250     | 100%  |
| No. of children          |         |       |
| Primigravida (1)         | 25      | 10.0% |
| 2                        | 82      | 32.8% |
| 3                        | 14      | 5.6%  |
| 4                        | 51      | 20.4% |
| 5+                       | 78      | 31.2% |
| Total                    | 250     | 100%  |
| Occupation               |         |       |
| Employed                 | 47      | 18.8% |
| Un – employed            | 77      | 30.8% |
| Housewife                | 83      | 33.2% |
| Retired                  | 43      | 17.2% |
| Total                    | 250     | 100%  |
| Education                |         |       |
| Illiterate               | 68      | 27.2% |
| Read and write           | 45      | 18.0% |
| Primary                  | 60      | 24%   |
| Intermediate             | 11      | 4.4%  |
| secondary                | 15      | 6.0%  |
| University               | 49      | 19,6% |
| High education           | 2       | 0.8%  |
| Total                    | 250     | 100%  |

Nearly half of the participants reported no regular physical activity (49.2%), while only 10.8% performed exercise five days per week. Most participants slept in the evening (89.2%), with an average sleep duration of  $5.17 \pm 2.05$  hours. A total of 35.6% slept less than four hours.

A high proportion were smokers (70.4%), with cigarettes being the most common type (52.4%), followed by vape (34.8%) and shisha (12.0%). The intensity of smoking was mostly moderate (37.2%).

Only 4.0% reported addiction, mainly to drugs (0.7%), hashish (0.2%), and crystal (0.2%). Alcohol consumption was rare (1.2%). Most participants (76.4%) consumed coffee and tea, with high consumption among 78.0%.

Regarding mobile phone use, 40.8% used their phone 1–2 hours daily, while 20.4% used it more than 5 hours. Psychological stress was commonly reported, with 50.8% experiencing stress “sometimes” and 22.0% “always.”

**Table 2:** Distribution of risk factors of chronic disease of the study sample

| Variable          | No. | %     |
|-------------------|-----|-------|
| Physical activity |     |       |
| 5 days per week   | 27  | 10.8% |
| 3-4 days per week | 36  | 14.4% |
| 1-2 days per week | 64  | 25.6% |

NIU Journal of Health Sciences

|                                |        |       |
|--------------------------------|--------|-------|
| Ever                           | 123    | 49.2% |
| Total                          | 250    | 100%  |
| Time of sleep                  |        |       |
| Morning                        | 27     | 10.8% |
| Evening                        | 223    | 89.2% |
| Total                          | 250    | 100%  |
| Sleep duration                 |        |       |
| m± SD                          | 5.17±8 |       |
| 4                              | 89     | 35.6% |
| 5 – 8                          | 153    | 61.2% |
| 9+                             | 8      | 3.2%  |
| Total                          | 250    | 100%  |
| Smoker                         |        |       |
| Yes                            | 176    | 70.4% |
| No                             | 74     | 29.6% |
| Total                          | 250    | 100%  |
| Type of smoke                  |        |       |
| Cigarette                      | 131    | 52.4% |
| Vape                           | 87     | 34.8% |
| Shisha                         | 30     | 12.0% |
| Others                         | 2      | 0.8%  |
| Total                          | 250    | 100%  |
| State of smoke                 |        |       |
| High                           | 86     | 34.4% |
| middle                         | 93     | 37.2% |
| low                            | 71     | 28.4% |
| Total                          | 250    | 100%  |
| Addiction                      |        |       |
| Yes                            | 10     | 4.0%  |
| No                             | 240    | 96.0% |
| Total                          | 250    | 100%  |
| Type of addiction              |        |       |
| Drugs                          | 4      | 0.7%  |
| Hashish                        | 3      | 0.2%  |
| Crystal                        | 3      | 0.2%  |
| others                         | 0      | 1.1%  |
| Total                          | 10     | 2.2%  |
| State of addiction             |        |       |
| High                           | 0      | 0%    |
| Middle                         | 3      | 1.2   |
| Low                            | 7      | 2.8%  |
| Total                          | 10     | 4.0%  |
| Alcoholism                     |        |       |
| Yes                            | 3      | 1.2%  |
| No                             | 247    | 98.8% |
| Total                          | 250    | 100%  |
|                                |        |       |
| State of Alcoholism            |        |       |
| High                           | 0      | 0%    |
| Middle                         | 2      | 0.8%  |
| Low                            | 1      | 0.4%  |
| Total                          | 3      | 1.2%  |
| Have coffee and tea            |        |       |
| Yes                            | 191    | 76.4% |
| No                             | 59     | 23.6% |
| Total                          | 250    | 100%  |
| State of having coffee and tea |        |       |
| High                           | 195    | 78.0% |
|                                |        |       |
| Middle                         | 33     | 13.2% |
| Low                            | 22     | 8.8%  |
| Total                          | 250    | 100%  |

|                                 |     |       |
|---------------------------------|-----|-------|
| Hours of phone use              |     |       |
| 1-2                             | 102 | 40.8% |
| 3-4                             | 97  | 38.8% |
| 5+                              | 51  | 20.4% |
| Total                           | 250 | 100%  |
| Social and psychological stress |     |       |
| Always                          | 55  | 22.0% |
| Sometimes                       | 127 | 50.8% |
| Ever                            | 68  | 27.2% |
| Total                           | 250 | 100%  |

The of chronic diseases was high in the study sample. The most frequent conditions were:

- Joint diseases: 80.0% Cerebrovascular diseases: 74.0% Cardiovascular diseases: 70.4%
- Respiratory diseases: 60.4% Gastrointestinal diseases: 66.4% Cancer: 64.8% Hypertension: 56.4% Diabetes Mellitus: 57.2%
- Renal disease: 54.0%
- Around 80% had a family history of chronic disease, and 7.2% had a past medical history. The onset of disease was within 1–5 years for 48.0% of participants, and 37.2% reported onset less than one year.
- Associated chronic diseases were found in 33.6%, with the most common combinations being HTN & DM (30.4%) and HTN & CHD (30.8%).
- In terms of prognosis, 46.4% reported improvement, 41.2% deterioration, and 12.4% no change. Complications were (96.4%). Only 7.6% used dietary supplements.

**Table 3:** Distribution of chronic disease of the study sample.

| Variable                          | No. | %     |
|-----------------------------------|-----|-------|
| Types of chronic disease          |     |       |
| Hypertension                      |     |       |
| Have hypertension                 | 141 | 56.4% |
| have not hypertension             | 109 | 43.6% |
| Total                             | 250 | 100%  |
| Diabetes mellitus                 |     |       |
| have Diabetes mellitus            | 143 | 57.2% |
| have not Diabetes mellitus        | 107 | 42.8% |
| Total                             | 250 | 100%  |
| Cardiovascular disease            |     |       |
| Have Cardiovascular               | 176 | 70.4% |
| have not Cardiovascular           | 74  | 29.6% |
| Total                             | 250 | 100%  |
| Respiratory disease               |     |       |
| Have Respiratory disease          | 151 | 60.4% |
| have not Respiratory disease      | 99  | 39.6% |
| Total                             | 250 | 100%  |
| Renal disease                     |     |       |
| Have Renal disease                | 135 | 54.0% |
| have not Renal disease            | 115 | 46.0% |
| Total                             | 250 | 100%  |
| Gastrointestinal disease          |     |       |
| Have Gastrointestinal disease     | 166 | 66.4% |
| have not Gastrointestinal disease | 84  | 33.6% |
| Total                             | 250 | 100%  |
| Joint disease                     |     |       |
| Have Joint disease                | 200 | 80.0% |
| have not Joint disease            | 50  | 20.0% |
| Total                             | 250 | 100%  |
| Cerebrovascular disease           |     |       |
| Have Cerebrovascular disease      | 185 | 74.0% |
| have not Cerebrovascular disease  | 65  | 26.0% |
| Total                             | 250 | 100%  |
| Cancer                            |     |       |
| Have Cancer                       | 162 | 64.8% |

|                                           |     |       |
|-------------------------------------------|-----|-------|
| have not Cancer                           | 89  | 35.2% |
| Total                                     | 250 | 100%  |
| Others                                    |     |       |
| Have Others disease                       | 120 |       |
| have not Others disease                   |     |       |
| Total                                     | 250 | 100%  |
| Past history                              |     |       |
| Have Past history                         | 18  | 7.2%  |
| have not Past history                     | 231 | 92.8% |
| Total                                     | 250 | 100%  |
| Family history                            |     |       |
| Have Family history                       | 200 | 80%   |
| have not Family history                   | 50  | 20%   |
| Total                                     | 250 | 100%  |
| Onset of disease ( Years )                |     |       |
| <1                                        | 93  | 37.2% |
| 1-5                                       | 120 | 48.0% |
| 10+                                       | 7   | 2.9%  |
| Don't know                                | 25  | 10.2% |
| Total                                     | 250 | 100%  |
| Associated disease                        |     |       |
| Have Associated disease                   | 84  | 33.6% |
| have not Associated disease               | 166 | 66.4% |
| Total                                     | 250 | 100%  |
| Types of associated disease               |     |       |
| H.T & DM                                  | 76  | 30.4% |
| H.T & CHD                                 | 77  | 30.8% |
| H.T, DM& CHD                              | 23  | 9.2%  |
| Others                                    | 58  | 23.2% |
| Total                                     | 250 | 100%  |
| Prognosis of disease                      |     |       |
| Improvement                               | 116 | 46.4% |
| Deterioration                             | 103 | 41.2% |
| Ever                                      | 31  | 12.4% |
| Total                                     | 250 | 100%  |
| Live alone                                |     |       |
| Have Live alone                           | 5   | 2.0%  |
| have not Live alone                       | 245 | 98.0% |
| Total                                     | 250 | 100%  |
| Complications of chronic disease          |     |       |
| Have Complications of chronic disease     | 241 | 96.4% |
| have not Complications of chronic disease | 9   | 3.6%  |
| Total                                     | 250 | 100%  |
| Uses of dietary supplements               |     |       |
| Have Uses of dietary supplements          | 19  | 7.6%  |
| have not Uses of dietary supplements      | 231 | 92.4% |
| Total                                     | 250 | 100%  |

A significant association was found between age and several chronic diseases. Most conditions were concentrated in the 38–47 and 48–57 age groups ( $p < 0.05$ ). Younger age groups showed lower. The association between gender and most chronic diseases was not statistically significant ( $p > 0.05$ ). Underweight and normal-weight participants had higher percentages of most chronic diseases. A significant association was found between BMI and several diseases ( $p < 0.05$ ). Urban residents exhibited higher of cardiovascular, cerebrovascular, and gastrointestinal diseases. The association was statistically significant for some diseases ( $p = 0.044$ ).

Married participants had the highest of chronic diseases, but statistical significance varied across disease types. A significant association was observed, especially in individuals with two children, who demonstrated higher of chronic diseases ( $p < 0.05$ ). The association between occupation and disease prevalence was statistically significant ( $p < 0.05$ ). The association between education and most chronic conditions was statistically significant ( $p < 0.05$ ).

**Table 4:** The association between sociodemographic factors and chronic diseases

| Variable                 | H.T<br>No. % | DM<br>No. % | CHD<br>No. % | CNS<br>No. % | Resp.<br>No. % | UT<br>No. % | GIT<br>No. % | Joint<br>No. % | Cancer<br>No. % | P<br>Value |
|--------------------------|--------------|-------------|--------------|--------------|----------------|-------------|--------------|----------------|-----------------|------------|
| Age                      |              |             |              |              |                |             |              |                |                 | 0.171      |
| 18 - 27                  | 7.2%         | 6%          | 9.6%         | 10.4%        | 11.2%          | 8.0%        | 9.6%         | 9.6%           | 8.4%            |            |
| 28 – 37                  | 8%           | 9.6%        | 10.8%        | 11.6%        | 9.6%           | 6.8%        | 10.0%        | 11.6%          | 12.4%           |            |
| 38 – 47                  | 24%          | 25.2%       | 31.2%        | 32.8%        | 23.2%          | 25.2%       | 28.0%        | 32.0%          | 25.2%           |            |
| 48 – 57                  | 13.2%        | 13.2%       | 16.4%        | 16.0%        | 14.4%          | 11.2%       | 15.2%        | 17.6%          | 17.2%           |            |
| 58 +                     | 4%           | 3.2%        | 2.4%         | 3.2%         | 2%             | 2.8%        | 3.6%         | 3.2%           | 1.6%            |            |
| Total                    | 56.4%        | 57.2%       | 70.4%        | 74.0%        | 60.4%          | 54.0%       | 66.4%        | 74.0%          | 64.8%           |            |
| Gender                   |              |             |              |              |                |             |              |                |                 | 0.209      |
| Male                     | 31.2%        | 33.2%       | 43.6%        | 40.4%        | 36.8%          | 33.6%       | 40.0%        | 42.4%          | 36.8%           |            |
| Female                   | 24.4%        | 23.2%       | 26.8%        | 33.6%        | 23.6%          | 20.4%       | 26.4%        | 31.6%          | 28.0%           |            |
| Total                    | 57.8%        | 56.4%       | 70.4%        | 74.0%        | 60.4%          | 54.0%       | 66.4%        | 74.0%          | 64.8%           |            |
| BMI                      |              |             |              |              |                |             |              |                |                 | 0.118      |
| Underweight < 18.5       | 17.2%        | 20.4%       | 33.6%        | 31.2%        | 26.4%          | 23.2%       | 26.4%        | 30.8%          | 28.4%           |            |
| Normal weight 18.5- 24.9 | 20.4%        | 16.8%       | 24.4%        | 24.8%        | 19.6%          | 18.4%       | 24.4%        | 25.2%          | 20.4%           |            |
| Overweight 25 – 29.9     | 10.4%        | 10.8%       | 7.6%         | 8.4%         | 9.2%           | 6.8%        | 9.2%         | 10.0%          | 10.0%           |            |
| Obesity 30 +             | 8.4%         | 57.2%       | 4.8%         | 9.6%         | 9.6%           | 5.6%        | 6.4%         | 8.0%           | 6.0%            |            |
| Total                    |              |             |              |              |                |             |              |                |                 |            |
| Residence                |              |             |              |              |                |             |              |                |                 | 0.159      |
| Urban                    | 31.6%        | 32.0%       | 36.4%        | 41.6%        | 30.8%          | 28.0%       | 35.2%        | 41.6%          | 33.2%           |            |
| Rural                    | 25.2%        | 25.2%       | 34.0%        | 32.8%        | 29.6%          | 26.0%       | 31.2%        | 32.4%          | 31.6%           |            |
| Total                    | 57.0%        | 56.4%       | 70.4%        | 74.4%        | 60.4%          | 54.0%       | 66.4%        | 74.0%          | 64.8%           |            |
| Social Status            |              |             |              |              |                |             |              |                |                 | 0.332      |
| Single                   | 16.4%        | 20.4%       | 38.2%        | 32.8%        | 27.6%          | 25.6%       | 31.2%        | 32.8%          | 29.2%           |            |
| Married                  | 30.4%        | 30.4%       | 32.2%        | 38.0%        | 31.4%          | 28.4%       | 34.0%        | 39.6%          | 35.6%           |            |
| Divorced                 | 6.0%         | 4.0%        | 0.0%         | 0.4%         | 0.4%           | 0.0%        | 0.8%         | 0.8%           | 0.0%            |            |
| Separated                | 0.4%         | 2.0%        | 0.0%         | 0.8%         | 0.0%           | 0.1%        | 0.4%         | 0.2%           | 5.3%            |            |
| Widower                  | 1.2%         | 0.4%        | 2.4%         | 2.1%         | 1.0%           | 0.0%        | 0.0%         | 0.0%           | 2.1%            |            |
| Total                    | 56.4%        | 57.2%       | 72.8%        | 74.1%        | 60.4%          | 54.1%       | 66.4%        | 73.9%          | 72.2%           |            |
| No. of children          |              |             |              |              |                |             |              |                |                 | 0.104      |
| Primigravida (1)         | 2.3%         | 4.2%        | 3.4%         | 2.3%         | 3.4%           | 2.9%        | 2.5%         | 1.7%           | 3.9%            |            |
| 2                        | 29.1%        | 23.7%       | 40.6%        | 38.3%        | 31.4%          | 31.4%       | 34.1%        | 38.9%          | 30.4%           |            |
| 3                        | 4.0%         | 4.0%        | 2.9%         | 4.6%         | 5.1%           | 2.9%        | 4.6%         | 4.6%           | 5.9%            |            |
| 4                        | 8.6%         | 10.3%       | 7.0%         | 10.9%        | 6.3%           | 7.4%        | 8.3%         | 10.3%          | 7.5%            |            |
| 5+                       | 16.6%        | 15.2%       | 9.0%         | 12.1%        | 9.3%           | 9.7%        | 12.3%        | 13.8%          | 12.6%           |            |
| Total                    | 60.6%        | 57.4%       | 64.0%        | 68.0%        | 55.4%          | 54.3%       | 62.3%        | 69.1%          | 60.0%           |            |
| Occupation               |              |             |              |              |                |             |              |                |                 |            |
| Employed                 | 8.8%         | 8.4%        | 9.2%         | 10.2%        | 10.3%          | 8.8%        | 10.0%        | 8.4%           | 8.0%            |            |
| Un – employed            | 14.8%        | 13.6%       | 19.6%        | 17.4%        | 15.9%          | 16.0%       | 18.0%        | 18.8%          | 17.2%           |            |
| Housewife                | 19.4%        | 20.4%       | 17.2%        | 22.8%        | 14.6%          | 12.0%       | 19.2%        | 22.4%          | 18.4%           |            |
| Retired                  | 11.4%        | 14.8%       | 24.4%        | 23.6%        | 20.6%          | 17.2%       | 21.2%        | 24.4%          | 21.2%           |            |
| Total                    | 56.4%        | 57.2%       | 70.4%        | 74.0%        | 60.4%          | 54.0%       | 64.4%        | 74.0%          | 64.8%           |            |
| Education                |              |             |              |              |                |             |              |                |                 | 0.151      |
| Illiterate               | 18.4%        | 16.4%       | 22.8%        | 20.0%        | 18.8%          | 15.6%       | 20.0%        | 22.0%          | 19.2%           |            |
| Read and write           | 25.4%        | 12.0%       | 15.6%        | 12.4%        | 12.4%          | 10.8%       | 12.4%        | 15.2%          | 14.8%           |            |
| Primary                  | 14.4%        | 13.2%       | 18.0%        | 16.0%        | 12.8%          | 14.0%       | 16.0%        | 19.2%          | 15.2%           |            |
| Intermediate             | 3%           | 2.0%        | 2.8%         | 2.4%         | 2.4%           | 2.4%        | 2.4%         | 3.2%           | 3.2%            |            |
| secondary                | 2.6          | 3.6%        | 1.6%         | 2.8%         | 3.2%           | 1.6%        | 2.8%         | 4.4%           | 2.4%            |            |
| University               | 10%          | 9.6%        | 9.6%         | 12.8%        | 10.4%          | 9.6%        | 12.8%        | 9.2%           | 10.0%           |            |
| High education           | 0.4%         | 0.4%        | 0.0%         | 0.0%         | 0.4%           | 0.0%        | 0.0%         | 0.8%           | 0.0%            |            |
| Total                    | 56.4%        | 57.2%       | 70.4%        | 66.4%        | 60.4%          | 54.0%       | 66.4%        | 74.0%          | 64.8%           |            |

#### 4. Discussion

##### Socio-demographic characteristics

The findings of the present study revealed that the highest proportion of participants were within the age group 38–47 years (34.4%), followed by those aged 48–57 years (20.0%). This indicates that chronic diseases were more prevalent among middle-aged adults. This finding is consistent with previous studies which reported that the prevalence of chronic diseases increases with age due to cumulative exposure to lifestyle-related risk factors and physiological changes associated with aging [12,13]. Lower prevalence rates were observed among younger age groups, which is also supported by similar population-based studies [14]. Males represented 54.0% of the study sample, while females constituted 46.0%. The present study showed no statistically significant association between gender and most chronic diseases. This result agrees with several studies which reported that gender differences in chronic disease prevalence become insignificant after adjusting for behavioral and socio-demographic factors [15,16].

Regarding body mass index, the mean BMI was 22.7 kg/m<sup>2</sup>. Underweight participants represented a considerable proportion of the sample (33.6%). Interestingly, underweight and normal-weight individuals showed higher prevalence of several chronic diseases. This finding is lower than what is commonly reported in studies emphasizing obesity as a major risk factor [17], but similar results have been observed in populations where chronic illness, malnutrition, and disease-related weight loss are common [18]. More than half of the participants resided in urban areas (56.4%). Urban residents exhibited higher prevalence of cardiovascular, cerebrovascular, and gastrointestinal diseases. This finding is in agreement with other studies that reported higher chronic disease burden in urban settings due to sedentary lifestyle, unhealthy dietary habits, psychological stress, and environmental pollution [19,20].

Married participants represented the majority of the sample (55.2%) and showed higher prevalence of chronic diseases compared to other social groups. This result is consistent with studies suggesting that marital responsibilities, increased stress, and age-related factors may contribute to higher disease prevalence among married individuals [21]. Participants with two or more children demonstrated higher rates of chronic diseases, which may reflect cumulative stress, reduced self-care, and limited time for healthy behaviors, as reported in previous studies [22].

With regard to occupation, housewives and unemployed participants constituted the largest proportions of the study sample. The association between occupation and chronic disease prevalence was statistically significant. Similar findings were reported in studies indicating that unemployment and retirement are associated with increased risk of chronic diseases due to physical inactivity, psychological stress, and limited access to health care services [23].

Educational level showed a significant association with chronic diseases, where participants with lower educational attainment exhibited higher prevalence. This finding agrees with studies reporting that low education is associated with poor health literacy, unhealthy lifestyle practices, and delayed health-seeking behavior [24,25].

##### Lifestyle-related risk factors

The present study revealed a high prevalence of physical inactivity, as nearly half of the participants (49.2%) reported no regular physical activity. This finding is consistent with WHO reports and other studies that identified physical inactivity as one of the leading risk factors for non-communicable diseases worldwide [12,26].

Sleep pattern analysis showed that most participants slept in the evening (89.2%), with a mean sleep duration of 5.17 ± 2.05 hours. More than one-third of participants slept less than four hours per day. This finding agrees with studies that linked short sleep duration to increased risk of hypertension, diabetes mellitus, cardiovascular disease, and psychological stress [27,28]. Smoking prevalence was remarkably high in the present study (70.4%). Cigarette smoking was the most common type, followed by vaping and shisha. Moderate smoking intensity was predominant. Similar high smoking rates and patterns have been reported in regional studies, which demonstrated strong associations between smoking and respiratory, cardiovascular, and cerebrovascular diseases [29,30].

Alcohol consumption and substance addiction were reported by a small proportion of participants. Although the prevalence was low, previous studies have shown that even limited exposure to alcohol and drugs may worsen disease outcomes and increase the risk of complications among patients with chronic diseases [31]. The majority of participants consumed coffee and tea (76.4%), with a high level of consumption reported by most of them. Excessive caffeine intake has been associated with sleep disturbance, anxiety, and increased cardiovascular risk in some studies [32].

Regarding mobile phone use, a considerable proportion of participants used their phones for more than three hours daily. Prolonged screen time has been associated with sedentary behavior, sleep disturbance, and increased psychological stress [33]. Psychological stress was commonly reported, with 50.8% experiencing stress sometimes and 22.0% always. This finding is consistent with studies identifying chronic stress as a major contributor to hypertension, cardiovascular disease, poor glycemic control, and unfavorable prognosis [34].

### **Prevalence and Pattern of Chronic Diseases**

The present study demonstrated a high prevalence of chronic diseases among the study sample. Joint diseases were the most frequently reported condition (80.0%), followed by cerebrovascular diseases (74.0%), cardiovascular diseases (70.4%), gastrointestinal diseases (66.4%), diabetes mellitus (57.2%), and hypertension (56.4%). Similar disease patterns were reported in other community-based studies where musculoskeletal and cardiovascular diseases were among the most prevalent chronic conditions [35,36].

A high proportion of participants reported a family history of chronic disease (80.0%), which is consistent with studies emphasizing the combined role of genetic susceptibility and shared environmental factors in chronic disease development [37]. Only 7.2% reported a past medical history, which may indicate underdiagnoses or late detection of chronic conditions.

Most participants reported disease onset within the last five years, suggesting a recent increase in chronic disease burden. This finding agrees with studies reporting a rising incidence of chronic diseases due to lifestyle transitions and urbanization [38]. Associated chronic diseases were present in one-third of participants, with hypertension combined with diabetes mellitus and coronary heart disease being the most common combinations. This clustering has been widely reported and reflects shared risk factors such as physical inactivity, smoking, stress, and unhealthy diet [39].

Despite the high prevalence of complications (96.4%), only a small proportion of participants reported using dietary supplements. Similar findings were reported in studies suggesting low awareness and inadequate

The present study demonstrated a statistically significant association between age and the prevalence of several chronic diseases, with the highest proportions observed among participants aged 38–47 and 48–57 years. This finding is consistent with previous studies which reported that middle-aged adults carry a higher burden of chronic diseases due to cumulative exposure to behavioral risk factors and progressive physiological decline [12,13]. Younger age groups showed lower prevalence rates, which is in agreement with findings reported in developing countries [14].

Regarding gender, males showed slightly higher percentages of most chronic diseases compared to females; however, the association was not statistically significant. This result agrees with several studies indicating that gender alone is not a strong determinant of chronic disease prevalence when lifestyle and socioeconomic factors are considered [15,16].

Body mass index was significantly associated with several chronic diseases. Interestingly, underweight and normal-weight participants demonstrated higher proportions of chronic diseases compared to overweight and obese individuals. Similar findings have been reported in studies suggesting that chronic disease-related weight loss, malnutrition, and long-standing illness may explain higher disease prevalence among underweight individuals in certain populations [17,18].

Residence was another important factor, as urban residents exhibited higher prevalence of cardiovascular, cerebrovascular, and gastrointestinal diseases compared to rural residents. This finding is consistent with studies reporting that urbanization is associated with sedentary behavior, unhealthy diet, psychological stress, and environmental pollution, all of which contribute to chronic disease development [19,20].

Marital status showed varying associations with chronic diseases. Married participants demonstrated the highest prevalence of most chronic conditions. This result agrees with studies suggesting that increased responsibilities, psychosocial stress, and age-related factors among married individuals may contribute to higher disease prevalence [21]. A statistically significant association was observed between the number of children and chronic diseases, particularly among participants with two children, who showed the highest prevalence. This finding is consistent with studies reporting that increasing family responsibilities may negatively affect self-care practices and increase stress levels [22].

Occupation was significantly associated with chronic disease prevalence. Housewives, unemployed, and retired participants showed higher proportions of chronic diseases compared to employed individuals. Similar findings were reported in studies indicating that unemployment and retirement are associated with reduced physical activity, increased stress, and poorer access to health care services [23].

Educational level showed a significant association with most chronic diseases. Participants with lower educational attainment had higher prevalence rates, which agrees with studies demonstrating that low education is associated with limited health literacy, unhealthy lifestyle behaviors, and delayed utilization of health services [24,25].

### **Lifestyle factors and chronic disease associations**

Physical inactivity was significantly prevalent, with nearly half of participants reporting no regular exercise. This finding is consistent with WHO reports identifying physical inactivity as a major contributor to non-communicable diseases worldwide [12,26].

Sleep duration and sleep timing were also important factors. Short sleep duration (<4 hours) was common and has been associated with increased risk of hypertension, diabetes, cardiovascular disease, and psychological stress, as reported in previous studies [27,28].

Smoking showed a strong association with several chronic diseases. Cigarette smoking was the most common form, followed by vaping and shisha, with moderate smoking intensity being predominant. These findings are consistent with regional and international studies linking smoking to respiratory, cardiovascular, and cerebrovascular diseases [29,30]. Alcohol consumption and substance addiction were reported by a small proportion of participants; however, previous studies indicate that even low levels of alcohol and drug use may worsen disease prognosis and increase complications among patients with chronic diseases [31]. High consumption of coffee and tea was common among participants. Excessive caffeine intake has been associated with sleep disturbance, anxiety, and cardiovascular effects in some populations [32]. Prolonged mobile phone use was also prevalent and has been linked to sedentary behavior, sleep disturbance, and increased psychological stress [33]. Psychological stress was frequently reported and showed a strong association with chronic diseases, which is consistent with studies identifying stress as a key

## **5. Conclusions**

This cross-sectional study of 250 adults attending Al-Ramadi hospital demonstrated a high burden of chronic disease within the study sample. Joint disease was the most frequently reported condition (80.0%), followed by cerebrovascular disease (74.0%), cardiovascular disease (70.4%), gastrointestinal disease (66.4%), cancer (64.8%), respiratory disease (60.4%), diabetes mellitus (57.2%), hypertension (56.4%), and renal disease (54.0%). A family history of chronic disease was reported by 80.0% of participants, while associated chronic diseases were present in 33.6%. The high proportion of reported complications further indicates the substantial clinical burden carried by this hospital-based sample.

Chronic disease prevalence varied across several sociodemographic characteristics, including age, body mass index, residence, marital and family characteristics, occupation, and educational level, whereas gender showed less consistent differences. At the same time, modifiable lifestyle risks were common: 49.2% of participants reported no regular physical activity, 70.4% were smokers, 35.6% reported sleeping less than four hours, and psychological stress was frequently reported. These findings show that the observed chronic disease burden occurred alongside a combination of social, demographic, familial, and behavioral factors rather than a single isolated determinant.

The findings support strengthening prevention and early-detection strategies that address smoking, physical inactivity, inadequate sleep, and psychological stress while also considering education, occupation, residence, and family history when planning health services. Targeted screening and health education may be particularly useful for groups with a higher observed disease burden. Because the study used a cross-sectional design and convenience sampling in a hospital setting, the results should be interpreted as associations within the study sample and should not be used to infer causality or precise population prevalence for all adults in Iraq.

## References

- WHO - Noncommunicable Diseases (NCDs)](<https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases>)
- Collins O. Airhihenbuwa ; Tung-Sung Tseng; Victor D. Sutton; LeShawndra Price, Global Perspectives on Improving Chronic Disease Prevention and Management in Diverse Settings , PREVENTING CHRONIC DISEASE P U B L I C H E A L T H R E S E A R C H , P R A C T I C E , A N D P O L I C Y V o l u m e 1 8 , E 3 3 A P R I L 2 0 2 1
- Inger Benkel , Maria Arnby and Ulla Molander, Living with a chronic disease: A quantitative study of the views of patients with a chronic disease on the change in their life situation,SAGE Open Medicine2020; 8: 1-7 .
- GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020; 396: 1204–1222.
- Davidson's Principles and Practice of Medicine, 24th Edition,2022, Elsevier, Chapters on Cardiovascular Disease, Diabetes, Obesity, Hypertension, Lipid Disorders, and Lifestyle Disease Prevention.
- World Health Organization. (2018). Noncommunicable diseases country profiles 2018. Geneva: WHO.
- Iraq; World Health Organization - Noncommunicable Diseases (NCD) Country Profiles, 2016
- Kidney Disease: Improving Global Outcomes (KDIGO). (2022). KDIGO 2022 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. <https://kdigo.org/guidelines/ckd-evaluation-and-management/>
- Petra Maresova, Ehsan Javanmardil, Sabina Barakovic, Jasmina Barakovic Husic, Signe Tomsone, Ondrej Krejcar and Kamil Kuca, Consequences of chronic diseases and other limitations associated with old age – A scoping review, *BMC Public Health* (2019) 19:1431
- Henk ten Have, Bert Gordijn, Having a chronic disease and being chronically ill, *Medicine, Health Care and Philosophy* (2025) 28:175–176
- Barbara Stacherl, Odile Sauzet Chronic disease onset and wellbeing development: longitudinal analysis and the role of healthcare access. *European Journal of Public Health* ,2023, 34; 1: 29–34
- World Health Organization. Noncommunicable diseases. WHO; 2023.
- Prince MJ, et al. The burden of disease in middle-aged and older adults. *Lancet*. 2015; 385:549–562.
- Nugent R. Chronic diseases in developing countries. *Ann N Y Acad Sci*. 2008; 1136:70–79.
- Bener A, et al. Gender differences in chronic diseases. *Int J Public Health*. 2013; 58:173–183.
- Al-Raddadi R, et al. Noncommunicable disease risk factors. *BMC Public Health*. 2019; 19:1–10.
- Landi F, et al. Underweight status and chronic disease outcomes. *Clin Nutr*. 2016; 35:509–514.
- Kim JY, et al. BMI and chronic disease risk. *J Epidemiol*. 2017; 27:405–412.
- Allender S, et al. Urbanization and chronic disease. *J Urban Health*. 2010; 87:938–951.
- Yusuf S, et al. Global cardiovascular risk factors. *Lancet*. 2004;364: 937–952.
- Robards J, et al. Marital status and health outcomes. *Soc Sci Med*. 2012; 74:1340–1347.
- Nomaguchi KM, Milkie MA. Parenthood and health. *J Health Soc Behav*. 2003; 44:121–135.
- Marmot M, et al. Employment status and health. *Lancet*. 2008; 372:1661–1669.
- Cutler DM, Lleras-Muney A. Education and health. *J Econ Perspect*. 2006; 20:77–98.

- Berkman ND, et al. Health literacy and health outcomes. *Ann Intern Med.* 2011; 155:97–107.
- Lee IM, et al. Physical inactivity and disease burden. *Lancet.* 2012; 380:219–229.
- Cappuccio FP, et al. Sleep duration and health. *Eur Heart J.* 2011; 32:1484–1492.
- Shan Z, et al. Sleep duration and diabetes risk. *Diabetes Care.* 2015; 38:529–537.
- WHO. Tobacco and chronic disease. WHO; 2022.
- U.S. Department of Health and Human Services. The health consequences of smoking. 2014.
- Rehm J, et al. Alcohol use and chronic disease. *Lancet.* 2009; 373:2223–2233.
- Grosso G, et al. Coffee consumption and health. *Nutrients.* 2017; 9:1–20.
- Thomee S, et al. Mobile phone use and mental health. *BMC Public Health.* 2011; 11:66.
- Steptoe A, Kivimäki M. Stress and cardiovascular disease. *Nat Rev Cardiol.* 2012; 9:360–370.



## The Awareness Study of Thyroid Diseases among Iraqi Patients

BADEA' A THAMER YAHYA

Department of Family and Community Medicine, Medical College, University of Anbar, Iraq  
[Med.badeaa.thamer@unanbar.edu.iq](mailto:Med.badeaa.thamer@unanbar.edu.iq)

### Abstract.

**Background:** Thyroid diseases are more common worldwide among chronic illnesses, a clinical condition that coexists with anemia. Even though anemia and thyroid problems frequently coexist, it is still unclear what causes the two conditions. Even in the 21st century, very few people really know much about the small thyroid gland located at the back of the neck and its vitally important function. Any dysfunction of the thyroid has profound impact on health and well-being. Iron-deficient anemia adversely affects thyroid hormone status, whereas thyroid hormones directly and indirectly promote the proliferation of erythrocyte precursors through the augmentation of erythropoietin synthesis. As a result, many types of anemia may arise after thyroid failure.

**Material and Method:** A Descriptive cross-sectional study for awareness of thyroid diseases and anemia was carried out in Al-Ramadi city at the Al-Andalus health center from 18th of November to the end of March 2023. Two hundred fifty participants at age 18- >65 years were enrolled in the study. They were selected by non-probability convenient sampling technique. Data were analyzed using.

**Results:** The illiterate and secondary were (32%). The knowledge regarding thyroid gland was (30.4%). About (10%) knew that the function of thyroid hormone affects the menstrual cycle in women.

**Conclusion:** Knowledge regarding thyroid gland was fairly worse.

The knowledge of prevention of thyroid disease regarding early thyroid testing can protect you from thyroid disease was unsatisfactory. The awareness about pregnancy and the postpartum period are risk factors for developing thyroid diseases was also fairly unsatisfactory. However, more strategies are needed to increase the knowledge about the thyroid and thyroid disease.

**Keywords:** Thyroid diseases; awareness; anemia; prevention; risk factors; Iraqi patients.

### 1. Introduction

The thyroid gland is butterfly-shaped and the largest endocrine gland in the human body, and it is positioned in the anterior portion of the neck. It produces thyroid hormones, which have a significant impact on protein synthesis and the basal metabolic rate (BMR) (1) (2). Furthermore, these hormones are essential for the neurocognitive development of young children and adolescents, as well as for adults to maintain normal physiological functioning (3).

In the past few years, abnormal thyroid function has become a major problem in clinical practice, raising health issues among patients. Thyroid hormones are essential for growth, neuronal development, reproduction and regulation of energy metabolism. Hypothyroidism and hyperthyroidism are common conditions with potentially devastating health consequences that affect all populations worldwide. Iodine nutrition is a key determinant of thyroid disease risk; however, other factors, such as ageing, smoking status, genetic susceptibility, ethnicity, endocrine disruptors and the advent of novel therapeutics, including immune checkpoint inhibitors, also influence thyroid disease epidemiology. (4). Thyroid illness is caused by a deficiency in iodine or by autoimmune diseases. The thyroid gland plays a significant role in the metabolism and proliferation of blood cells; hematological disorders are frequently observed in patients with thyroid disorders, and the most frequent problem is anemia. Thyroid dysfunction is one of the leading endocrine disorders. It represents around 30% to 40% of the patients seen in an endocrine practice. (5) (6).

Thyroid problems are mostly caused by iodine insufficiency. Over 190 million people worldwide have been estimated to suffer from iodine deficiency illnesses, and approximately one-third of all people live in areas that are iodine deficient (7) Thyroid disease is becoming increasingly common worldwide, and females are more likely to be affected than males. The prevalence of thyroid among middle-aged women was 7%. The major cause of (subclinical) hypothyroidism in Europe and is more prevalent in women with idiopathic subfertility and polycystic ovarian syndrome (PCOS) than in fertile women (8) The thyroid nodule is the most common type of the condition, accounting for 19-68% of the general population. It is more frequently seen in women. THYROID NODULES ARE A common clinical problem. Epidemiologic studies have shown the prevalence of palpable thyroid nodules to be approximately 5% in women and 1% in men living in iodine-sufficient parts of the world (9).

Thyroid diseases are one of the most under-diagnosed and neglected medical illnesses, and patients' lack of general awareness may be an important concern. In general, lack of knowledge and comprehension of the thyroid gland and its symptoms will lead patients to go undiagnosed. Some of the factors may be lack of qualified physicians treating such patients, less time spent by doctors for patient education, lack of awareness and use of available electronic media to harness information, poor knowledge about reliable sources of information. Educational needs are much greater in diseased population with poor health literacy. Any dysfunction of the thyroid has profound impact on health and well-being. Thyroid disorders are believed to be a common health issue in India, as it is worldwide. Globally, thyroid disorders continue to be common yet one of the most under- diagnosed and neglected chronic health conditions (10).

Anemia and low thyroid function are common, especially in older individuals. Thyroid function measurements are often included in the clinical examination of anemia. The symptoms of (subclinical) hypothyroidism and anemia are similar, which not only frequently presents a challenging diagnostic problem, but may also point to a causal relationship between low thyroid function and anemia (11). Both hyperthyroidism and hypothyroidism were associated with reduced hemoglobin levels in a population-based cohort in cross-sectional analyses but not in longitudinal analyses (12).

Aim of the study: To study the awareness study of thyroid diseases and anemia

## **2. Research Methodology**

### **2.1 Study design**

A cross-sectional study for awareness of thyroid diseases and anemia among Iraqi patients. This study was carried out in Al – Ramadi city at the Al – Andalus health center. For the period 18th of November to end of March 2023. The study was conducted on women who visit antenatal care. The study was approved by College of Medicine University of Anbar. The sample size was two hundred and fifty, selected by non-probability convenient sampling technique.

### **2.2 Data collection**

After taking informed verbal consent, the specially designed questionnaire was administered through interviewing women with closed ended questions related to the awareness study of thyroid diseases and anemia. The questionnaire was divided into

Three parts: part one was demographic data included:

Gender, age, education, occupation...etc.; part two: Knowledge about thyroid disease. Part three for awareness about the prevention of thyroid disorders.

### **2.3 Statistical analysis**

The statistical analysis was conducted in the form of percentages. The statistical analysis was carried out by using Statistical Analysis System (SAS) version 9.6

### 3. Results

The study sample is two hundred and fifty participants who were interviewed. They were from Al – Andalus health center. Table 1 shows the gender distribution; the highest frequency were females (76.4%). The age range of participants were ranges from 18 to more than 65. The highest proportion were 18 – 40 years old (55.2 %), while the lowest frequency (2.8 %) at the age more than 65 years. The Table also shows education that ranges from the lowest frequency (0.8%) among post graduate

And the illiterate and secondary groups have the same highest frequency (32%).

Occupation wise, the study sample was composed of house wives that representatives (62%) and the employed (14.8%). Regarding marital status, the highest frequency (72%) was married, while the lowest frequency was divorce that representatives (2.4%). About (60.4%) of participants were having one to four number of family member. Table 1 also shows the distribution for participants having thyroid disease, the lowest proportion were hypothyroidism (11.6%) while those who didn't know about thyroid disease (49.6%). About (80.4%) of participants were haven't had thyroid test. About (27.2%) of participants were usage medication to treat thyroid problem. Only (8%) of participants have family history of throid disease

| Variables                                                | No. | %    |
|----------------------------------------------------------|-----|------|
| <b>Sex</b>                                               |     |      |
| Male                                                     | 59  | 23.6 |
| Female                                                   | 191 | 76.4 |
| <b>Age (years)</b>                                       |     |      |
| (18 – 40)                                                | 138 | 55.2 |
| (41 – 65)                                                | 105 | 42   |
| (>65)                                                    | 7   | 2.8  |
| <b>Education</b>                                         |     |      |
| Illiterate                                               | 80  | 32   |
| Primary                                                  | 72  | 28.8 |
| Secondary                                                | 80  | 32   |
| University                                               | 16  | 6.4  |
| Postgraduate                                             | 2   | 0.8  |
| <b>Occupation</b>                                        |     |      |
| Housewife                                                | 155 | 62   |
| Employee                                                 | 37  | 14.8 |
| Student                                                  | 58  | 23.2 |
| <b>Married status</b>                                    |     |      |
| Married                                                  | 180 | 72   |
| Single                                                   | 64  | 25.6 |
| Divorce                                                  | 6   | 2.4  |
| <b>Number of family members</b>                          |     |      |
| (1 – 4)                                                  | 151 | 60.4 |
| (5 – 8)                                                  | 95  | 38   |
| (> 8)                                                    | 4   | 1.6  |
| <b>You have thyroid disease?</b>                         |     |      |
| No                                                       | 67  | 26.8 |
| Hypothyroidism                                           | 29  | 11.6 |
| Hyperthyroidism                                          | 30  | 12   |
| I don't know                                             | 124 | 49.6 |
| <b>Have you had thyroid tests?</b>                       |     |      |
| Yes                                                      | 49  | 19.6 |
| No                                                       | 201 | 80.4 |
| <b>Do you use medications to treat thvroid problems?</b> |     |      |
| Yes                                                      | 68  | 27.2 |
| No                                                       | 182 | 72.8 |
| <b>You have a family history of thyroid disease?</b>     |     |      |
| Yes                                                      | 20  | 8    |
| No                                                       | 101 | 40.4 |
| I don't Know                                             | 129 | 51.6 |

**Table 1:** The distribution of socio-demographic characteristics of the study sample

From two hundred and fifty participants interviewed, (30.4%) responded that they have heard of thyroid disease and (58.4%) didn't hear of the function of thyroid hormone affects the menstrual cycle in women. While those who knew the function of thyroid hormone affects the menstrual cycle in women have the lowest frequency (10%) as shown in Table 2.

Table 2 also shows the knowledge of hyperthyroidism, most of participants (70%) did not hear about hyperthyroidism. Only (9.6%) knew hyperthyroidism and (20.4%) didn't know there. knowledge of hypothyroidism, most of participants (70%) did not hear about hypothyroidism. Only (8%) knew hypothyroidism and (21.6%) didn't know there. Less than three quarter of participants (71.6%) didn't hear about unstopped treatment of hypothyroidism when thyroid test is normally. More than half (66.8%) didn't hear about unstopped treatment for thyroid disease when test are normal.

The participants who didn't hear that women with hypothyroidism get pregnant (57.6%), and those who knew the women with hypothyroidism can get pregnant (1.6 %). Almost (41.6%) did not know the euthyroid.

More than half (56%) didn't hear that the thyroid gland is an endocrine gland. About (68.8%), didn't hear that thyroid dysfunction affected brain. Only (2%) knew that thyroid dysfunction affects cholesterol levels and (42%) didn't know them. More than half (55.2%) didn't hear that thyroid dysfunction affects heart disease, while less than half (42%) didn't know that thyroid dysfunction affects heart disease. The participants who knew that exercise affects thyroid dysfunction have constituted (4%) and those who didn't hear that exercise affect thyroid function. About (6.8%) knew that iodized salt has a role in treating hypothyroidism, and (8%) know that iodine helps secrete thyroxine. Less than half (49.2%) Did not hear that the cause of thyroid disease can be hereditary, while (6%) knew them as shown in table 2.

Table 2 also shows that (10%) of study sample who were know weight gain is a symptom of hypothyroidism, (38.4%) did not know, while (51.6%) had not hear. The knowledge for presence of a lump in the neck could be a sign of thyroid disease or thyroid cancer (10%), while difficulty swallowing may be a sign of thyroid cancer (6.8%). More than half (52%) did not hear that skin changes, nails or hair loss could be signs of thyroid disease, and only (2%) knew them. Most of participants (56%) did not hear that stomach pain could be a symptom of thyroid disease. The knowledge for Feeling hot, losing weight despite a good appetite, insomnia, and lack of sleep are symptoms of hyperthyroidism that (2%). Almost (7.6%) knew that increased heart rate symptoms of hyperthyroidism, the knowledge of interruption of the menstrual cycle is a symptom of hyperthyroidism (3.2%). Only (6.8%, 2%) knew hyperthyroidism and hypothyroidism are accompanied by anemia respectively. More than half (56%) did not hear that feeling cold is a symptom of hypothyroidism, while (41.6%) did not know them. About (6%) of participants knew that miscarriage is a symptom of hypothyroidism, while (41.6%) did not know. Almost (2%) knew that bulging eyes could be a sign of thyroid disease. Medical health center considered the major source of knowledge of thyroid disease (46.8%), while the lowest source included learning (3.2%) as shown in Table 2.

**Table 2:** The distribution of knowledge about thyroid diseases among study sample

| Variable                                                                                       | No. | %    |
|------------------------------------------------------------------------------------------------|-----|------|
| <u>Have you heard of the thyroid gland?</u>                                                    |     |      |
| I know                                                                                         | 76  | 30.4 |
| I don't know                                                                                   | 62  | 24.8 |
| I don't hear                                                                                   | 112 | 44.8 |
| <u>Did you know that the function of thyroid hormone affects the menstrual cycle in women?</u> |     |      |
| I know                                                                                         | 25  | 10   |
| I don't know                                                                                   | 79  | 31.6 |
| I don't hear                                                                                   | 146 | 58.4 |
| <u>Do you know what hyperthyroidism is?</u>                                                    |     |      |
| I know                                                                                         | 24  | 9.6  |
| I don't know                                                                                   | 51  | 20.4 |
| I don't hear                                                                                   | 175 | 70   |

|                                                                                            |     |      |
|--------------------------------------------------------------------------------------------|-----|------|
| Do you know what hypothyroidism is?                                                        |     |      |
| I know                                                                                     | 20  | 8    |
| I don't know                                                                               | 54  | 21.6 |
| I don't hear                                                                               | 175 | 70   |
| Can you stop treating hypothyroidism when the tests are normal?                            |     |      |
| I know                                                                                     | 3   | 1.2  |
| I don't know                                                                               | 68  | 27.2 |
| I don't hear                                                                               | 179 | 71.6 |
| Can you stop treating your thyroid when tests are normal?                                  |     |      |
| I know                                                                                     | 6   | 2.4  |
| I don't know                                                                               | 77  | 30.8 |
| I don't hear                                                                               | 167 | 66.8 |
| Can women with hypothyroidism get pregnant normally?                                       |     |      |
| I know                                                                                     | 4   | 1.6  |
| I don't know                                                                               | 102 | 40.8 |
| I don't hear                                                                               | 144 | 57.6 |
| Do you know what the euthyroid activity?                                                   |     |      |
| I know                                                                                     | 46  | 18.4 |
| I don't know                                                                               | 104 | 41.6 |
| I don't hear                                                                               | 100 | 40   |
| Did you know that the thyroid gland is an endocrine gland?                                 |     |      |
| I know                                                                                     | 5   | 2    |
| I don't know                                                                               | 105 | 42   |
| I don't hear                                                                               | 140 | 56   |
| Did you know that thyroid dysfunction affects the brain?                                   |     |      |
| I know                                                                                     | 15  | 6    |
| I don't know                                                                               | 63  | 25.2 |
| I don't hear                                                                               | 172 | 68.8 |
| Did you know that thyroid dysfunction affects cholesterol levels?                          |     |      |
| I know                                                                                     | 5   | 2    |
| I don't know                                                                               | 105 | 42   |
| I don't hear                                                                               | 140 | 56   |
| Did you know that thyroid dysfunction affects heart disease?                               |     |      |
| I know                                                                                     | 7   | 2.8  |
| I don't know                                                                               | 105 | 42   |
| I don't hear                                                                               | 138 | 55.2 |
| Did you know that exercise affects thyroid dysfunction?                                    |     |      |
| I know                                                                                     | 10  | 4    |
| I don't know                                                                               | 105 | 42   |
| I don't hear                                                                               | 135 | 54   |
| Does iodized salt have a role in treating hypothyroidism?                                  |     |      |
| I know                                                                                     | 17  | 6.8  |
| I don't know                                                                               | 105 | 42   |
| I don't hear                                                                               | 128 | 51.2 |
| Did you know that iodine helps secrete the hormone thyroxine?                              |     |      |
| I know                                                                                     | 20  | 8    |
| I don't know                                                                               | 104 | 41.6 |
| I don't hear                                                                               | 126 | 50.4 |
| Did you know that the cause of thyroid disease can be hereditary?                          |     |      |
| I know                                                                                     | 15  | 6    |
| I don't know                                                                               | 112 | 44.8 |
| I don't hear                                                                               | 123 | 49.2 |
| Did you know that weight gain is a symptom of hypothyroidism?                              |     |      |
| I know                                                                                     | 25  | 10   |
| I don't know                                                                               | 96  | 38.4 |
| I don't hear                                                                               | 129 | 51.6 |
| Do you think that a lump in the neck could be a sign of thyroid disease or thyroid cancer? |     |      |
| I know                                                                                     | 25  | 10   |
| I don't know                                                                               | 96  | 38.4 |
| I don't hear                                                                               | 129 | 51.6 |

|                                                                                                          |     |      |
|----------------------------------------------------------------------------------------------------------|-----|------|
| Did you know that persistent difficulty swallowing may be a sign of thyroid cancer?                      |     |      |
| I know                                                                                                   | 17  | 6.8  |
| I don't know                                                                                             | 104 | 41.6 |
| I don't hear                                                                                             | 129 | 51.6 |
| Do you think that skin changes, nails or hair loss could be signs of thyroid disease?                    |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 115 | 46   |
| I don't hear                                                                                             | 130 | 52   |
| Do you believe that stomach pain could be a symptom of thyroid disease?                                  |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 105 | 42   |
| I don't hear                                                                                             | 140 | 56   |
| Did you know that feeling hot and losing weight despite a good appetite is a symptom of hyperthyroidism? |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 106 | 42.4 |
| I don't hear                                                                                             | 139 | 55.6 |
| Did you know that insomnia and lack of sleep are symptoms of hyperthyroidism?                            |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 108 | 43.2 |
| I don't hear                                                                                             | 137 | 54.8 |
| Did you know that increased heart rate is a symptom of hyperthyroidism?                                  |     |      |
| I know                                                                                                   | 19  | 7.6  |
| I don't know                                                                                             | 115 | 46   |
| I don't hear                                                                                             | 116 | 46.4 |
| Did you know that interruption of the menstrual cycle is a symptom of hyperthyroidism?                   |     |      |
| I know                                                                                                   | 8   | 3.2  |
| I don't know                                                                                             | 110 | 44   |
| I don't hear                                                                                             | 132 | 52.8 |
| Did you know that hyperthyroidism is accompanied by anemia?                                              |     |      |
| I know                                                                                                   | 17  | 6.8  |
| I don't know                                                                                             | 113 | 45.2 |
| I don't hear                                                                                             | 120 | 48   |
| Did you know that hypothyroidism is accompanied by anemia?                                               |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 127 | 50.8 |
| I don't hear                                                                                             | 123 | 49.2 |
| Did you know that feeling cold is a symptom of hypothyroidism?                                           |     |      |
| I know                                                                                                   | 16  | 6.4  |
| I don't know                                                                                             | 94  | 37.6 |
| I don't hear                                                                                             | 140 | 56   |
| You know that miscarriage is a symptom of hypothyroidism?                                                |     |      |
| I know                                                                                                   | 15  | 6    |
| I don't know                                                                                             | 104 | 41.6 |
| I don't hear                                                                                             | 131 | 52.4 |
| You think that bulging eyes could be a sign of thyroid disease?                                          |     |      |
| I know                                                                                                   | 5   | 2    |
| I don't know                                                                                             | 105 | 42   |
| I don't hear                                                                                             | 140 | 56   |
| Sources of knowledge                                                                                     |     |      |
| TV                                                                                                       | 30  | 12   |
| Social media                                                                                             | 45  | 18   |
| Friends                                                                                                  | 50  | 20   |
| Medical health center                                                                                    | 117 | 46.8 |
| Learning                                                                                                 | 8   | 3.2  |

Regarding the awareness about the prevention of thyroid disorders, that a balanced diet protects you from thyroid disease, don't hear by 130 (52%). 35 (14%) of participants knew that early thyroid testing and avoiding smoking and stimulants protected them from thyroid demands as shown in Table 3.

**Table 3:** Awareness regarding preventive behaviors of participants

| Variable                                                                                                              | No. | %    |
|-----------------------------------------------------------------------------------------------------------------------|-----|------|
| <u>Did you know that a balanced diet protects you from thyroid disease?</u>                                           |     |      |
| I know                                                                                                                | 16  | 6.4  |
| I don't know                                                                                                          | 104 | 41.6 |
| I don't heard                                                                                                         | 130 | 52   |
| <u>Did you know that early thyroid testing can protect you from thyroid disease?</u>                                  |     |      |
| I know                                                                                                                | 35  | 14   |
| I don't know                                                                                                          | 77  | 30.8 |
| I don't heard                                                                                                         | 138 | 55.2 |
| <u>Did you know that reducing foods containing soy protein protects you from thyroid demands?</u>                     |     |      |
| I know                                                                                                                | 5   | 2    |
| I don't know                                                                                                          | 77  | 30.8 |
| I don't heard                                                                                                         | 168 | 67.2 |
| <u>Did you know that avoiding large amounts of iodine in foods and medications protects you from thyroid demands?</u> |     |      |
| I know                                                                                                                | 10  | 4    |
| I don't know                                                                                                          | 85  | 34   |
| I don't heard                                                                                                         | 155 | 62   |
| <u>Did you know that avoiding smoking and stimulants protects you from thyroid disorders?</u>                         |     |      |
| I know                                                                                                                | 35  | 14   |
| I don't know                                                                                                          | 87  | 34.8 |
| I don't heard                                                                                                         | 128 | 51.2 |

Awareness about the risk factors for thyroid disorders 125 (50%), 130 (52%), 120 (48%), and 104 (41.6%) of individuals don't hear about smoking & women, exposure to radiation, insufficient or excess iodine intake, pregnancy, or the postpartum period, respectively as shown in Table 4.

**Table 4:** Displays the participants of their risk factor.

| Variable                                                                                                       | No. | %    |
|----------------------------------------------------------------------------------------------------------------|-----|------|
| <u>Do you think that smoking is a risk factor for thyroid disease?</u>                                         |     |      |
| I know                                                                                                         | 35  | 14   |
| I don't know                                                                                                   | 90  | 36   |
| I don't heard                                                                                                  | 125 | 50   |
| <u>Do you believe that exposure to radiation is a risk factor for developing thyroid diseases?</u>             |     |      |
| I know                                                                                                         | 15  | 6    |
| I don't know                                                                                                   | 105 | 42   |
| I don't heard                                                                                                  | 130 | 52   |
| <u>Do you think that insufficient or excess iodine intake is a risk factor for thyroid disease?</u>            |     |      |
| I know                                                                                                         | 20  | 8    |
| I don't know                                                                                                   | 110 | 44   |
| I don't heard                                                                                                  | 120 | 48   |
| <u>Do you think that women are more susceptible to thyroid diseases?</u>                                       |     |      |
| I know                                                                                                         | 20  | 8    |
| I don't know                                                                                                   | 105 | 42   |
| I don't heard                                                                                                  | 125 | 50   |
| <u>Do you think that pregnancy and the postpartum period are risk factors for developing thyroid diseases?</u> |     |      |
| I know                                                                                                         | 41  | 16.4 |
| I don't know                                                                                                   | 105 | 42   |
| I don't heard                                                                                                  | 104 | 41.6 |

#### 4. Discussion

The current study has focused on the awareness study of thyroid diseases and anemia. The findings of this study revealed a highest proportion at age 18- 40 years old (55.2%), this result higher than that reported by other studies (13). Most participants were female, this finding agree with (13).

The present study showed that (44.8%) of participants don't hear the thyroid gland, less than (1.6%) know women with hypothyroidism can get pregnant normally, according to a study done in Delhi that shows similar results to this study (13) but in a study by Singh *et al.*, 60% patients knew the correct meaning of the term "thyroid." (14) .

Weight gain is a symptom of hypothyroidism but don't know by 38.4%; the presence of a lump in the neck could be a sign of thyroid disease or thyroid cancer; and difficulty swallowing may be a sign of thyroid cancer (51.6%) of individuals don't hear that and that similar study in Saudi Arabia that found (39.4%) had no idea about thyroid cancer (15).

The current study demonstrates that there is no significant difference between genders in the level of awareness regarding thyroid diseases and that, similar to the study by Almuzaini A *et al.*, there is no significant difference in knowledge levels among genders in the level of knowledge (16).

Knowledge the risk factors associated with thyroid diseases is an essential aspect, smoking is thought to increase the risk of thyroid disorders in a variety of physiological and pathological contexts (17).

According to the present study, just 8% of respondents thought that being female increased the possibility of developing thyroid disease, that's acceptable, according to a study done in Saudi Arabia, 21% of participants strongly agreed that having a female partner is a risk factor. These findings suggest that most people are unaware of this, which could delay getting a diagnosis (18).

Past medical history and past family history were both significantly correlated with better knowledge regarding thyroid disorders. This study shows that 8% thought that past family history was correlated with thyroid dysfunction, and only about 8.8% thought that thyroid dysfunction was associated with anemia. This corresponds with what Canaris *et al.* described in their article, which showed that a higher prevalence of thyroid diseases is associated with an increase in the level of knowledge about the manifestation of these conditions and a higher willingness to participate in thyroid screening efforts (19).

Consistent with the present findings, that smoking is a risk factor for thyroid disease (14%). This result disagree with other study (20). The findings of exposure to radiation is a risk factor for developing thyroid diseases revealed a lowest proportion (6%), this result lowest than that reported by (20).

#### 5. Conclusion

- The study revealed that the knowledge of participants regarding heard of thyroid disease is fairly worse 30.4%.
- The knowledge of prevention of thyroid disease regarding early thyroid testing can protect you from thyroid disease was unsatisfactory as 14%.
- The awareness about pregnancy and the postpartum period are risk factors for developing thyroid diseases was also fairly unsatisfactory as 16.4%.
- Most participants had not heard of either hyperthyroidism or hypothyroidism (70% for each), indicating limited awareness of the main thyroid disorders.
- Awareness of the relationship between thyroid dysfunction and anemia was very low: only 6.8% recognized an association with hyperthyroidism and 2% with hypothyroidism.
- Most participants (80.4%) had never undergone thyroid testing, while medical health centers were the leading source of information about thyroid disease (46.8%).

## 6. Recommendations

- Further studies are needed including follow up studies for non-heard of thyroid disease.
  - Government involvement in fornication of food like our, salt with iodine is the need of the day.
  - There is a need for a public health education initiative
- And more strategies to increase the awareness of thyroid disease.

## References

1. Alyahya A, AlNaim A, AlBahr AW, Almansour F, Elshebiny A. Knowledge of thyroid disease manifestations and risk factors among residents of the Eastern Province, Saudi Arabia. *Cureus*. 2021, p. 13:e13035. 10.7759/cureus.13035.
2. Meng Z, Liu M, Zhang Q, Liu L, Song K, Tan J, et al. Gender and age impacts on the association between thyroid function and metabolic syndrome in Chinese. *Medicine*. 2015, p. 94:e2193.
3. AM., Abdulrahman Ibrahim. Survey of awareness of thyroid disorders among the Riyadh population, Central Region of Saudi Arabia. 2018, pp. ;72:4039–4044.
4. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, et al. Global epidemiology of hyperthyroidism and hypothyroidism. *Nat Rev Endocrinol*. 2018, pp. 14:301–16. 10.1038/nrendo.2018.18.
5. SA., Alqahtani. Prevalence and characteristics of thyroid abnormalities and its association with anemia in ASIR Region of Saudi Arabia. 2021, pp. 11:494–504.
6. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. 2014, pp. 99:923–31. 10.1210/jc.2013-2409.
7. Assessment of the iodine deficiency disorders and monitoring their elimination. [https://apps.who.int/iris/bitstream/handle/10665/43781/9789241595827\\_eng.pdf](https://apps.who.int/iris/bitstream/handle/10665/43781/9789241595827_eng.pdf). 2021.
8. Poppe K, Bisschop P, Fugazzola L, Mintziori G, Unuane D, Weghofer A. Erratum. European thyroid association guideline on thyroid disorders prior to and during assisted reproduction. 2021, p. 10: 268.
9. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. 2016, pp. 26:1–133.
10. Rai S, Sirohi S, Khatri AK, Dixit S, Saroshe S. Assessment of knowledge and awareness regarding thyroid disorders among women of a cosmopolitan city of Central India. 2016, pp. ;7:219–222.
11. den Elzen WP, de Craen AJ Mooijaart SP, Gussekloo J. Low thyroid function and anemia in old age: the Leiden 85-plus study. 2015, pp. 63:407–409.
12. Floriani C, Feller M, Aubert CE, M'Rabet-Bensalah K, Collet TH, den Elzen WPJ, Bauer DC, Angelillo-Scherrer A, Aujesky D, Rodondi N. Thyroid dysfunction and anemia: a prospective cohort study and a systematic review. 2018, pp. ;28(5):575–582.
13. Pradeep Kumar, Deepak Khandelwal, Suresh Mittal, Deep Dutta, Sanjay Kalra, Pratima Katiyar, and Vivek Aggarwal. Knowledge, Awareness, Practices and Adherence to Treatment of Patients with Primary Hypothyroidism in Delhi 2017 May-Jun; 21(3): 429–433.
14. Singh A, Sachan B, Malik NP, Sharma VK, Verma N, Singh CP. Knowledge, awareness and practices (KAP) among patients with thyroid swelling attending Cytology Clinic in a Medical College 2013;1:793–5.
15. A. Alhazmi, Abdullah M. Alobaid, Saqer Mohammed Althunayyan, Wajid Syed and Mahmood Basil A. Al-Rawi. A cross-sectional assessment of knowledge, awareness of risk factors, and perceptions of thyroid disease (TD) among adults living in Saudi Arabia – A community based study. 2022
16. Almuzaini A, Alshareef B, Alghamdi S. Assessment of knowledge and awareness regarding thyroid disorders among Saudi people. pp. et al. *IJMDC*. 2019; 3:100–106.
17. WM., Wiersinga. Smoking and thyroid. pp. 2013; 79:145–151.
18. Aladwani S, Alosaimi M, Muammar M. A cross-sectional survey to assess knowledge, attitude, and practices in patients with hypothyroidism in Riyadh, Saudi Arabia. pp. 2019; 8:153–160.
19. Canaris GJ, Tape TG, Wigton RS. Thyroid disease awareness is associated with high rates of identifying subjects with previously undiagnosed thyroid dysfunction. 2013; 13:351.

20. Syed W, Samarkandi OA, Alsadoun A, Harbi MKA, Al-Rawi MBA. Evaluation of clinical knowledge and perceptions about the development of thyroid cancer-An observational study of healthcare undergraduates in Saudi Arabia. *Front Public Health*. 2022, p. 10:912424.



## Maternal-Infant Bonding in the Postpartum Period

ASALA RIYADH MOHAMMED

College of Nursing, University of Thi-Qar, Dhi Qar, Iraq

asala.riyadh@utq.edu.iq

<https://orcid.org/0009-0001-5927-4116>

### Abstract

**Background:** One of the most important predictors of a child's emotional, cognitive, and social development is the relationship between mother and child during the postnatal period; any disruptions in this process may cause long-term bonding issues for both the mother and their child.

**Aim:** To determine the level of maternal-infant bonding among Iraqi postpartum mothers and to look at the correlations between bonding and specific demographic characteristics such as age, education, economic position, and birth order.

**Method:** Study was a descriptive cross-sectional, which carried out at Bint Al-Huda Teaching Hospital in Nasiriyah, Iraq, extended from July to December 2025. The Postpartum Bonding Questionnaire (PBQ), translated into Arabic, was administered to the carefully chosen sample of 108 postpartum women in Nasiriyah. SPSS version 26 was used to analyze the data by using descriptive and inferential statistics.

**Results:** The study's findings which related to mothers' bonding, showed that 74.1% had excellent, 22.2% were moderate, and 3.7% of mothers had low attachment. In addition, the economic status ( $P = 0.04$ ), maternal age ( $P = 0.02$ ), the child's birth order ( $P = 0.01$ ), and were all statistically substantially correlated with bonding levels. There were no significant correlations with residence or education.

**Conclusion:** The current study referred that the level of Maternal-Infant Bonding was generally adequate, a small percentage of mothers exhibited low levels of this bond, attributed to young age, first birth, and economic status. These findings suggest the need for early screening and tailored psychological interventions in postpartum care to maintain healthy mother-infant bonds and prevent any potential problems in this bond.

**Keywords:** Infant, Bonding, Nursing, Maternity, Postpartum, Pediatric.

### 1. Introduction

The mother and infant's bond developed throughout the postpartum phase is arguably one of the most crucial foundations of early-age child development. The mother-infant bond is one part of the more general attachment system. It forms early within hours or days after delivery and helps the infant securely attach, manage emotions, and develop both socially and cognitively (Liu et al., 2023; Keller and Kärtner, 2021). During this sensitive period, the elevation of the maternal hormone oxytocin triggers soothing and calming behaviors such as gentle stroking and soft vocalizations, which strengthen the mother's and infant's emotional attachment (Scatliffe et al., 2022; Vittner and McGrath, 2021).

Furthermore, the bond between mother and infant influences the infant's development as well as the mother's psychological health. Emotional connection between the mother and child improves the intimacy and sensitivity of the mother's caregiving (Dubber et al., 2022; Prevatt and Desmarais, 2023). On the other hand, bonding disruptions due to trauma experienced during birth, postpartum mental health issues, hormonal dysregulations, or social support deficits can lead to postpartum bonding disorder. The mother may in these cases feel overwhelmed, emotionally and

socially distant, which deteriorates the quality of caregiving and infant development (Wang et al., 2022; Pilkington et al., 2021).

The advanced technique in neuro-imaging have showed the brain regions involved in maternal attachment such as the amygdala, prefrontal cortex, and striatum. These findings suggest that maternal-infant attachment is not simply a behavioral-emotional process but is also neurobiological and influenced by intrinsic and extrinsic factors (Feldman et al., 2022; Swain and Ho, 2021). Understanding these neural pathways could offer valuable insight into the enormous effect of maternal bonding on the mother's and child's brain development.

Maternal-infant bonding does not have the same quality in all societies and even differs within the same society depending on healthcare access. Iraq and many other low- to middle-income countries (LMICs) have restricted or unequal access to perinatal care. Mental health stigma, culture, and barriers to healthcare all shape the maternal experience (Ahmed and Al-Jawadi, 2023; Al-Hamad and Al-Nashmi, 2021). Understanding these barriers is vital for formatting the bonding and maternal mental health-focused interventions that are sensitive to the culture.

## **2. Methodology**

### **2.1 Study Design**

A descriptive cross-sectional approach used in the current study to assess postpartum maternal-infant bonding and evaluate the presence of bonding disorders. Data collection was conducted at Bint Al-Huda Teaching Hospital in Al-Nasiriyah City, Iraq, during the period extended from 1st July to 31st December 2025. This extended duration allowed for a more varied sample and reduced the potential for bias, thus enhancing the generalizability of the outcomes.

### **2.2 Study Sample**

The researchers used to select 108 postpartum mothers, a non-probability purposive sampling technique. Participants were included according to the following criteria:

- Mothers who had recently given birth (within 6 weeks postpartum).
- Mothers who consented to participate voluntarily.

### **Study Instrument**

The main tool used in this study was a structured, then Arabic-language questionnaire developed after an extensive literature review. The questionnaire consisted of two parts:

Part I: The demographic characteristics include (e.g., age, education, economic status, number and order of children, residence).

Part II: Items which used to assess the bonding between mothers and their infants were adopted from the Postpartum Bonding Questionnaire (PBQ) by Brockington et al. (2006), adapted and validated for the current study.

The scoring items was interpreted as follows:

Low bonding: Mean score less than 1.66.

Modest bonding: Mean score between 1.67 and 2.33.

High bonding: Mean score more or equal to 2.34.

### **Pilot Study:**

To evaluate the feasibility, clarity, timing, and reliability of the questionnaire, ten individuals were included in a pilot study. Some modifications were made to ensure content adequacy and ease of administration, and based on the results and expert feedback.

### **Validity and Reliability:**

A committee from the College of Nursing at Thi-Qar University, consist of seven specialists, was evaluating the content to determine its validity. Cronbach's alpha coefficient was used to confirm the instrument's reliability, the result was a coefficient of 0.87, representative a strong internal consistency.

### Data Collection Procedure:

The data was collected via face-to-face interviews directed by the researcher. Each interview taken approximately 20-25 minutes in each interview, and written consent was obtained from all participants. The researcher clarified the study's objective and assured participants of data confidentiality before distributing the questionnaire.

### Statistical Analysis

By using SPSS version 26, the data were analyzed. The analysis included:  
Descriptive statistics: mean, standard deviation, frequencies, and percentages.

### Inferential statistics:

To examine associations between bonding levels and nominal demographic variables; the Chi-square test was used. To compare bonding scores amongst more than two groups, ANOVA test was used. While the independent t-test used to assess differences between two independent groups.

### Ethical Consideration:

This work received ethical approval from the Research Ethics Committee at Middle Euphrates Technical University (Ref. No. AATUCMHTLAB-June. 6225). After participants were fully informed of the study's objectives and methodology and their right to withdraw at any time without affecting their care, each participant provided written informed consent. Privacy and confidentiality were maintained throughout the study.

### Study Results:

**Table 1:** Participants' Demographic Characteristics

| Variables            | Category                        | Frequency | Percentage |
|----------------------|---------------------------------|-----------|------------|
| Age Group            | 17 - 22 years                   | 28        | 25.9       |
|                      | 23 - 28 years                   | 42        | 38.9       |
|                      | 29 - 34 years                   | 26        | 24.1       |
|                      | 35 years and above              | 12        | 11.1       |
|                      | Mean $\pm$ SD: 26.46 $\pm$ 4.76 |           |            |
|                      | Range: 17 - 43 years            |           |            |
| Education Level      | Not reading & writing           | 15        | 13.9       |
|                      | Primary school                  | 37        | 34.3       |
|                      | High school                     | 39        | 36.1       |
|                      | College / University            | 17        | 15.7       |
| Residence            | Urban                           | 84        | 77.8       |
|                      | Rural                           | 24        | 22.2       |
| Economic Status      | Not enough                      | 12        | 11.1       |
|                      | Enough                          | 57        | 52.8       |
|                      | Very enough                     | 39        | 36.1       |
| Birth Order of Child | First                           | 36        | 33.3       |
|                      | Second                          | 32        | 29.6       |
|                      | Third                           | 21        | 19.4       |
|                      | Fourth or more                  | 19        | 17.6       |

The table overhead shows the demographic characteristics of the participating mothers. where 38.9% of them were 23–28 years old, with the mean age of 26.46 years (standard deviation = 4.76), and ages arranged between 17 and 43 years. 77.8% of the sample lived in urban areas, and 70.4% had completed at least one primary or secondary education. The majority of the participants were of the economic status of “acceptable” (52.8%). For birth order, the first-time mothers had the largest percent by birth order (33.3%).

**Table 2:** Overall Bonding Level Distribution

| Bonding Level | Frequency | Percentage | Mean | SD   | Result    |
|---------------|-----------|------------|------|------|-----------|
| Low           | 4         | 3.7%       | 2.52 | 0.28 | High      |
| Moderate      | 24        | 22.2%      |      |      | (Overall) |
| High          | 80        | 74.1%      |      |      |           |
| Total         | 108       | 100%       |      |      |           |

The table presents all classifications of bonding levels. The high bonding levels with children were demonstrated by the majority of participants (74.1%), while the moderate bonding was 22.2%, and 3.7% showed low bonding levels. In general, this distribution indicates a strong mother-child bond, while approving that a minority may experience bonding difficulties.

**Table 3:** The relation between Bonding Level and all Demographic Variables:

| Variables            | Chi-Square Value | df | Asymptotic Significance (2-sided) |
|----------------------|------------------|----|-----------------------------------|
| Age Group            | 32.45            | 18 | 0.02                              |
| Education Level      | 15.78            | 15 | 0.47                              |
| Economic Status      | 20.34            | 12 | 0.04                              |
| Birth Order of Child | 28.67            | 15 | 0.01                              |
| Residence            | 8.92             | 6  | 0.18                              |

This table shows the results of the chi-square test, which explores the relationships between levels of correlation and demographic variables. Significant associations were found for the age group ( $P = 0.02$ ), economic status ( $P = 0.04$ ), and birth order of child ( $P = 0.01$ ). This indicates that the younger mothers, with difficulties in economic status, and mothers with a first-born child were more likely to experience bonding challenges. In addition, there is no significant association between the other demographic variables and bonding levels.

### 3. Discussion

The current study aimed to assess the levels of bonding between mothers and their children, as well as the relations between demographic factors and the bonding quality. The results highlighted the pivotal role of maternal age, socio-economic status, and birth order in forming emotional bonds.

In alignment with previous studies, the maternal age was significantly associated with the correlation ( $P=0.02$ ); particularly, those younger mothers aged 17-22 years appeared to have greater difficulty building close emotional relationships with their children. This may be related to the lower emotional maturity and higher stress levels, in addition to a few life experiences that can interfere with emotional availability and supple caregiving. often more emotionally mature and financially secure, founded in the older mothers, may hold caregiving skills and emotional self-regulation, which reflecting in facilitating a stronger connection with their infants (Aas and Nordheim, 2021; Bozzatello et al., 2021).

Remarkably, the mothers' educational level did not show a statistically significant relation to mother-child bonding ( $p < 0.05$ ). While higher education may provide mothers with better problem-solving skills and the ability to search for information about child growth and development, the findings suggest that bonding is fundamentally an innate emotional process which is not enhanced by formal education as automatically. This finding is supported by a study which demonstrated that educational attainment alone is a weaker determinant of attachment than emotional availability and inherent nurturing behaviors (Harding and Paulsell, 2020; Tichelman et al., 2022).

The significant connotation between economic status and bonding ( $P = 0.04$ ), was one of the most important findings of this study. The mothers who reported "not enough" for their economic status were more probable to exhibit moderate or low bonding levels. This supports by a body of literature which indicate that financial difficulties can cause a chronic stress, anxiety, and depression, which in turn reduce a mother's emotional abilities and responsiveness in caregiving (Giallo et al., 2020; Kim and Bianco, 2021). Economic stress may shift a mother's focus towards meeting basic survival needs, causing less emotional energy to nurture the subtle mother-infant's bond.

In the line of bonding, the study found a strong connection between the child's birth order and bonding ( $P = 0.01$ ), where the mothers displayed a higher tendency to build a strong bond emotionally with their firstborn child. This is because first-time mothers can give over-focused and intentional attention to their first child. The mother's attention and caregiving become distributed with subsequent children, which may lead to a qualitatively different form of attachment that may be not necessarily insecure. This concurs with Baranowska and Tataj-Puzyna (2021), who found that first-time mothers experience a lot of emotional intense issues, novelty-oriented by bonding process.

Finally, the current study institute no significant association between urban vs. rural residence and bonding ( $p = 0.18$ ). This result proposes that in the specific socio-cultural line of this sample, other factors, such as access to healthcare in cities or family support centers in rural areas, might counterbalance one another. It promotes the idea that proximal factors like maternal mental health and caregiving behavior are more predictive of attachment than location-based on geographic alone (Hussein et al., 2022; Li and Liu, 2020).

The distribution of attachment levels (74.1% high, 22.2% medium, and 3.7% low) is encouraging, indicating that the majority of mothers are able to establish stable attachment bonds. Nevertheless, there is a clinical significance when identifying the group with moderate attachment and a small group with low attachment. Those mothers, particularly young mothers from low socio-economic backgrounds or first-time mothers, may benefit from aimed interventions. Medium attachment may represent a suboptimal emotional connection that can be, with appropriate support, strengthened, potentially preventing the long-term relational difficulties (Tichelman et al., 2022).

#### **4. Limitations of the Current Study**

There are some restrictions to be aware of, while the study provides valuable insights into maternal-infant bonding in this population. At first, bonding is taken at a single moment in time through the cross-sectional design, causing a reduced ability to understand how bonding develops or changes through the postpartum period. Second, the results may not be applicable to all Iraqi postpartum women due to the sample technique which was used from a single hospital in Al-Nasiriyah. Third, data collection was dependent on a self-report questionnaire (PBQ), This could cause bias due to social desirability, where mothers might over-report positive bonding experiences. Fourth, the factors related to the mother's mental health status (such as depression and anxiety) and other variables which may have significantly influenced the study were not assessed, nor was the quality level of the marital rapport or the availability of social care, which mostly influences bonding. Future research is useful to employ longitudinal designs, include multiple sites across Iraq, and incorporate objective measures and assessments of mental health to build upon this study's results.

#### **5. Nursing Role**

The nurses considered as the first line of support for evaluating, fostering, and nourishing mother-child attachment, particularly in the delicate postpartum phase. Nurses are ideally positioned to conduct initial assessments using reliable tools like the PBQ (Brockington et al., 2006). In addition, it's crucial for nurses to educate mothers about typical infant behaviors and the ways to encourage responsive caregiving, which is essential to fostering secure attachment (Tichelman et al., 2022). The nurses as well provide essential emotional support, offering a compassionate care and counselling to relieve maternal worries and enhancing comfort. Furthermore, the nurses help the child bonding by connecting families with further resources, such as lactation consultants, social workers, or mental health services, ensuring a comprehensive support system (Dubber et al., 2022). At last, nurses act as guardians, which can accessible through careful assessment, skilled intervention, and a caring presence, helping to preserve the vital bond between mother and infant, in addition to supporting the children develop mentally and emotionally.

#### **6. Conclusion**

The current study proves the complex nature of the normal mother-infant bonding during the postpartum period. The findings showed that although the majority (74.1%) of participants mothers developed a good bond with their newborns, a sizeable minority (22.2% moderate, 3.7% low) struggle with bonding. Definitely, younger maternal age ( $P = 0.02$ ), economic level difficulties ( $P = 0.04$ ), and having a first-born child ( $P = 0.01$ ) were identified as vital risk

factors associated with lower bonding levels. In addition, the maternal education and urban/rural residence did not show a significant correlation. These results underscore the need for targeted, culturally sensitive interventions. Healthcare providers should prioritize early screening for bonding difficulties, particularly for mothers who exhibit recognized risk factors. The supporting young mothers, reducing the economic stress through the convenient social support referrals, and providing extra guidance to first-time mothers, were the effective strategies to promote secure attachment and foster healthy emotional, social, and cognitive development in children.

## 7. Recommendations

Considering the results of the current study, which carried out in Iraq, the PBQ tool can be used for maternal-infant bonding to routinely examine young, primipara, and economically disadvantaged moms. Also, the support programmers should be initiated by healthcare centers and nursing counselling services as supported ways that enhance maternal-infant attachment. Training courses in bonding assessment and intervention should be included in nursing education. Postnatal care facilities should be provided by policymakers and funds invested in the support of maternal mental health. In addition, for future research the longitudinal study designs and an examination of the underlying psychological and cultural mechanisms influencing bonding are required.

## References

- Aas, M., & Nordheim, L. V. (2021). Maternal age and child outcomes: A systematic review. *BMJ Open*, 11(5), e045678. <https://doi.org/10.1136/bmjopen-2020-045678>
- Ahmed, H. M., & Al-Jawadi, A. A. (2023). Maternal mental health and bonding in postpartum women in Iraq: A cross-sectional study. *Iraqi Journal of Medical Sciences*, 21(1), 45-53. <https://doi.org/10.22578/IJMS.21.1.6>
- Al-Hamad, A., & Al-Nashmi, E. (2021). Cultural beliefs and practices affecting maternal-infant bonding in Arab countries: A scoping review. *Journal of Transcultural Nursing*, 32(4), 389-398. <https://doi.org/10.1177/10436596211002984>
- Baranowska, B., & Tataj-Puzyna, U. (2021). First-time versus experienced mothers: Differences in bonding and parenting stress. *Midwifery*, 95, 102934. <https://doi.org/10.1016/j.midw.2021.102934>
- Bozzatello, P., Brignolo, E., De Grandi, E., & Bellino, S. (2021). Mother-infant bonding in the postpartum period: The role of maternal age. *Journal of Clinical Medicine*, 10(15), 3341. <https://doi.org/10.3390/jcm10153341>
- Brockington, I. F., Fraser, C., & Wilson, D. (2006). The postpartum bonding questionnaire: A validation study. *Archives of Women's Mental Health*, 9(5), 233-242. <https://doi.org/10.1007/s00737-006-0132-1>
- Dubber, S., Reck, C., Müller, M., & Gawlik, S. (2022). Postpartum bonding: The role of maternal depression, anxiety, and stress. *Archives of Women's Mental Health*, 25(3), 421-430. <https://doi.org/10.1007/s00737-022-01221-7>
- Feldman, R., & Bakermans-Kranenburg, M. J. (2022). The neurobiology of human attachment: Insights from imaging studies. *Current Opinion in Psychology*, 43, 45-51. <https://doi.org/10.1016/j.copsyc.2021.06.008>
- Giallo, R., Gartland, D., Woolhouse, H., & Brown, S. (2020). Economic hardship and maternal mental health: A longitudinal study. *Journal of Affective Disorders*, 265, 112-119. <https://doi.org/10.1016/j.jad.2020.01.045>
- Harding, J. F., & Paulsell, D. (2020). Maternal education and parenting practices: A longitudinal analysis. *Early Childhood Research Quarterly*, 51, 234-245. <https://doi.org/10.1016/j.ecresq.2019.12.002>
- Hussein, A. A., Bachi, D. M., & Kamil, H. F. (2022). Teachers' Awareness about Epilepsy in Children at Al Basra Primary Schools. *Bahrain Medical Bulletin*, 44(3), 1045-1049. Retrieved from: <https://search.emarefa.net/detail/BIM-1439756>
- Kamil, H. F., Dawood, S. B., & Hussein, A. A. (2023). Quality of Life for Child under Chemotherapy at Al Basra Specialized Hospital for Children. *Bahrain Medical Bulletin*, 45(2). Retrieved from: <https://search.emarefa.net/detail/BIM-1635487>
- Keller, H., & Kärtner, J. (2021). Cultural variations in mother-infant interactions: A cross-cultural study. *Culture and Psychology*, 27(3), 345-362. <https://doi.org/10.1177/1354067X20984325>
- Kim, P., & Bianco, H. (2021). How economic hardship affects maternal emotional availability and infant attachment. *Frontiers in Psychology*, 12, 678432. <https://doi.org/10.3389/fpsyg.2021.678432>
- Li, Q., & Liu, H. (2020). Urban-rural differences in parenting and child development: A systematic review. *International Journal of Environmental Research and Public Health*, 17(15), 5567. <https://doi.org/10.3390/ijerph17155567>

- Liu, X., Wang, Z., & Zhang, Y. (2023). Postpartum depression and maternal-infant bonding: A longitudinal study. *BMC Psychiatry*, 23, 456. <https://doi.org/10.1186/s12888-023-04892-3>
- Pilkington, P., Milne, L., Cairns, K., & Whelan, T. (2021). The role of partner support in postpartum bonding: A longitudinal study. *Journal of Family Psychology*, 35(5), 678-687. <https://doi.org/10.1037/fam0000834>
- Prevatt, B. S., & Desmarais, S. L. (2023). Maternal mental health and infant bonding: A meta-analysis. *Infant Mental Health Journal*, 44(2), 198-215. <https://doi.org/10.1002/imhj.22045>
- Scatliffe, N., Casavant, S., Vittner, D., & Cong, X. (2022). Oxytocin and early parent-infant interactions: A systematic review. *International Journal of Nursing Sciences*, 9(2), 234-245. <https://doi.org/10.1016/j.ijnss.2022.03.004>
- Swain, J. E., & Ho, S. S. (2021). Neurobiology of mother-infant bonding: A neuroimaging perspective. *Current Opinion in Psychiatry*, 34(3), 273-281. <https://doi.org/10.1097/YCO.0000000000000694>
- Tichelman, E., Westerneng, M., Witteveen, A. B., & van der Wal, M. F. (2022). Correlates of mother-infant bonding: A systematic review. *Journal of Reproductive and Infant Psychology*, 40(3), 245-268. <https://doi.org/10.1080/02646838.2021.1892047>
- Vittner, D., & McGrath, J. (2021). Oxytocin and the parent-infant relationship: A systematic review. *Journal of Perinatal & Neonatal Nursing*, 35(2), 167-178. <https://doi.org/10.1097/JPN.0000000000000556>
- Wang, S., Liu, Y., & Zhou, X. (2022). Risk factors for postpartum bonding disorders: A meta-analysis. *Journal of Affective Disorders*, 305, 234-245. <https://doi.org/10.1016/j.jad.2022.03.012>





## Factors Affecting Young Medical Students' Choice for Future Medical Specialty

SALWA SH. ABDUL-WAHID

Community Medicine, Al-Farabi University, Iraq

[s\\_sh\\_abdulwahid@yahoo.co.uk](mailto:s_sh_abdulwahid@yahoo.co.uk)

HANAN ABDULHAQ KALAF

Diyala Medical College, Iraq

### Abstract

**Background:** Young doctors' choice of medical specialty is essential to maintaining a sufficient health workforce and developing a sustainable healthcare system.

**Aim:** The aim of this article is to explore the factors taken into consideration by young medical students in choosing their future specialty.

**Materials and Methods:** A cross-sectional study was conducted, for the period from 1st of September to the 31st of December /2025. The study sample were (176) medical students, (47), males and (129) females, selected randomly from the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> grades of Diyala Medical College. A questionnaire was designed by the researchers included personal, educational level' variables in regard to the medical students in addition to socio-demographic variables related to the students' family. The students were asked to complete the questionnaire following the explanation of the subject and objectives of the research with each grade.

**Results:** Revealed that highest preference rate among males' undergraduates for Surgery (31.3%), followed by internal Medicine and Pediatrics (14.8% & 10.8%) respectively. While for females the choice for Obstetrics & Gynecology following surgery choice. There is choice' variation according to the grade. The results also revealed a relation between personal interest, social and economic factors with the choice of medical specialty.

**Conclusion & Recommendations:** The overall two preferred specialties were general surgery (27%), internal medicine, ophthalmology and pediatrics (8.3%). While the choice among female it was surgery, gyne and obstetrics. Deficient choice for Family and Community Medicine, this is against the term of "Tomorrow Doctor" in its emphasis on Primary health care, Family doctors and Public Health. The study recommends Career counseling should be offered to students about each specialty's challenges, /opportunities with an ultimate goal to match the country-specific demand and supply of physicians. Another points focusing on spreading the importance of the principles of primary health care, family doctor and public health to be choices for future specialty, as these are the pillars of tomorrow's doctor.

**Keywords:** Medical students, Undergraduates, Medical Specialties, Diyala Medical College.

### 1. Introduction

After graduating from medical school, medical students have to decide a career path to pursue for further specialization (1). The future career specialties chosen by medical students and the factors involved in making these choices are of great significance for balanced distribution of doctors in the different specialties and for planning of the workforce of healthcare services (1, 2). Clinical specialties were statistically rated higher than basic medical sciences specialties

(3). Therefore, there is strong evidence that students decide their future medical career during or even before medical school [4, 5]. Their choice, however, can change during the course of medical school [6-7]. Many factors influence the medical students' choice of future career [7-8]. Medical students' choices have evolved throughout time, with a growing interest in quality of life or in technological excellence (9, 10). Generally, these factors are classified into two groups; intrinsic factors, for example those related to personal attributes, interest and preferences, and extrinsic factors like those related to work environment social or economic effect (11). Although studies conducted around the world have attempted to identify the factors that affect medical students' choice of specialty, data is scarce on the real and deep factors that influence the choice of specialty (12). The impact of these factors varies from setting to setting. In United Kingdom, other peoples' perception of the job and fewer practice hours were considered the most important factors by students, whereas in Turkey, financials and prestige were more important [13, 14]. Gender is another important factor that influences career choice (12, 15, 16). Specialty choice for their future medical practice (12, 15, 16). On the other hand, students in Jordan considered the intellectual content of the specialty and the individual's competencies most influential while a personal interest in the specialty was the deriving factor for students in Kingdom of Saudi Arabia, UAE, Lebanon, Kuwait, Australia, Hong Kong, Rawanda Korea and India, (2,3, 5, 6, 9, 10, 16, 17 & 23). This study aims to determine the factors that influence medical student' choice of future medical specialty.

## 2. Persons & Method

Cross sectional study was conducted for the period from 1st September to the 31st of December. 2025. The study sample were medical student from Diyala Medical College. Questionnaire was designed by researcher sent to (176) medical student to collect data for the current study. The questionnaire covered demographic characteristics, specialty preferences, and factors that influenced career preferences. Demographic characteristics included the student's age, gender, grade, and residency. Students were asked to select the most preferred career preference. Specialties listed were surgery, obstetrics & gynecology, pediatrics, internal medicine, ophthalmology, dermatology, radiology, otolaryngology (ENT), and other specialties. The questionnaire influences on specialty preferences were indicated in order to assess the factors to which students attach importance when choosing their first specialty preferences. Responses to the influences were categorized to interesting subject, intellectual challenge, good income, prestige and good social life, advice from faculty practicing physician, parents or friends.

### 2.1 Inclusion and Exclusion Criteria

The study had to focus on medical students after their selection year and before their graduation, to ask about the detail the choice of specialty of each student, and look for factors influencing their choice. Juniors and Specialist's physicians excluded from this study.

## 3. Result

**Table 1:** Frequency of specialization preference between males and females Medical students

| Medical Specialty       | Males<br>No. (%) | Females<br>No. (%) | Total<br>No. (%) |
|-------------------------|------------------|--------------------|------------------|
| -Pediatric              | 3 (15,78%)       | 16 (84,21%)        | 19 11%           |
| -Internal medicine      | 9 (34,61%)       | 17 (65,38%)        | 26 15%           |
| Surgery                 | 19 (34,54%)      | 36 (65,45%)        | 55 31%           |
| Radiology               | 1 (9,09%)        | 10 (90,90%)        | 11 6%            |
| Gynecology & Obstetrics | 0 (0%)           | 18 (100%)          | 18 10%           |
| Dermatology             | 5 (27,77%)       | 13 (72,22%)        | 18 10%           |
| -ENT                    | 2 (10%)          | 0 (0%)             | 2 1%             |
| Ophthalmology           | 4 (33,33%)       | 8 (66,66%)         | 12 7%            |
| - Others                | 4 (26,66%)       | 11 (73,33%)        | 15 9%            |

**Table 2:** Frequency distribution of medical specialists' preference among medical students according to the grade

| Medical Specialty  | 1 <sup>st</sup> grade<br>No. (%) | 2 <sup>nd</sup> grade<br>No. (%) | 3 <sup>rd</sup> grade<br>No. (%) | 4 <sup>th</sup> grade<br>No. (%) | 5 <sup>th</sup> grade<br>No. (%) | 6 <sup>th</sup> grade<br>No. (%) |
|--------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| 1-pediatric        | 5,26%                            | 21,05%                           | 21,05%                           | 31,57%                           | 10,52%                           | (10,52%                          |
| 2-internal medicin | 0%                               | 86,92%                           | 23,07%                           | 3,84%                            | 19,23%                           | 26,92%                           |
| 3-surgery          | 23,72%                           | 21,81%                           | 14,54%                           | 9,09%                            | 10,95                            | 10,9%                            |

|                |        |        |        |        |        |        |
|----------------|--------|--------|--------|--------|--------|--------|
| 4-Radiology    | 9,09%  | 9,09%  | 18,18% | 18,18% | 36,36% | 0%     |
| 5-Gyncoylogy   | 27,77% | 0%     | 0%     | 22,22% | 16,66% | 33,33% |
| 6-Dermatology  | 11,11% | 16,66% | 22,22% | 22,22% | 16,66% | 11,11% |
| 7-ENT          | 0%     | 0%     | 0%     | 50%    | 50%    | 0%     |
| 8-opthalmology | 25%    | 16,66% | 16,66% | 25%    | 8,33%  | 8,33%  |
| 9- other       | 13,33  | 0%     | 20%    | 13,33% | 20%    | 33,3%  |

**Table 3:** Frequency distribution of the factors affecting choice of medical specialty among Medical students according to gender

| Factors affecting choice                 | Males<br>No. (%) | Females<br>No. (%) | Total<br>No. (%) |
|------------------------------------------|------------------|--------------------|------------------|
| -Interesting subject & personal interest | 33 ( 21,29%)     | 122 ( 78,70%)      | 155 ( 88%)       |
| -Good in come, & work-life balance       | 4 ( 80%)         | 1 ( 20%)           | 5 (3%)           |
| -Prestige and social life requirements   | 14 ( 87,5%)      | 2 ( 12,5%)         | 16 ( 9%)         |
| Total                                    | 51 (29 %)        | 125 (71%)          | 176 ( 100%)      |

#### 4. Discussions

Career preferences made by medical students and factors influencing these preferences are of importance to medical workforce planners especially in times of over-supply or under-supply of doctors. Surgery, internal medicine, pediatrics, and obstetrics and gynecology were the most specialty preferred preferences among medical students at Diyala medical college. Factors influencing students' choices were studied depending on each discipline. The most frequent factor was their real interest in that specialty. Prestige and social life requirements second affecting factor followed by income factor and work-life balance. Clinical specialties more than basic medical sciences specialties as specialty preferences. Personal interest and an anticipated higher income were other influential factors contributing to specialty preference. (1) Saudi medical students and interns at the University of Dammam were influenced mostly by lifestyle when they considered their future specialty. Internal medicine, family medicine, general surgery, pediatrics, and emergency medicine were the preferred specialties. (3, 4).

Table (1) show the variation of specialties among student about 3(15,78%) of male chose pediatric but in female about 16(84,21%). Some of student chose internal medicine about 9(34,61%) of male and in female about 17(65,38%). While about 19(34,54%) of male and 36(65,45%) of female chose the surgery. But some student chose radiology about 1(9,09%) of male and 10(90,90%) of female. About (0%) of male and 18(100) of female chose gynecology & Obestetrics. While some student about 5(27,77%) of male and 13(72,22%) of female chose the dermatology. About 2(100%) of male and 0(0%) of female chose ENT. While some student about 4(33,33%) of male and 8(66,66%) of female chose Ophthalmology. Furthermore, some student chose other specialties about 4(26,66%) of male and 11(73,33%) of female.

Not only thoughtful individual factors such as interest for the discipline, work-life balance or role modeling can influence the specialization choice, but also sociobiological aspects. For example, gender can modify factors associated with the choice of several medical specialties: in the US, men-to-female ratio was 4.9:1 in obstetrics and gynecology [6], 24.6% of last-year male medical students would chose internal medicine compared with 11.8% female in Rwanda [7], or in Korea, where 2.9% of male would be interested in pediatrics for 10.7% of woman [8]. Men are more interested by technical challenges, salary, career and prestige, women by time related aspects and societal orientation [9], [10], [11]. Gender influences an important variable, A shift in the gender ratio in medical students have occurred at the end of the 20th century [12] from a male to a female over-representation, leading to difficulties for policymakers to adapt incentives, learning programs, schemes for managing physicians' flows and the specialization's issue. If many papers have investigated the gender imbalance among physicians [6], [7], [8],

Table (2) Show frequency of specialization preference among grade of students. First grade about 1(5,26%), Second grade about 4(21,05%), Third grade about 4(21,05%), Fourth grade about 6(%), Fifth grade about 2(10,52%), And

sixth stage about 2(10,52%) chose pediatric. Other chose Internal medicine about 0(0%) of first grade, 7(86,92%) of second grade, 6(23,07%) of third grade, 1(3,84%) of fourth grade, 5(19,23%) of fifth grade, And 7(26,92%) of sixth grade. While about 18 (23,72%) of first grade, 12(21,81%) of second grade, 8(14,54%) of third grade, 5(9,09%) of fourth grade, 6(10,95%) of fifth grade, and 6(10,95%) of sixth grade chose the surgery. Some student in first grade about 1(9,09%), 1(9,09%) in second grade, 2(18,18%) in third stage, 2(18,18%) in fourth grade, 4(36,36%) in fifth grade, And 0(0%) in sixth grade chose the radiology. The current and projected nationwide shortage of primary care physicians necessitates studying factors that could influence students' specialty choice for their future medical practice (11,12).

First grade about 5(27,77%), second grade about 0(0%), third grade about 0(0%), fourth grade about 4(22,22%), fifth grade about 3(16,66%), And sixth grade about 6(33,33%) chose the gynecology. About 2(11,11%) of first grade, 3(16,66%) of second grade, 4(22,22%) of third grade, 4(22,22%) of fourth grade, 3(16,66%) of fifth grade, and 2(11,11%) of sixth grade chose the dermatology. While some student of first grade about 0(0%), 0(0%) of second grade, 0(0%) of third grade, 1(50%) of fourth grade, 1(50%) of fifth grade, and 0(0%) of sixth grade chose ENT. About 3(25%) of first grade, 2(16,66%) of second grade, 2(16,66%) of third grade, 3(25%) of fourth grade, 1(8,33%) of fifth grade, and 1(8,33%) of sixth grade chose Ophthalmology. About 2(13,3%) of first grade, 0(0%) of second grade, 3(20%) of third grade, 2(13,33%) of fourth grade, 3(20%) of fifth grade, and 5(33,3%) of sixth grade chose other. Table(3) show frequency of factor affecting the choice of male and female students. About 33(21,29%) of male, 122(78,70%) of female affecting by interesting subject. While some student about 4(80%) of male and 1(20%) of female affecting by good income. Other about 14(87,5%) of male and 2(12,5%) of female affecting by prestige and good life social life.

## 5. Conclusions & Recommendations

The overall two preferred specialties were general surgery (27%), internal medicine and pediatrics (8.3%) among males. While it was for surgery, gynecology and dermatology for females. Deficient choice for Family and Community Medicine, this is against the term of "Tomorrow Doctor" in its emphasis on Primary health care, Family doctors and public health. The study recommends Career counseling should be offered to students about each specialty's challenges, /opportunities with an ultimate goal to match the country-specific demand and supply of physicians. Knowledge about doctors' preferences regarding their medical specialty could support the development of targeted policies to increase the attractiveness of deficit specialties. The study recommends Focusing on spreading the importance of the principles of primary health care, family doctor and public health to be choices for future specialty, as these are the pillars of tomorrow's doctor.

## References

- 1-M.D. Al-Mendalawi. Specialty preferences of Iraqi medical students. Clin Teach, 7 (3) (2010 Sep), pp. 175-179. View in Scopus Google Scholar
- 2-M. Abdulrahman, M. Makki, S. Shaaban, M.A. Shamsi, M. Venkatramana, N. Sulaiman, et al. Specialty preferences and the factors influencing them among pre-clerkship medical students: the first study from alfaisal university-college of medicine, Saudi Arabia. Cureus [Internet] (2016 Nov 23), [cited 2019 Jul 4]; Available from: <http://www.cureus.com/articles/5799-specialty-preferences-and-the-factors-influencing-them-among-pre-clerkship-medical-students-the-first-study-from-alfaisal-university-college-of-medicine-saudi-arabia/> Google Scholar
- 3-M. Abdulrahman, M. Makki, S. Shaaban, M.A. Shamsi, M. Venkatramana, N. Sulaiman, et al. Specialty preferences and motivating factors: a national survey on medical students from five UAE medical schools. Educ Health, 29 (3) (2016), p. 14. Google Scholar
- 4- R. AlKhilawi, A. Alatassi, A. Almohawis, T. Alhumaid, K. Almazyad, R Bustami. Medical students' attitude toward anesthesia as a future career. Saudi J Anaesth, 12 (2) (2018), p. 215
- 5-Wu SM, Chu TK, Chan ML, Liang J, Chen JY, Wong SYS. A study on what influence medical undergraduates in Hong Kong to choose family medicine as a career. 2014 [cited 2019 Aug 9]; Available from: <http://hub.hku.hk/handle/10722/214278> / Google Scholar
- 6-B. Khater-Menassa, S. Major. Factors influencing the choice of specialty among medical students in Lebanon. J Med Liban, 53 (1) (2005 Mar), pp. 16-20. View in Scopus Google Scholar

- 7-Beniamin Michalik, Mateusz Kulbat, Alicja Domagała. Factors affecting young doctors' choice of medical specialty—A qualitative study. *Plos one*: February 1, 2024  
<https://doi.org/10.1371/journal.pone.0297927>
8. Mathieu Levailant; Lucie Levailant, Nicolas Lerolle; Benoît Vallet, Jean-François Hamel-Broza. Factors influencing medical students' choice of specialization: A gender based systematic review. *Ethical Medicine*, Volume 28, November 2020, 100589. <https://doi.org/10.1016/j.eclinm.2020.100589>
- 9- R. Al-Fouzan, S. Al-Ajlan, Y. Marwan, M Al-Saleh. Factors affecting future specialty choice among medical students in Kuwait. *Med Educ Online*, 17 (1) (2012 Jan), p. 19587. Google Scholar
10. B. Kaur, A. Carberry, N. Hogan, D. Robertson, J Beilby. The medical schools' outcomes database project: australian medical student characteristics. *BMC Med Educ*, 14 (2014 Aug 29), p. 180
- 11- Jonathan Ryan 1, Christian Palacios 1, Ghaith Al-Eyd. Factors Influencing Medical Students' Specialty Choice: Insights to Address the Evolving Primary Care Gap. *Cureus* 2025 Jul 25;17(7): e88748. doi: 10.7759/cureus.88748
- 12- T. J. Kadium1, S. Sh. Abdulwahid. Central Admission to Medical Colleges and its impact on the rates of medical specialties in Iraq. *Iraqi Journal of Public Health* (2017) 1:1. DOI 10.22317/ijph.06201702
- 13-E. Svirko, M.J. Goldacre, T Lambert. Career choices of the United Kingdom medical graduates of 2005, 2008 and 2009: questionnaire surveys. *Med Teach*, 35 (5) (2013 May), pp. 365-375
- 14-S.S. Budhathoki, P.A.C. Zwanikken, P.K. Pokharel, A.J Scherpbier. Factors influencing medical students' motivation to practise in rural areas in low-income and middle-income countries: a systematic review. *BMJ Open*, 7 (2) (2017 Feb 1), Article e013501 View at publisherCrossrefView in ScopusGoogle Scholar
15. H. Alkhaneen, F. Alhusain, K. Alshehri, N Al Jerian. Factors influencing medical students' choice of emergency medicine as a career specialty—A descriptive study of Saudi medical students. *Int J Emerg Med*, 11 (1) (2018 Dec), p. 14  
View in ScopusGoogle ScholarAna Angeli
- 16- G. Kansayisa, S. Yi, Y. Lin, A Costas-Chavarri. Gender-based analysis of factors affecting junior medical students' career selection: addressing the shortage of surgical workforce in Rwanda. *Hum Resour Health*, 16 (1) (2018 Dec), pp. 1-8. Google Scholar
17. Y.-Y. Kim, U.-N. Kim, Y.S. Kim, J.-S Lee. Factors associated with the specialty choice of Korean medical students: a cross-sectional survey. *Hum Resour Health*, 14 (1) (2016 Dec), pp. 1-8
18. S. Evans, B. Sarani. The modern medical school graduate and general surgical training: are they compatible? *Arch Surg*, 137 (3) (2002 Mar 1), pp. 274-277
19. J. Bickel. Gender equity in undergraduate medical education: a status report  
*J Womens Health Gend Based Med*, 10 (3) (2001 Apr), pp. 261-270
- 20 - J.H. Lefevre, M. Roupret, S. Kerneis, L Karila. Career choices of medical students: A national survey of 1780 students: motivation in medical student career choices. *Med Educ*, 44 (6) (2010 Jun), pp. 603-612
21. M.B.M. Soethout, ThJ ten Cate, G van der Wal. Factors associated with the nature, timing and stability of the specialty career choices of recently graduated doctors in European countries, a literature review. *Med Educ Online*, 9 (1) (2004 Dec), p. 4360
22. H.M.S. Alahwal, F. Al Sayes, B.S. El-deek, B. Kurdi, N.A. Al-Hamayel, A.S Barefah. Career counseling activities and choice of specialties among medical interns  
*Bahrain Med Bull*, 32 (4) (2010). Google Scholar
23. R. Anand, P.S. Sankaran. Factors influencing the career preferences of medical students and interns: a cross-sectional, questionnaire-based survey from India  
*J Educ Eval Health Prof*, 16 (2019 May 15), p. 12





## Pulmonary Injury Biomarkers, Oxidative Stress and Systemic Inflammation in Conventional Cigarette, Waterpipe and Electronic Cigarette Users: A Cross-Sectional Comparative Study

NERAN THAAR KLEAB

Department of Pharmacy, Al-Dour Technical Institute, Northern Technical University, Iraq  
neran.tk@ntu.edu.iq

### Abstract

**Background:** Tobacco smoke injures the airway epithelium, imposes an oxidant load and activates innate immunity, but the biological footprint of waterpipe smoking and electronic cigarette (e-cigarette) use is less well characterised, particularly in Middle Eastern populations where all three modalities coexist.

**Objective:** To compare a panel of pulmonary injury biomarkers (CC16, KL-6, YKL-40, galectin-3, TREM-1), oxidative stress indices (malondialdehyde [MDA], superoxide dismutase-1 [SOD-1] protein) and the systemic inflammatory cytokine interleukin-6 (IL-6) among conventional cigarette smokers, waterpipe smokers, exclusive e-cigarette users and non-smokers. **Methods:** In this cross-sectional study, 120 apparently healthy men aged 18–45 years were classified by smoking status into four equal groups (n = 30 each): non-smokers, cigarette smokers, waterpipe smokers and exclusive e-cigarette users. All eight analytes were quantified in serum by enzyme-linked immunosorbent assay. Group differences were tested by one-way ANOVA with Tukey HSD post-hoc comparisons, and associations by Pearson correlation.

**Results:** Serum CC16 was significantly lower, and KL-6, YKL-40, IL-6 and MDA significantly higher, in every smoking group than in non-smokers (all  $p < 0.001$ ;  $\eta^2 = 0.64\text{--}0.74$ ). The largest deviations occurred in the two combustible groups, which did not differ significantly from each other, whereas e-cigarette users showed an intermediate profile lying between non-smokers and combustible smokers. Galectin-3, TREM-1 and SOD-1 protein did not differ among groups ( $p > 0.20$ ). CC16 correlated inversely with the injury, oxidation and inflammation markers ( $r = -0.63$  to  $-0.71$ ), which were positively inter-correlated ( $r = 0.69\text{--}0.73$ ; all  $p < 0.001$ ).

**Conclusion:** A coherent signature of airway epithelial injury, lipid peroxidation and IL-6-mediated inflammation separates smokers from non-smokers. The signature is most pronounced with combustible products, including the waterpipe, and is attenuated but clearly present in e-cigarette users. CC16, KL-6, YKL-40, IL-6 and MDA were the most discriminating circulating markers in this population.

**Keywords:** club cell protein 16; Krebs von den Lungen-6; YKL-40; lipid peroxidation; interleukin-6; waterpipe; electronic cigarettes; tobacco harm reduction.

### 1. Introduction

Tobacco use remains one of the leading preventable causes of morbidity and mortality worldwide, accounting for more than seven million deaths each year from direct use and over one million further deaths attributable to second-hand smoke [1,2]. Although the age-standardised burden has declined in many high-income countries, the absolute number of tobacco-related deaths continues to rise, and the industry's marketing has shifted towards low- and middle-income countries with weaker regulatory systems [3]. The Eastern Mediterranean region has a distinctive profile: smoking prevalence among men remains high, and while cigarette smoking has fallen by roughly 7% over the past decade, waterpipe smoking has increased by almost 8% over the same period [4]. This shift is clinically relevant

because a single waterpipe session delivers substantially more carbon monoxide, particulate matter and several other toxicants than a single cigarette [5].

Three interconnected mechanisms underlie the respiratory effects of inhaled tobacco toxicants: disruption of the airway epithelial barrier, oxidative injury by reactive oxygen species, and activation of innate and cytokine-mediated immunity [6]. Several circulating proteins index these processes with increasing specificity. Club cell secretory protein-16 (CC16) is an anti-inflammatory pneumoprotein produced by non-ciliated bronchiolar epithelial cells; its serum concentration falls as club cell populations are depleted by chronic smoke exposure, and low CC16 predicts accelerated lung-function decline and incident chronic obstructive pulmonary disease (COPD) [7–10]. CC16 is also implicated in acute epithelial injury and viral lung damage, underscoring its sensitivity as a marker of barrier integrity [11,12]. Krebs von den Lungen-6 (KL-6) is a high-molecular-weight MUC1 glycoprotein released into the circulation when alveolar epithelial cells are damaged [13,14]. YKL-40 (chitinase-3-like protein 1) is secreted by epithelial cells, macrophages and neutrophils, is elevated in smokers, and correlates with airway inflammation, remodelling and neutrophilic disease phenotypes [15–17]. Galectin-3, a  $\beta$ -galactoside-binding lectin involved in tissue remodelling and fibrosis [18,19], and the triggering receptor expressed on myeloid cells-1 (TREM-1), an amplifier of innate immune responses [20,21], have also been proposed as lung injury markers, but their behaviour in otherwise healthy smokers is poorly defined. Large population analyses further indicate that smoking perturbs several systemic immune and inflammatory markers simultaneously, which argues for a multi-marker approach [22].

Oxidative stress is the common biochemical substrate of these processes. Malondialdehyde (MDA), an end-product of membrane lipid peroxidation, reflects the oxidant load imposed by smoke, whereas superoxide dismutase (SOD), a first-line antioxidant enzyme, may increase, decrease or remain unchanged depending on the intensity and duration of oxidant exposure [5,23]. Interleukin-6 (IL-6) links local airway events to low-grade systemic inflammation and is consistently elevated in current smokers [24,25].

Whereas the literature on conventional cigarettes is extensive, few studies have examined such a biomarker panel in cigarette, waterpipe and e-cigarette users drawn from the same demographically matched population. Comparative data are scarcer still for Middle Eastern cohorts, where waterpipe smoking is culturally entrenched and e-cigarettes are gaining popularity among younger adults.

### 1.1 Previous studies and research gap

Regional and international studies provide the context for the present analysis. Al-Tameemi and colleagues, in a cross-sectional study of 108 smokers and 51 non-smokers in Iraq, reported dose-dependent increases in plasma IL-6 (from 1.86 to 2.58 pg/mL) and TNF- $\alpha$  (from 4.93 to 6.29 pg/mL) among smokers [26]. The direction and magnitude of that effect resemble the present findings, although absolute IL-6 concentrations here are higher, a difference most plausibly attributable to the assay platform, sample matrix and population characteristics rather than to a different biological pattern. On the oxidative side, a 2024 pilot study documented a significant rise in MDA together with reduced activities of SOD, catalase and glutathione peroxidase in chronic smokers, confirming the importance of lipid peroxidation and suggesting that antioxidant enzyme activity may decline with heavier or longer exposure [23]. Most directly relevant, a 2025 Saudi study compared respiratory, haematological and inflammatory profiles across cigarette, shisha, e-cigarette and non-smoking groups and found that lung function was more impaired with combustible products than with e-cigarettes, while cigarette and shisha users shared a similar inflammatory profile [27].

This body of work exposes two gaps. First, most regional studies have relied on a conventional oxidative–inflammatory panel (MDA, SOD, catalase, glutathione peroxidase, IL-6, TNF- $\alpha$ , C-reactive protein) rather than on epithelium-specific markers of lung injury. Second, very few have compared cigarette, waterpipe and e-cigarette exposure within a single matched cohort. The present study addresses both gaps by adding CC16, KL-6, YKL-40, galectin-3 and TREM-1 to the panel, thereby focusing on early, subclinical airway injury by smoking modality. Specifically, we aimed to compare these markers of pulmonary injury, oxidative stress and systemic inflammation across four matched male groups, and to identify which circulating markers best discriminate smokers from non-smokers.

## 2. Materials and Methods

### 2.1 Study Design and Setting

This cross-sectional comparative study was conducted at Tikrit Teaching Hospital and its affiliated primary healthcare centres in Salah Al-Din Governorate, Iraq. Recruitment, sampling and laboratory analysis were carried out between January and April 2026.

### 2.2 Participants and Grouping

A total of 120 apparently healthy men aged 18–45 years were enrolled consecutively and classified according to their self-reported smoking status into four equal groups (n = 30 each): non-smokers (controls), conventional cigarette smokers, waterpipe smokers and exclusive e-cigarette users. Participants in the smoking groups were required to have used their respective product for at least one year. Only men were enrolled in order to minimise sex-related variation in IL-6 and oxidative markers [24].

Exclusion criteria were chronic respiratory disease, asthma, tuberculosis, COVID-19 within the preceding three months, autoimmune disease, malignancy, chronic kidney disease, chronic liver disease, and dual or poly-use of tobacco or nicotine products. These criteria were applied to remove the confounding influence of established pulmonary disease, altered renal clearance of the analytes, and the additive effects of multiple exposures.

### 2.3 Sample collection and biomarker assays

Venous blood (5 mL) was drawn from each participant, allowed to clot, and centrifuged at 3000 rpm for 10 min. Serum was separated and stored at –80 °C until analysis. Serum concentrations of the eight analytes (CC16, KL-6, YKL-40/CHI3L1, galectin-3, TREM-1, IL-6, MDA and SOD-1) were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co., Ltd, Hangzhou, China) according to the manufacturer's instructions (Table 1). Protein analytes were quantified by quantitative sandwich ELISA and MDA by competitive ELISA. Samples were diluted five-fold as specified in each kit insert, absorbance was read at 450 nm, and concentrations were interpolated from each kit's standard curve and expressed in the kit's calibrated units. It should be noted that the SOD-1 assay measures the concentration of SOD-1 protein rather than its catalytic activity.

**Table 1.** ELISA kits used for serum biomarker quantification (Sunlong Biotech Co., Ltd, Hangzhou, China).

| Biomarker       | Kit                                                              | Catalogue no. | Assay format |
|-----------------|------------------------------------------------------------------|---------------|--------------|
| CC16            | Human Clara cell protein (CC16) ELISA Kit                        | QS0462Hu      | Sandwich     |
| KL-6            | Human Krebs von den Lungen-6 (KL-6) ELISA Kit                    | SL2082Hu      | Sandwich     |
| YKL-40 (CHI3L1) | Human Chitinase-3-like Protein 1 (YKL-40/CHI3L1) ELISA Kit       | SL2399Hu      | Sandwich     |
| Galectin-3      | Human Galectin-3 ELISA Kit                                       | SL2065Hu      | Sandwich     |
| TREM-1          | Human Triggering Receptor Expressed on Myeloid Cells-1 ELISA Kit | QS1742Hu      | Sandwich     |
| IL-6            | Human Interleukin-6 (IL-6) ELISA Kit                             | SL1001Hu      | Sandwich     |
| MDA             | Human Malondialdehyde (MDA) ELISA Kit                            | SL1135Hu      | Competitive  |
| SOD-1           | Human Superoxide Dismutase 1 (SOD-1) ELISA Kit                   | QS2309Hu      | Sandwich     |

**Note:** All assays were performed according to the manufacturer's protocols, with absorbance read at 450 nm and concentrations interpolated from kit-specific standard curves. The SOD-1 assay quantifies SOD-1 protein concentration rather than enzymatic activity.

### 2.4 Ethical considerations

The study protocol was approved by the Scientific Research Ethics Committee of Northern Technical University (approval no. 127A/26, dated 25 February 2026). All participants provided written informed consent, confidentiality was maintained at every stage, and the study was conducted in accordance with the Declaration of Helsinki.

## 2.5 Statistical analysis

Data were analysed with SPSS version 27 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean  $\pm$  standard deviation (SD). Normality was assessed with the Shapiro–Wilk test and homogeneity of variance with Levene's test before parametric analysis. Between-group differences were tested by one-way analysis of variance (ANOVA), with eta-squared ( $\eta^2$ ) reported as the effect size and Tukey's honestly significant difference (HSD) test used for post-hoc pairwise comparisons, which controls the family-wise error rate within each marker. Where variances were unequal, results were confirmed with Welch's ANOVA and Games–Howell post-hoc tests. Eight separate ANOVAs were performed (one per biomarker) and no formal correction was applied across markers; however, all significant omnibus tests had  $p < 0.001$  and would remain significant after a Bonferroni correction for eight tests ( $\alpha = 0.00625$ ). Pearson correlation coefficients were calculated among the biomarkers that showed significant group effects. No a priori sample-size calculation was performed; the fixed group size of 30 is acknowledged as a design limitation, and the observed effect sizes are reported descriptively rather than being used to compute retrospective power. A two-sided  $p$  value  $< 0.05$  was considered statistically significant.

## 3. Results

### 3.1 Demographic comparability

The four groups did not differ significantly in age (one-way ANOVA,  $F = 0.93$ ,  $p = 0.428$ ). Mean ages were  $33.3 \pm 8.6$  years in non-smokers,  $30.8 \pm 8.2$  years in cigarette smokers,  $34.2 \pm 8.3$  years in waterpipe smokers and  $33.5 \pm 8.1$  years in e-cigarette users, supporting the validity of between-group comparison of the biomarker panel.

### 3.2 Pulmonary injury biomarkers

Serum CC16 differed markedly across groups ( $F = 69.85$ ,  $p < 0.001$ ,  $\eta^2 = 0.644$ ; Table 2). The highest concentration was observed in non-smokers ( $15.91 \pm 1.59$  pg/mL). All three smoking groups had significantly lower values: waterpipe smokers showed the lowest concentration ( $10.97 \pm 1.57$  pg/mL), closely followed by cigarette smokers ( $11.17 \pm 1.24$  pg/mL), while e-cigarette users occupied an intermediate position ( $13.09 \pm 1.59$  pg/mL). Each smoking group differed from controls, and e-cigarette users differed from both combustible groups, whereas cigarette and waterpipe smokers did not differ from each other (Tukey  $p = 0.955$ ; Table 3).

The alveolar damage marker KL-6 showed the strongest overall group effect ( $F = 111.28$ ,  $p < 0.001$ ,  $\eta^2 = 0.742$ ), rising from  $220.48 \pm 17.68$  U/mL in non-smokers to  $326.70 \pm 33.45$  U/mL in cigarette smokers and  $346.12 \pm 36.65$  U/mL in waterpipe smokers, with e-cigarette users again intermediate ( $279.35 \pm 24.61$  U/mL). YKL-40 followed a parallel pattern ( $F = 104.69$ ,  $p < 0.001$ ,  $\eta^2 = 0.730$ ), increasing from  $39.94 \pm 4.74$  pg/mL in non-smokers to  $62.76 \pm 6.10$  and  $59.87 \pm 6.47$  pg/mL in the cigarette and waterpipe groups, respectively. The e-cigarette group mean for YKL-40 ( $45.70 \pm 6.08$  pg/mL) lay closer to that of non-smokers than to either combustible group, and the non-smoker versus e-cigarette contrast was the least pronounced of the smoking comparisons ( $p = 0.001$ ). For both KL-6 and YKL-40, the combustible groups did not differ significantly from each other, both exceeded the e-cigarette group, and the e-cigarette group exceeded non-smokers.

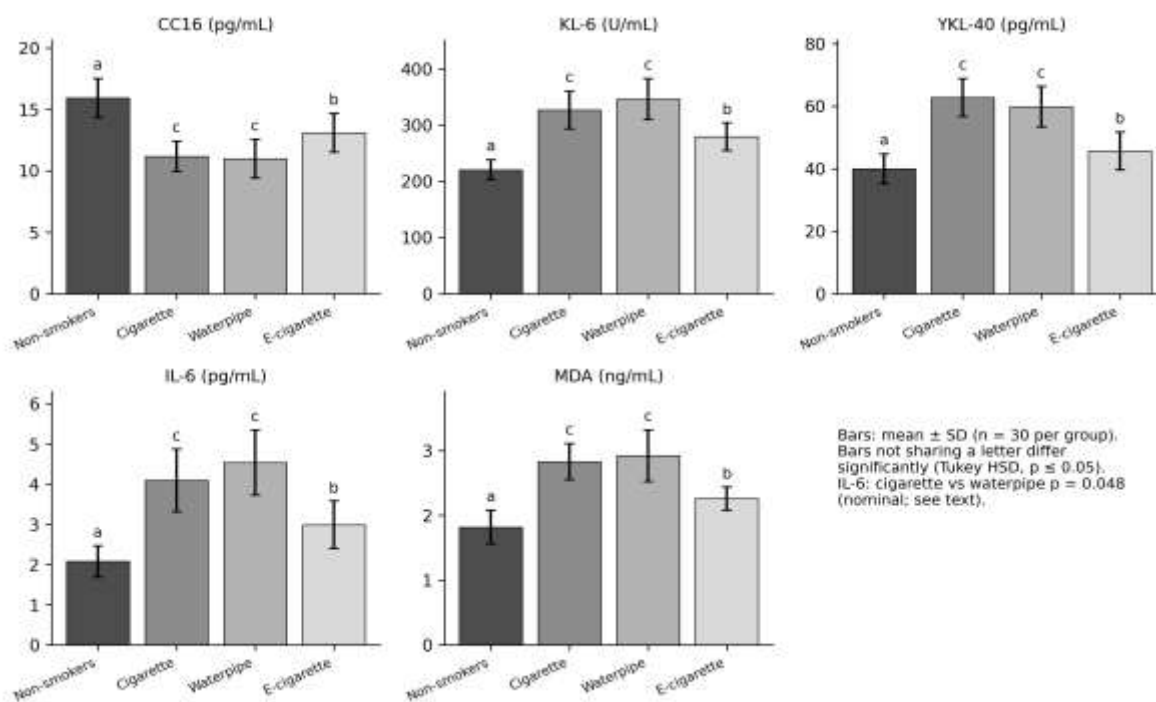
Neither galectin-3 ( $F = 1.48$ ,  $p = 0.223$ ) nor TREM-1 ( $F = 0.15$ ,  $p = 0.927$ ) differed among groups; mean values clustered around 8 ng/mL and 26 pg/mL, respectively, in all four groups. These two markers therefore did not distinguish smokers from non-smokers in this cohort.

**Table 2:** Serum pulmonary injury biomarkers, oxidative stress indices and IL-6 (mean  $\pm$  SD) by study group.

| Biomarker          | Non-smokers        | Cigarette          | Waterpipe          | E-cigarette        | F      | p       | $\eta^2$ |
|--------------------|--------------------|--------------------|--------------------|--------------------|--------|---------|----------|
| CC16 (pg/mL)       | 15.91 $\pm$ 1.59   | 11.17 $\pm$ 1.24   | 10.97 $\pm$ 1.57   | 13.09 $\pm$ 1.59   | 69.85  | < 0.001 | 0.644    |
| KL-6 (U/mL)        | 220.48 $\pm$ 17.68 | 326.70 $\pm$ 33.45 | 346.12 $\pm$ 36.65 | 279.35 $\pm$ 24.61 | 111.28 | < 0.001 | 0.742    |
| YKL-40 (pg/mL)     | 39.94 $\pm$ 4.74   | 62.76 $\pm$ 6.10   | 59.87 $\pm$ 6.47   | 45.70 $\pm$ 6.08   | 104.69 | < 0.001 | 0.730    |
| Galectin-3 (ng/mL) | 7.98 $\pm$ 0.97    | 8.43 $\pm$ 0.95    | 8.49 $\pm$ 1.05    | 8.43 $\pm$ 1.31    | 1.48   | 0.223   | 0.037    |

| Biomarker      | Non-smokers       | Cigarette          | Waterpipe          | E-cigarette       | F     | p       | $\eta^2$ |
|----------------|-------------------|--------------------|--------------------|-------------------|-------|---------|----------|
| TREM-1 (pg/mL) | 26.05 $\pm$ 2.76  | 26.41 $\pm$ 2.94   | 26.48 $\pm$ 2.65   | 26.45 $\pm$ 2.91  | 0.15  | 0.927   | 0.004    |
| IL-6 (pg/mL)   | 2.08 $\pm$ 0.39   | 4.09 $\pm$ 0.78    | 4.54 $\pm$ 0.81    | 2.99 $\pm$ 0.59   | 83.67 | < 0.001 | 0.684    |
| MDA (ng/mL)    | 1.82 $\pm$ 0.26   | 2.83 $\pm$ 0.28    | 2.92 $\pm$ 0.40    | 2.26 $\pm$ 0.18   | 94.91 | < 0.001 | 0.711    |
| SOD-1 (pg/mL)  | 121.97 $\pm$ 8.16 | 120.72 $\pm$ 10.31 | 117.78 $\pm$ 12.54 | 118.29 $\pm$ 8.37 | 1.18  | 0.319   | 0.030    |

**Note:** Values are mean  $\pm$  SD;  $n = 30$  per group.  $F$  and  $p$  from one-way ANOVA ( $df = 3, 116$ );  $\eta^2 = \text{eta-squared}$ . Units are those of each kit's calibrated standard curve with the manufacturer's five-fold dilution applied. CC16, club cell secretory protein-16; KL-6, Krebs von den Lungen-6; TREM-1, triggering receptor expressed on myeloid cells-1; IL-6, interleukin-6; MDA, malondialdehyde; SOD-1, superoxide dismutase-1.



**Figure 1.** Mean ( $\pm$  SD) serum concentrations of the five discriminating biomarkers in the four study groups ( $n = 30$  per group). Bars not sharing a common letter differ significantly by Tukey HSD ( $p \leq 0.05$ ). Cigarette and waterpipe groups share a letter for every marker, indicating comparable measured effects of the two combustible products, with e-cigarette users occupying an intermediate position.

### 3.3 Oxidative stress indices and systemic inflammation

Lipid peroxidation, indexed by MDA, increased significantly across groups ( $F = 94.91$ ,  $p < 0.001$ ,  $\eta^2 = 0.711$ ), from  $1.82 \pm 0.26$  ng/mL in non-smokers to  $2.83 \pm 0.28$  and  $2.92 \pm 0.40$  ng/mL in cigarette and waterpipe smokers, with e-cigarette users intermediate ( $2.26 \pm 0.18$  ng/mL). Systemic IL-6 mirrored this gradient ( $F = 83.67$ ,  $p < 0.001$ ,  $\eta^2 = 0.684$ ), rising from  $2.08 \pm 0.39$  pg/mL in non-smokers to  $4.09 \pm 0.78$  and  $4.54 \pm 0.81$  pg/mL in cigarette and waterpipe smokers, and  $2.99 \pm 0.59$  pg/mL in e-cigarette users. Waterpipe smokers had the numerically highest IL-6 concentration; the contrast with cigarette smokers reached nominal significance (Tukey-adjusted  $p = 0.048$ ) but lies at the margin of the threshold and would not survive correction for the multiple markers tested, so it is interpreted cautiously rather than as evidence of a substantive difference between the two combustible products.

In contrast, SOD-1 protein concentration was closely similar across groups (117.8–122.0 pg/mL) and did not differ significantly ( $F = 1.18$ ,  $p = 0.319$ ). The combination of a clear, graded rise in MDA with unchanged SOD-1 protein indicates a dissociation between the oxidant load imposed by smoking and the expression of this first-line antioxidant protein in these relatively young smokers.

**Table 3:** Tukey HSD post-hoc pairwise comparisons (adjusted p values) for the five biomarkers with significant group effects.

| Comparison                 | CC16    | KL-6    | YKL-40  | IL-6    | MDA     |
|----------------------------|---------|---------|---------|---------|---------|
| Non-smokers vs cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Non-smokers vs waterpipe   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Non-smokers vs e-cigarette | < 0.001 | < 0.001 | 0.001   | < 0.001 | < 0.001 |
| Cigarette vs waterpipe     | 0.955   | 0.053   | 0.233   | 0.048   | 0.603   |
| Cigarette vs e-cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Waterpipe vs e-cigarette   | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

**Note:** Significance threshold  $p \leq 0.05$ . The recurrent non-significant cigarette-versus-waterpipe contrast indicates a comparable measured effect of the two combustible modalities under the present sampling; in the absence of quantitative exposure data it should not be read as proof of biological equivalence. The IL-6 cigarette-versus-waterpipe contrast ( $p = 0.048$ ) is nominally significant only.

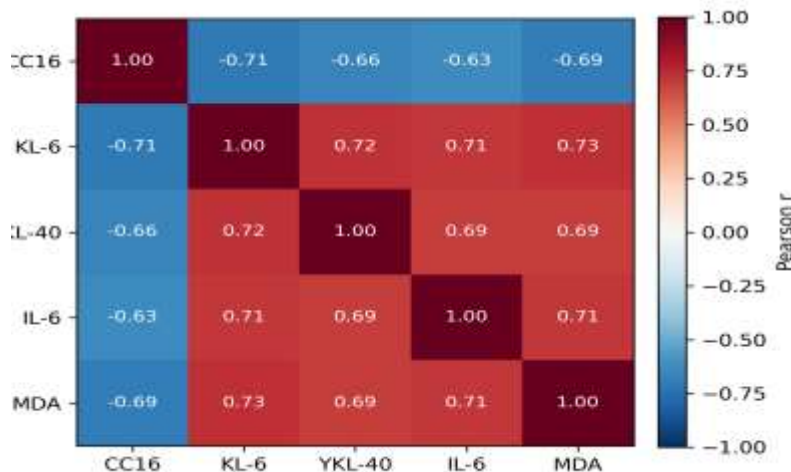
### 3.4 Correlations among biomarkers

Across the whole sample ( $n = 120$ ), the five discriminating biomarkers were strongly inter-correlated in a biologically coherent direction (Table 4, Figure 2). CC16 correlated negatively with every injury, oxidation and inflammation marker ( $r = -0.63$  to  $-0.71$ ; all  $p < 0.001$ ), reflecting loss of the protective epithelial protein as injury, oxidation and inflammation increase. The remaining markers correlated positively with one another ( $r = 0.69$ – $0.73$ ; all  $p < 0.001$ ), the strongest association being between KL-6 and MDA ( $r = 0.73$ ). This pattern is consistent with a single underlying axis linking epithelial injury, lipid peroxidation and IL-6-mediated inflammation.

**Table 4:** Pearson correlation matrix among the five biomarkers with significant group effects (whole sample,  $n = 120$ ).

|        | CC16  | KL-6  | YKL-40 | IL-6  | MDA   |
|--------|-------|-------|--------|-------|-------|
| CC16   | 1.00  | −0.71 | −0.66  | −0.63 | −0.69 |
| KL-6   | −0.71 | 1.00  | 0.72   | 0.71  | 0.73  |
| YKL-40 | −0.66 | 0.72  | 1.00   | 0.69  | 0.69  |
| IL-6   | −0.63 | 0.71  | 0.69   | 1.00  | 0.71  |
| MDA    | −0.69 | 0.73  | 0.69   | 0.71  | 1.00  |

**Note:** All correlations significant at  $p < 0.001$ . Negative coefficients for CC16 reflect its inverse relationship with the injury, oxidation and inflammation markers.



**Figure 2:** Pearson correlation heatmap among the five discriminating biomarkers (whole sample,  $n = 120$ ). The inverse loading of CC16 against the positively co-varying injury, oxidation and inflammation markers is consistent with a single exposure-responsive axis. All correlations were significant at  $p < 0.001$ .

#### 4. Discussion

This study compared a multidimensional biomarker panel among conventional cigarette, waterpipe and e-cigarette users within a single age-matched male cohort. Three findings stand out. First, a consistent and biologically coherent signature (reduced CC16 with elevated KL-6, YKL-40, IL-6 and MDA) discriminated all smokers from non-smokers. Second, the two combustible modalities produced statistically indistinguishable derangements on almost every measure, whereas e-cigarette use produced a consistently intermediate profile. Third, three of the markers examined (galectin-3, TREM-1 and SOD-1 protein) did not discriminate smoking status, a negative finding that carries its own interpretation.

The reduction in CC16 mirrors observations from prospective cohorts in which low circulating CC16 precedes and predicts lung-function decline and incident COPD [9,28]. Because club cells both secrete CC16 and participate in the detoxification of inhaled xenobiotics, depletion of these cells in chronic smokers offers a parsimonious explanation for the observed fall [8,10]; the partial preservation of CC16 in e-cigarette users is consistent with a smaller, though not trivial, epithelial insult. The elevation of KL-6 accords with its established role as a marker of alveolar epithelial damage [13,14,29], and the rise in YKL-40 agrees with earlier reports of increased serum chitinase-like protein in both combustible smokers and e-cigarette users [15,16,30].

It is noteworthy that waterpipe smoking produced derangements statistically similar to, and in the case of IL-6 numerically greater than, those of cigarette smoking, although the IL-6 contrast ( $p = 0.048$ ) is inconclusive. The waterpipe is widely perceived as a benign social alternative to cigarettes, yet toxicant dosimetry shows that users may inhale greater quantities of carbon monoxide and particulate matter than cigarette smokers and are therefore exposed to comparable or greater biological hazard [5,31,32]. Experimental and human studies likewise indicate that waterpipe smoke is at least as pro-inflammatory and pro-oxidant for the lung as cigarette smoke [31,33]. An important caveat applies to this comparison: exposure was not quantified (no pack-years, cotinine or exhaled carbon monoxide data were collected), so the statistical similarity of the two combustible groups may reflect the doses actually delivered rather than the intrinsic toxicity of the products. The finding is therefore best described as a similar measured effect rather than as proof of biological equivalence.

The intermediate position of e-cigarette users is in keeping with an emerging body of evidence. A recent meta-analysis of approximately 24,000 adults found that combustible smokers had higher inflammatory marker concentrations than both non-smokers and e-cigarette users, with the latter group typically falling between the two [34]. The 2025 Saudi comparison of cigarette, shisha, e-cigarette and non-smoking groups reached a convergent conclusion, with combustible products impairing lung function more than e-cigarettes while cigarette and shisha users displayed similar inflammatory profiles [27]. Controlled comparisons of combustible and electronic hookah also show a stronger oxidative and inflammatory signal with the combustible product, yet vaping is far from inert [35]. E-cigarette aerosols induce oxidant generation, epithelial toxicity and IL-6/IL-8 release in vitro and in vivo [36–39], cause vascular oxidative stress and endothelial dysfunction after even short-term exposure [40], and have recognised cardiovascular and systemic effects [41,42]. The present data therefore support a graded interpretation: on these indicators, e-cigarettes are less harmful than combustible products but are not equivalent to non-exposure.

The unchanged galectin-3 and TREM-1 concentrations warrant cautious interpretation. Their stability in a young, clinically healthy cohort suggests that the early, subclinical injury captured by CC16 and KL-6 had not yet progressed to the fibrotic remodelling or acute innate immune amplification that these proteins index [19,21]. The stable SOD-1 value requires even greater care. The present finding is not directly comparable with studies that measured SOD enzymatic activity, such as the 2024 pilot study of chronic smokers that reported reduced activity [23], because the assay used here quantifies the concentration of SOD-1 protein. An unchanged protein concentration does not exclude a loss of function: oxidative inactivation or post-translational modification can reduce catalytic activity without altering the amount of immunoreactive protein. The dissociation between clearly elevated MDA and stable SOD-1 protein should therefore be read as evidence of unchanged antioxidant protein expression, not of preserved antioxidant capacity, and direct activity assays would be needed to distinguish the two. Differences in age and exposure duration

between cohorts may also contribute. Overall, the null result for SOD-1 protein does not contradict the literature on SOD activity but adds a methodologically distinct observation.

The strong inter-correlations among the discriminating markers further support a single exposure-responsive pathway linking epithelial barrier injury, lipid peroxidation and IL-6-mediated inflammation in the systemic circulation. The inverse loading of CC16 on this axis is expected, given its anti-inflammatory and antioxidant properties and its contribution to the resolution of inflammation and repair of the airway epithelial barrier [8,43].

#### 4.1 Strengths and Limitations

The strengths of this study include the balanced group sizes, the age comparability across groups, the exclusion of dual users, and a panel that spans epithelial injury, oxidative stress and inflammation. Several limitations must be acknowledged. The cross-sectional design permits only associative inference, and the biomarker differences represent a single point in time. The most important limitation is the absence of a quantitative exposure measure; smoking was categorised by product only, so the statistical similarity of the combustible groups may reflect delivered dose rather than intrinsic toxicity, and no dose–response relationship could be modelled. Apart from age, potential confounders such as body mass index, socioeconomic status and the duration or intensity of use were not recorded, so no multivariable adjustment (for example by ANCOVA) was possible and residual confounding cannot be excluded. The young, single-sex, single-region sample limits generalisability, and the lack of spirometry prevents the biomarker changes from being linked to functional outcomes. Two analytical caveats also apply. First, the SOD-1 assay measured protein concentration rather than enzymatic activity, so stable SOD-1 does not imply preserved antioxidant activity. Second, all concentrations are expressed in the units of each manufacturer's calibrated standard curve, and the absolute values of some analytes lay near the nominal limits of their kits and depend on the dilution and calibration applied; the results should therefore be interpreted as relative comparisons between groups rather than as absolute reference concentrations, and confirmation on an orthogonal analytical platform would strengthen them.

### 5. Conclusion

In this matched male cohort, conventional cigarette, waterpipe and e-cigarette use were each associated with a clear biomarker profile of airway epithelial injury (reduced CC16, elevated KL-6 and YKL-40), lipid peroxidation (elevated MDA) and systemic inflammation (elevated IL-6). The two combustible products produced statistically similar and most severe derangements, contradicting the common perception of the waterpipe as a benign alternative, whereas e-cigarettes produced a measurable but consistently smaller effect. Because exposure intensity was not quantified, the similarity between the combustible groups should be regarded as a similarity of measured effect rather than of biological equivalence. CC16, KL-6, YKL-40, IL-6 and MDA behaved as sensitive and mutually consistent markers of early, subclinical lung injury, whereas galectin-3, TREM-1 and SOD-1 protein did not discriminate smoking status. These findings do not support the notion that e-cigarettes are a safe alternative to combustible products, while at the same time not overstating their lower biomarker burden. Longitudinal studies incorporating quantitative exposure metrics and lung-function testing are needed to establish causal pathways and clinical relevance.

#### 5.1 Future Directions

Future work should link these biomarkers to spirometric decline, include women and older adults, quantify exposure with cotinine or exhaled carbon monoxide, and follow e-cigarette users over time. Such studies would strengthen the evidence base and help resolve whether the intermediate profile of vaping represents a real reduction in risk or a delayed pathway to the same harm.

#### Declarations

**Ethics approval:** The study was approved by the Scientific Research Ethics Committee of Northern Technical University (no. 127A/26, 25 February 2026) and conducted in accordance with the Declaration of Helsinki.

**Consent to participate:** All participants gave written informed consent.

**Funding.** This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

**Conflicts of interest:** The author declares no conflict of interest.

**Data availability:** The data analysed in this study are available from the corresponding author upon reasonable request.

## References

1. World Health Organization. WHO Global Report on trends in prevalence of tobacco use 2000–2025, third edition. Geneva: World Health Organization; 2019.
2. World Health Organization. Tobacco: key facts. Geneva: World Health Organization; 2025.
3. Wang Y, Zhao Y, Liu Y, et al. Global burden of disease related to tobacco products and trends projected: 1990–2021. *Addict Behav.* 2025;167:108359.
4. Kargar S, Ansari-Moghaddam A. Prevalence of cigarette and waterpipe smoking and associated cancer incidence among adults in the Middle East. *East Mediterr Health J.* 2023;29(9):749–56.
5. Nakhaee N, Divsalar K, et al. Waterpipe (shisha, hookah) smoking, oxidative stress and hidden disease potential. *Redox Biol.* 2020;34:101545.
6. Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco smoke induces and alters immune responses in the lung triggering inflammation, allergy, asthma and other lung diseases. *Int J Environ Res Public Health.* 2018;15(5):1033.
7. Lange P, Celli B, Agustí A, et al. Lung-function trajectories leading to chronic obstructive pulmonary disease. *N Engl J Med.* 2015;373(2):111–22.
8. Laicho-Contreras ME, Polverino F, Tesfaigzi Y, et al. Club cell protein 16 (CC16) augmentation: a potential disease-modifying approach for chronic obstructive pulmonary disease (COPD). *Expert Opin Ther Targets.* 2016;20(7):869–83.
9. Guerra S, Halonen M, Vasquez MM, et al. Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. *Lancet Respir Med.* 2015;3(8):613–20.
10. Broeckaert F, Bernard A. Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. *Clin Exp Allergy.* 2000;30(4):469–75.
11. Zheng D, Chen H, Davids J, et al. Club cell secretory protein-16 (CC16) as a prognostic biomarker for COVID-19 and H1N1 viral infections. *Diagnostics (Basel).* 2024;14(16):1789.
12. Iwanaga T, Tohda Y, Sakurai H, et al. Diagnostic values for club cell secretory protein (CC16) in serum of patients of combined pulmonary fibrosis and emphysema. *COPD.* 2014;11(5):561–9.
13. Kohno N, Kyoizumi S, Awaya Y, et al. New serum indicator of interstitial pneumonitis activity: sialylated carbohydrate antigen KL-6. *Chest.* 1989;96(1):68–73.
14. Kobayashi J, Kitamura S. KL-6: a serum marker for interstitial pneumonia. *Chest.* 1995;108(2):311–5.
15. Létuvé S, Kozhich A, Arouche N, et al. YKL-40 is elevated in patients with chronic obstructive pulmonary disease and activates alveolar macrophages. *J Immunol.* 2008;181(7):5167–73.
16. Tsoumakidou M, Bouloukaki I, Thimaki K, et al. Overexpression of chitotriosidase and YKL-40 in peripheral blood and sputum of healthy smokers and patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis.* 2019;14:1457–66.
17. James BD, Buckley ST, et al. YKL-40 as a possible marker of neutrophilic asthma. *Front Med (Lausanne).* 2023;10:1115938.
18. de Boer RA, Yu L, van Veldhuisen DJ. Galectin-3 in cardiac remodeling and heart failure. *Curr Heart Fail Rep.* 2010;7(1):1–8.
19. Mackinnon AC, Gibbons MA, Farnworth SL, et al. Regulation of transforming growth factor- $\beta$ 1-driven lung fibrosis by galectin-3. *Am J Respir Crit Care Med.* 2012;185(5):537–46.
20. Bouchon A, Dietrich J, Colonna M. Cutting edge: inflammatory responses can be triggered by TREM-1, a novel receptor expressed on neutrophils and monocytes. *J Immunol.* 2000;164(10):4991–5.
21. Gibot S, Cravoisy A, Levy B, et al. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. *N Engl J Med.* 2004;350(5):451–8.
22. Shiels MS, Katki HA, Freedman ND, et al. Cigarette smoking and variations in systemic immune and inflammation markers. *J Natl Cancer Inst.* 2014;106(11):dju294.
23. Pandi A, Lal V, Chakraborty B, Kalappan VM. Evaluation of oxidative stress and antioxidant biomarkers in chronic cigarette smokers: a pilot study. *Cureus.* 2024;16(5):e60629.

24. Aldaham S, Foote JA, Chow HHS, Hakim IA. Smoking status effect on inflammatory markers in a randomized trial of current and former heavy smokers. *Int J Inflam*. 2015;2015:439396.
25. Wang Y, Yang J, Wang W, et al. Effects of smoking on inflammatory-related cytokine levels in human serum. *Molecules*. 2022;27(12):3715.
26. Al-Tameemi SA, Hameed NJ, Gomes KB, Abid HA. Cigarette smoking increases plasma levels of IL-6 and TNF- $\alpha$ . *Baghdad J Biochem Appl Biol Sci*. 2022;3(1):60–8.
27. Homoud MM, Qoutah R, Krishna G, et al. Comparative assessment of respiratory, hematological and inflammatory profiles of long-term users of cigarettes, shisha, and e-cigarettes in Saudi Arabia. *Tob Induc Dis*. 2025;23:61.
28. Zhai J, Insel M, Addison KJ, et al. Club cell secretory protein and lung function in COPD. *Respir Res*. 2022;23:248.
29. Ishikawa N, Hattori N, Yokoyama A, et al. Ageing and long-term smoking affects KL-6 levels in the lung, induced sputum and plasma. *BMC Pulm Med*. 2011;11:22.
30. Tan KS, Pukniyom Y, et al. The inflammatory biomarker YKL-40 is elevated in the serum, but not the sputum, of e-cigarette users. *Exp Lung Res*. 2021;47(2):85–93.
31. Khabour OF, Alzoubi KH, Bani-Ahmad M, et al. Acute and chronic effects of waterpipe tobacco smoke on the lung: inflammation and oxidative stress. *Inhal Toxicol*. 2018;30(3):103–10.
32. Münzel T, Hahad O, Kuntic M, et al. Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes. *Eur Heart J*. 2020;41(41):4057–70.
33. Khan NA, Lawyer G, McDonough S, et al. Systemic biomarkers of inflammation, oxidative stress and tissue injury and repair among waterpipe, cigarette and dual tobacco smokers. *Tob Control*. 2020;29(Suppl 5):s102–9.
34. Tobaldini E, Vassalini P, et al. Inflammatory and carcinogenic biomarker signatures in e-cigarette users: a meta-analysis. *Int J Public Health*. 2025;70:1608885.
35. Rezk-Hanna M, Mosenifar Z, Benowitz NL, et al. Differential effects of electronic hookah vaping and traditional combustible hookah smoking on oxidation, inflammation, and arterial stiffness. *Chest*. 2022;161(1):208–18.
36. Lerner CA, Sundar IK, Yao H, et al. Vapors produced by electronic cigarettes and e-juices with flavorings induce toxicity, oxidative stress, and inflammatory response in lung epithelial cells and in mouse lung. *PLoS One*. 2015;10(2):e0116732.
37. Muthumalage T, Lamb T, Friedman MR, Rahman I. E-cigarette flavored pods induce inflammation, epithelial barrier dysfunction, and DNA damage in lung epithelial cells. *Sci Rep*. 2019;9:19035.
38. Scott A, Lugg ST, Aldridge K, et al. Pro-inflammatory effects of e-cigarette vapour condensate on human alveolar macrophages. *Thorax*. 2018;73(12):1161–9.
39. Reidel B, Radicioni G, Clapp PW, et al. E-cigarette use causes a unique innate immune response in the lung, involving increased neutrophilic activation and altered mucin secretion. *Am J Respir Crit Care Med*. 2018;197(4):492–501.
40. Kuntic M, Oelze M, Steven S, et al. Short-term e-cigarette vapour exposure causes vascular oxidative stress and dysfunction. *Eur Heart J*. 2020;41(26):2472–83.
41. Almeida-da-Silva CLC, Matshik Dakafay H, O'Brien K, et al. Effects of electronic cigarette aerosol exposure on oral and systemic health. *Biomed J*. 2021;44(3):252–9.
42. Bhatnagar A. Cardiovascular effects of electronic cigarettes. *Circ Res*. 2024;134(11):1546–63.
43. Rennard SI, Pruzanski W, Hudig D. The club cell and its protein, CC16: time to shine. *Lancet Respir Med*. 2013;1(9):673–4.



## Testicular Gene Expression Response to *Icacina Trichantha* Root Ethanol Extract Therapy in Paroxetine-Induced Reproductive Dysfunction in Male Wistar Rats

O.C. UGBENI<sup>1\*</sup>

C.E. ESEINE-ALOJA<sup>2\*</sup>

<sup>1\*</sup>Department of Biochemistry, University of Benin, Edo State, Nigeria.

Email: osezele.ugbeni@uniben.edu

<sup>2\*</sup>College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Email: cee-aloja@aauekpoma.edu.ng

**Abstract.** Male infertility is increasingly linked to pharmacological agents, particularly selective serotonin reuptake inhibitors (SSRIs) such as paroxetine, which may disrupt spermatogenesis through endocrine and molecular mechanisms. Paroxetine is therefore used as an agent in studying some intervention strategy in male infertility in experimental animals. This study investigated the effects of paroxetine on the expression of key spermatogenic genes, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) in male Wistar rats and evaluated the remedial potential of *Icacina trichantha* root ethanol extract (ITREE) in comparison with tadalafil and clomiphene citrate. Twenty-five rats were randomly assigned to five groups: control (Group A), paroxetine only (Group B), paroxetine + ITREE (Group C), paroxetine + tadalafil (Group D), and paroxetine + clomiphene (Group E). The oral treatment regimen spans through 56 days. Paroxetine significantly increased CREM ( $92.64 \pm 2.35$  vs.  $28.80 \pm 2.94$ ), PRM I ( $58.17 \pm 1.73$  vs.  $21.64 \pm 1.29$ ), and PRM II ( $66.08 \pm 3.25$  vs.  $42.74 \pm 1.91$ ) compared with control, and elevated the PRM I/PRM II ratio from 0.51 in control to 0.88. Co-administration of ITREE partially restored expression levels (CREM  $68.87 \pm 1.46$ , PRM I  $42.15 \pm 1.63$ , PRM II  $55.71 \pm 3.28$ ) and attenuated the PRM I/PRM II ratio to 0.76. Tadalafil and clomiphene produced varying degrees of modulation, with CREM expression of  $66.96 \pm 2.07$  and  $105.12 \pm 3.85$ , respectively. These findings indicate that paroxetine disrupted spermatid differentiation and chromatin remodeling, while ITREE effectively mitigates these molecular alterations. The study highlights the therapeutic potential of *I. trichantha* roots in preventing SSRI-induced male reproductive toxicity and provides a molecular basis for plant-based interventions.

**Keywords:** Paroxetine, Spermatogenesis, CREM, Protamines, *Icacina trichantha*

### 1. Introduction

Male infertility is a growing global health concern, accounting for approximately 40–50% of infertility cases worldwide (Kumar & Singh, 2015). A wide range of factors including genetic abnormalities, endocrine disruption, environmental exposures, and pharmacological agents have been implicated in the impairment of male reproductive function (Agarwal *et al.*, 2021). Among pharmacological agents, antidepressants particularly selective serotonin reuptake inhibitors (SSRIs) have attracted increasing attention because of their potential adverse effects on reproductive physiology and sperm function. One of the most widely prescribed SSRIs is paroxetine, which is commonly used in the treatment of depression, anxiety disorders, obsessive–compulsive disorder, panic disorder, and post-traumatic stress disorder (Nevels *et al.*, 2016).

Paroxetine exerts its therapeutic action primarily through inhibition of the serotonin reuptake transporter (SERT), resulting in increased synaptic concentrations of serotonin in the central nervous system. While this mechanism is beneficial in the management of psychiatric disorders, excessive serotonergic activity has been associated with

disturbances in the hypothalamic–pituitary–gonadal (HPG) axis, leading to impaired reproductive hormone regulation (Mosbah *et al.*, 2019). Elevated serotonin levels may suppress gonadotropin-releasing hormone (GnRH) secretion and subsequently reduce follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone production, thereby disrupting spermatogenesis and sperm maturation (Schenker *et al.*, 1992; Mosbah *et al.*, 2019). Experimental and clinical studies have demonstrated that paroxetine administration can lead to reduced sperm count, impaired sperm motility, abnormal sperm morphology, and increased DNA fragmentation (Mosbah *et al.*, 2021; Yardimci *et al.*, 2019). Histopathological alterations including degeneration of seminiferous tubules, cellular disorganization, vacuolization, and reduced numbers of spermatozoa within the epididymal lumen have also been reported in animal models following paroxetine exposure (Elkelany & Kashef, 2023).

Beyond endocrine disruption and structural testicular damage, growing evidence suggests that male infertility may also result from alterations in the expression of genes essential for spermatogenesis. Spermatogenesis is a highly coordinated process involving mitotic proliferation of spermatogonia, meiotic division of spermatocytes, and differentiation of haploid spermatids into mature spermatozoa (Sánchez-Jasso *et al.*, 2023). This process is tightly regulated by transcription factors and signaling pathways that control stage-specific gene expression. Among these regulatory molecules, the cyclic adenosine monophosphate (cAMP) response element modulator (CREM) plays a central role in post-meiotic germ cell differentiation.

CREM is a cAMP-responsive transcription factor belonging to the CREB/ATF family of transcription regulators and is considered a key regulator of spermiogenesis. Through alternative promoter usage and extensive alternative splicing, the CREM gene produces multiple isoforms that function either as transcriptional activators or repressors (Behr & Weinbauer, 2001). During normal spermatogenesis, a developmental “switch” occurs in which CREM repressor isoforms expressed in pre-meiotic germ cells are replaced by activator isoforms, particularly CREM $\tau$ , in post-meiotic haploid spermatids (Peri & Serio, 2000). This switch is essential for the transcription of several spermatid-specific genes involved in sperm structural development and chromatin remodeling. Studies in CREM-deficient mice have demonstrated complete infertility characterized by arrest of germ cell maturation at the round spermatid stage, underscoring the indispensable role of CREM in spermatid differentiation (Blendy *et al.*, 1996; Nantel *et al.*, 1996). Similarly, abnormal CREM expression patterns have been observed in men with spermatogenic failure and round spermatid maturation arrest, indicating that disruption of CREM signaling contributes to male infertility (Steger, 1999; Sánchez-Jasso *et al.*, 2023).

Among the downstream targets regulated by CREM are the protamine genes, which encode sperm-specific nuclear proteins essential for chromatin condensation during the final stages of spermiogenesis. Protamines are small arginine-rich proteins that replace histones in elongating spermatids, leading to the formation of highly compacted sperm chromatin. In mammals, two major protamines, protamine 1 (PRM1) and protamine 2 (PRM2) are expressed and function cooperatively to achieve the extreme condensation of paternal DNA within the sperm head (Ren *et al.*, 2021). This histone-to-protamine transition is critical for maintaining genomic integrity, protecting sperm DNA from oxidative damage, and ensuring proper transmission of paternal genetic information during fertilization (Chioccarelli *et al.*, 2020; Kobayash *et al.*, 2021).

The expression of PRM1 and PRM2 is tightly regulated at transcriptional, translational, and post-translational levels during spermatogenesis. Any imbalance in the expression or ratio of PRM1 to PRM2 can disrupt chromatin packaging, resulting in abnormal sperm morphology, reduced motility, and increased DNA fragmentation (Pardede *et al.*, 2020; Ren *et al.*, 2021). Altered protamine expression has therefore been widely recognized as a biomarker of impaired spermatogenesis and male infertility. Environmental toxicants, pharmacological agents, and oxidative stress have all been reported to interfere with protamine expression and chromatin remodeling processes in germ cells (Bashiri *et al.*, 2021).

Given the increasing use of antidepressant medications and their potential reproductive toxicity, there is growing interest in identifying therapeutic agents capable of mitigating drug-induced reproductive dysfunction. Natural products and plant-derived bioactive compounds have been widely investigated for their protective effects on reproductive health due to their antioxidant, anti-inflammatory, and endocrine-modulating properties (Agarwal *et al.*,

2021). In this context, medicinal plants used in traditional medicine represent promising candidates for the management of male reproductive disorders.

Despite the documented reproductive toxicity of paroxetine, limited studies have explored its impact on the molecular regulators of spermatogenesis, particularly transcription factors such as CREM and downstream structural proteins such as protamines. Furthermore, the potential of phytotherapeutic agents to restore paroxetine-induced alterations in spermatogenic gene expression remains largely unexplored. Therefore, this study was designed to investigate the effects of paroxetine on the expression of CREM and protamine genes in the testes of male rats and to evaluate the remedial potential of *Icacina trichantha* root ethanolic extract in comparison with established therapeutic agents. Understanding the molecular mechanisms underlying these effects may provide valuable insight into novel strategies for the management of drug-induced male infertility.

Given the increasing incidence of antidepressant use and the growing concern about their potential reproductive side effects, there is a need to explore therapeutic strategies capable of mitigating drug-induced reproductive dysfunction. Plant-derived bioactive compounds have gained considerable attention in recent years due to their diverse pharmacological properties, including antioxidant, anti-inflammatory, and endocrine-modulating effects that may protect against reproductive toxicity (Agarwal *et al.*, 2021; Bashiri *et al.*, 2021). Medicinal plants used in traditional medicine therefore represent promising candidates for the management of male infertility associated with xenobiotic exposure.

*Icacina trichantha*, a medicinal plant widely used in African ethnomedicine, has been reported to possess several biological activities including antioxidant, anti-inflammatory, and cytoprotective properties (Nwankwo *et al.*, 2022). These pharmacological properties suggest that extracts derived from *I. trichantha* may have the potential to protect or restore reproductive function following chemical or drug-induced testicular damage. However, despite the increasing interest in medicinal plants as therapeutic agents, there is limited information regarding the potential role of *I. trichantha* in modulating molecular pathways involved in spermatogenesis.

In addition to phytotherapeutic agents, pharmacological interventions such as clomiphene citrate and tadalafil are commonly used in the management of male reproductive disorders. Clomiphene citrate is a selective estrogen receptor modulator that enhances endogenous testosterone production by stimulating gonadotropin secretion through blockade of estrogen-mediated negative feedback on the hypothalamic–pituitary axis (Krzastek *et al.*, 2019). Tadalafil, a phosphodiesterase type-5 inhibitor, is widely used in the treatment of erectile dysfunction and has also been shown to improve testicular blood flow, sperm parameters, and overall reproductive function (Corona *et al.*, 2020). These agents therefore serve as useful pharmacological comparators when evaluating the potential reproductive benefits of novel therapeutic compounds.

Although the adverse effects of paroxetine on sperm quality and testicular histology have been documented, there is limited information on its influence on key molecular regulators of spermatogenesis such as the cyclic AMP response element modulator (CREM) and protamine proteins. Since CREM acts as a critical transcription factor regulating post-meiotic germ cell differentiation and protamines are essential for chromatin condensation during spermiogenesis, alterations in the expression of these genes may contribute significantly to impaired sperm development and male infertility.

Therefore, the present study was designed to investigate the effects of paroxetine-induced reproductive toxicity on the expression of CREM and protamine genes in the testes of male Wistar rats and to evaluate the potential remedial effects of *Icacina trichantha* root ethanolic extract (ITREE). In addition, the therapeutic efficacy of ITREE was compared with established pharmacological agents, tadalafil and clomiphene citrate. It was hypothesized that paroxetine exposure would disrupt the expression of genes critical for spermatogenesis, particularly CREM and protamine, and that treatment with ITREE may ameliorate these molecular alterations and restore normal spermatogenic function.

## 2. Materials and Methods

### 2.1 Preparation of *Icacina trichantha* Root Ethanol Extract (ITREE):

Roots of *Icacina trichantha* were collected and washed thoroughly to remove soil and debris. They were then air-dried at room temperature and subsequently pulverized into coarse powder using a mechanical grinder. A measured quantity of the powdered root material was macerated in 40% ethanol at a ratio of 1:5 (w/v) and agitated on a mechanical shaker at room temperature for 72 hours, as previously described by Olumese *et al.* (2015). The mixture was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further lyophilized using a freeze dryer to obtain a dry ethanol extract, which was stored in a desiccator until use.

### 2.2 Experimental Animals

25 adult male Wistar rats weighing 180–220 g were used for this study. The animals were obtained from the Animal House Facility of the Faculty of Life Sciences, University of Benin, Nigeria. The rats were housed in standard plastic cages under controlled environmental conditions (temperature  $22 \pm 2^\circ\text{C}$ , relative humidity 50–60%, and a 12 h light/dark cycle). Animals had free access to standard laboratory chow and clean drinking water *ad libitum*. The animals were acclimatized for two weeks prior to the commencement of the experiment.

### 2.3 Experimental Design

The experimental animals were randomly assigned into five groups (n = 5 per group). Paroxetine was administered to induce reproductive toxicity in group B which served as negative control, while *Icacina trichantha* root ethanolic extract (ITREE) was evaluated for its potential remedial effects by co-administration with paroxetine in group C. Tadalafil and clomiphene citrate served as standard reference drugs and were co-administered with paroxetine in groups D and E respectively. All treatments were administered orally using an oral gavage once daily for 56 days. The experimental design is presented in Table 1.

**Table 1:** Experimental design for evaluation of the effects of ITREE on paroxetine-induced alterations in gene expression.

| Group                | Number of rats | Treatment                                                   | Duration |
|----------------------|----------------|-------------------------------------------------------------|----------|
| A (Control)          | 5              | Distilled water                                             | 56 days  |
| B (Negative control) | 5              | Paroxetine (10 mg/kg body weight)                           | 56 days  |
| C                    | 5              | Paroxetine (10 mg/kg bw) + ITREE (200 mg/kg bw)             | 56 days  |
| D                    | 5              | Paroxetine (10 mg/kg bw) + Tadalafil (5 mg/kg bw)           | 56 days  |
| E                    | 5              | Paroxetine (10 mg/kg bw) + Clomiphene citrate (10 mg/kg bw) | 56 days  |

### 2.4 Sample Collection

At the end of the experimental period (56 days), the rats were fasted overnight and sacrificed by cervical dislocation in accordance with established procedures for laboratory animals (Aguwa *et al.*, 2020). The animals were dissected and the testes were carefully excised.

### 2.5 Gene Expression Analysis

Gene expression of CREM, PRM I, PRM II and PDE5 in the testis of male Wistar rats was determined via Reverse Transcriptase Polymerase Chain Reaction Analysis (Elekofehinti *et al.* 2020; Olumegbon *et al.*, 2020) in the following steps;

### 2.6 RNA Isolation

Total RNA was extracted from testicular tissue using the Quick-RNA MiniPrep™ Kit (Zymo Research, USA) according to the manufacturer's instructions. To remove genomic DNA contamination, RNA samples were treated

with DNase I (New England Biolabs, USA). The concentration and purity of RNA were determined spectrophotometrically by measuring absorbance at 260 nm and the A260/A280 ratio.

## 2.7 cDNA Synthesis

Complementary DNA (cDNA) was synthesized from 1 µg of DNase-treated RNA using the ProtoScript® II First-Strand cDNA Synthesis Kit (New England Biolabs, USA). Reverse transcription was performed under the following conditions: 65°C for 5 min, 42°C for 60 min, and 80°C for 5 min to terminate the reaction.

## 2.8 Polymerase Chain Reaction (PCR) Amplification

PCR amplification of the target genes was carried out using OneTaq® 2X Master Mix (New England Biolabs, USA) and gene-specific primers synthesized by Inqaba Biotec (Pretoria, South Africa). The genes analyzed included cyclic AMP response element modulator (*Crem*), protamine 1 (*Prm1*), protamine 2 (*Prm2*), and phosphodiesterase 5A (*Pde5a*). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as the housekeeping gene. PCR reactions were performed in a final volume of 25 µL containing cDNA template, forward and reverse primers, nuclease-free water, and PCR master mix. Amplification was carried out under the following conditions: Initial denaturation: 95°C for 5 min

30 cycles of amplification: Denaturation at 95°C for 30 s, annealing for 30 s and extension at 72°C for 60 s, Final extension: 72°C for 10 min.

## 2.9 Agarose Gel Electrophoresis

The PCR products were separated on a 1.0% agarose gel containing ethidium bromide and visualized under ultraviolet illumination. Band intensities were quantified using ImageJ software (National Institutes of Health, USA). Relative gene expression levels were normalized to the housekeeping gene, *Gapdh* (Elekofehinti *et al.*, (Olumegbon *et al.*, 2020).

## 2.10 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 25. One-way analysis of variance (ANOVA) was used to determine differences among groups, followed by the Least Significant Difference (LSD) post hoc test for multiple comparisons. Statistical significance was considered at  $p < 0.05$ .

## 2.11 Ethical Approval

Ethical approval for the study was obtained from the Research Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria. All experimental procedures involving animals were conducted in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals (National Research Council, 2011).

## 3. Results

The effects of the various treatments on the expression of key genes involved in spermatogenesis and sperm chromatin remodeling in the testes of male Wistar rats are presented in Figures 1 to 4. The genes evaluated included cyclic AMP response element modulator (CREM), protamine I (PRM I), protamine II (PRM II), and the PRM I/PRM II ratio.

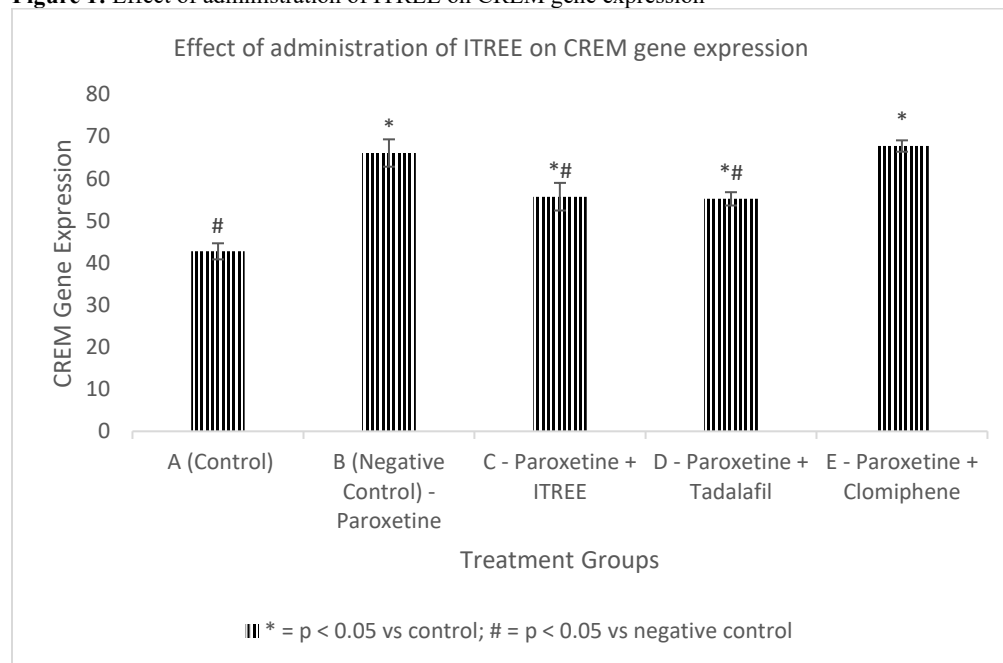
The negative control group (Group B), which received paroxetine alone, showed a significant ( $p < 0.05$ ) increase in the expression of CREM ( $92.64 \pm 2.35$ ), PRM I ( $58.17 \pm 1.73$ ), and PRM II ( $66.08 \pm 3.25$ ) compared with the control group (Group A) which recorded values of  $28.80 \pm 2.94$ ,  $21.64 \pm 1.29$ , and  $42.74 \pm 1.91$ , respectively. This indicates that administration of paroxetine markedly altered the expression of spermatogenesis-related genes in the testes of the experimental animals.

Treatment with the plant extract and standard drugs produced varying degrees of modulation of gene expression relative to the negative control group. Co-administration of paroxetine with ITREE (Group C) resulted in significant reduction in gene expression levels among the treatment groups, with CREM, PRM I, and PRM II values of  $68.87 \pm 1.46$ ,  $42.15 \pm 1.63$ , and  $55.71 \pm 3.28$ , respectively. These values were significantly different ( $p < 0.05$ ) from both the control and negative control groups. Similarly, Groups D co-administered Tadalafil and ITREE demonstrated partial normalization of gene expression levels compared with the negative control group, although the magnitude of reduction was less pronounced than that observed in Group C particularly for PRM I. In contrast, Group E co-administered Clomiphene and ITREE showed relatively unperturbed expression of PRM I and II compared to negative control but a particularly notable increase in CREM ( $105.12 \pm 3.85$ ) expression.

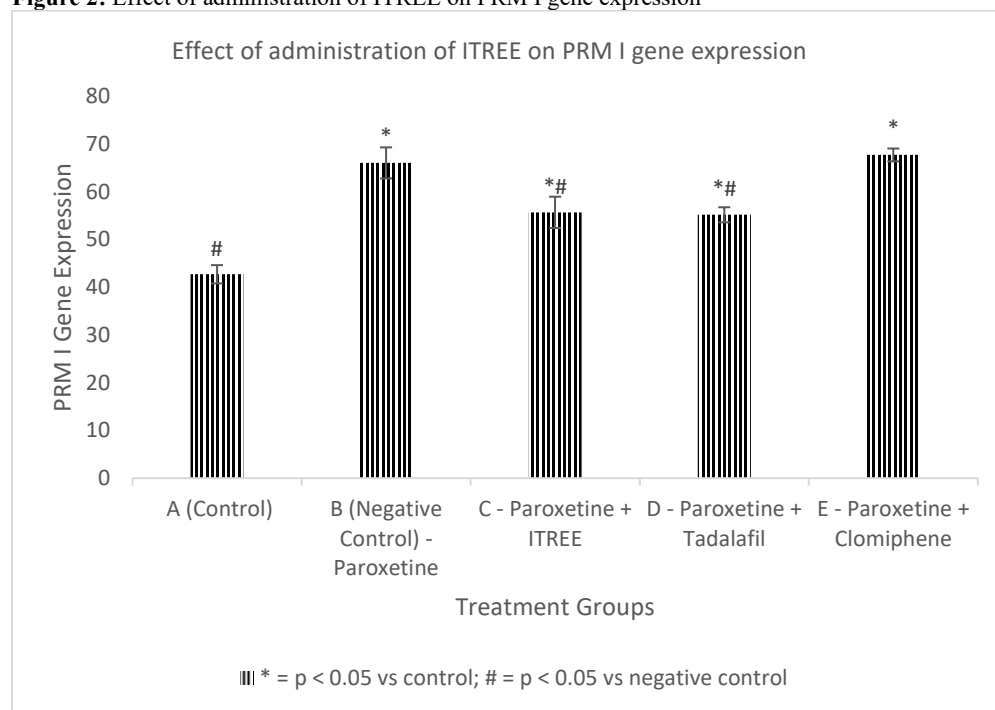
The PRM I/PRM II ratio, an important indicator of proper sperm chromatin packaging and spermatid maturation, was also altered across the experimental groups. The negative control group exhibited an increased ratio (0.88) compared with the control group (0.51), suggesting disruption of normal chromatin condensation dynamics. Among the treatment groups, the paroxetine + ITREE group (Group C) demonstrated the greatest restoration toward the control ratio (0.76). Groups E also showed a very partial improvement with ratio of 0.84, while Group E administered clomiphene exhibited an elevated ratio (1.23), indicating a greater imbalance in protamine expression.

Overall, the findings as presented in Figures 1 to 4 demonstrate that paroxetine administration significantly altered the expression of genes involved in spermatogenesis and chromatin remodeling, while co-administration with plant extracts and standard therapeutic agents resulted in varying degrees of modulation of these molecular markers.

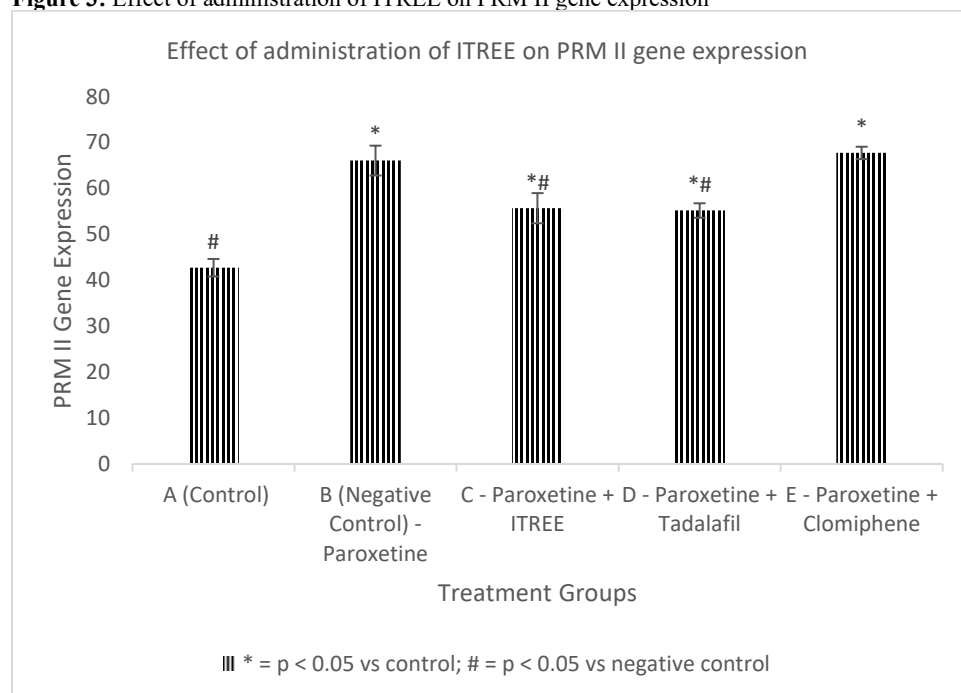
**Figure 1:** Effect of administration of ITREE on CREM gene expression



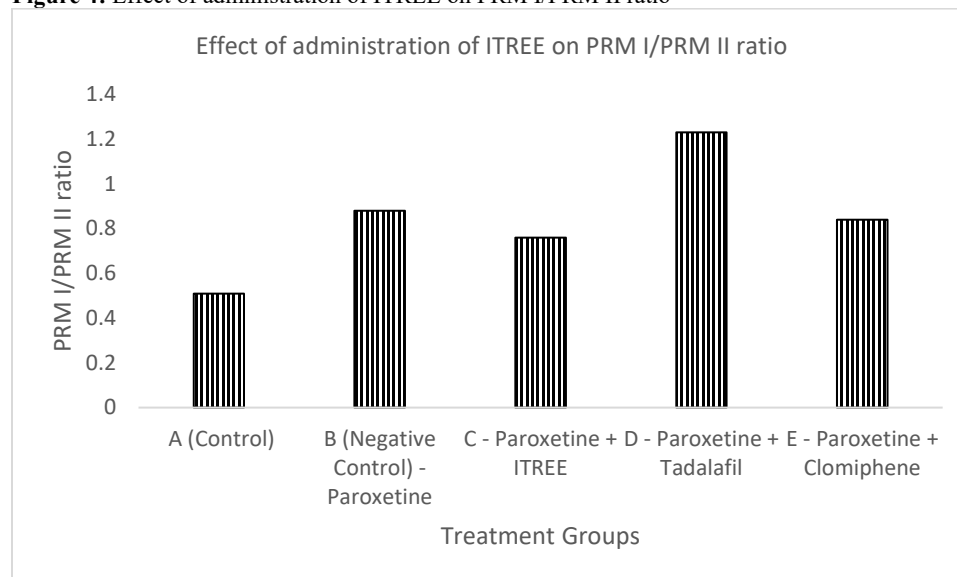
**Figure 2:** Effect of administration of ITREE on PRM I gene expression



**Figure 3:** Effect of administration of ITREE on PRM II gene expression



**Figure 4:** Effect of administration of ITREE on PRM I/PRM II ratio



#### 4. Discussion

The present study investigated the effect of paroxetine on the testicular expression of key genes involved in spermatogenesis, cyclic AMP response element modulator (CREM), protamine I (PRM I), and protamine II (PRM II) and evaluated the potential ameliorative role of ITREE in comparison with established therapeutic agents, tadalafil and clomiphene. These genes are central regulators of post-meiotic germ cell differentiation and chromatin remodeling during spermiogenesis; therefore, alterations in their expression may provide important molecular insights into mechanisms underlying drug-induced male reproductive dysfunction.

The findings of this study demonstrated that administration of paroxetine resulted in a marked dysregulation of spermatogenesis-related gene expression in the testes of male Wistar rats. Specifically, the negative control group (Group B) exhibited a significant elevation in the expression of CREM, PRM I, and PRM II compared with the control group (Group A). This observation suggests that paroxetine exposure disrupts the molecular processes governing spermatid differentiation and sperm chromatin packaging. Increasing evidence indicates that selective serotonin reuptake inhibitors (SSRIs), including paroxetine, may negatively affect male reproductive physiology through oxidative stress, endocrine disturbances, and disruption of testicular gene expression (Tanrikut & Schlegel, 2007). The significant overexpression of the investigated genes observed in the present study therefore supports the hypothesis that paroxetine can interfere with the transcriptional regulatory networks essential for normal spermatogenesis.

CREM is a pivotal transcription factor that mediates the expression of numerous genes required for the transition of round spermatids into elongated spermatids during spermiogenesis. It functions within the cyclic AMP signaling pathway and regulates genes involved in chromatin condensation, acrosome formation, and flagellar development. Previous studies have confirmed that CREM plays a critical role in both human and murine spermatogenesis (Peri & Serio, 2000; Behr & Weinbauer, 2001). Experimental evidence from knockout models has demonstrated that mice lacking the CREM gene are infertile due to an arrest of germ cell maturation at the round spermatid stage (Blendy *et al.*, 1996; Nantel *et al.*, 1996). In such models, the absence of CREM leads to the loss of expression of several post-meiotic genes essential for spermiogenesis, ultimately resulting in impaired sperm production (Sánchez-Jasso *et al.*, 2023).

Regulation of CREM expression is highly complex and occurs at multiple levels, including transcriptional control through the use of multiple promoters (Molina *et al.*, 1993; Stehle *et al.*, 1993; Daniel *et al.*, 2000), alternative transcript splicing (Foulkes *et al.*, 1991; Foulkes *et al.*, 1992), alternative polyadenylation sites (Foulkes *et al.*, 1991),

and translational regulation (Delmas *et al.*, 1992; Gellersen *et al.*, 1997). These regulatory mechanisms give rise to various CREM isoforms that may function either as transcriptional activators or repressors depending on the physiological context. Consequently, tight regulation of CREM expression is required to maintain the proper balance of gene activity during spermatogenesis.

In the present study, paroxetine administration resulted in a marked overexpression of CREM compared with the control group. Dysregulated or excessive expression of CREM may disturb the tightly controlled transcriptional network involved in germ cell differentiation. Recent evidence suggests that aberrant CREM expression may contribute to abnormal spermatid development, defective chromatin remodeling, and impaired sperm maturation (Sánchez-Jasso *et al.*, 2023). Furthermore, altered CREM expression has been associated with abnormal sperm morphology, reduced motility, and idiopathic male infertility (Karami Hezarcheshmeh *et al.*, 2024). Environmental and xenobiotic exposures have also been implicated in the disruption of CREM-regulated pathways (Sánchez-Jasso *et al.*, 2023). Therefore, the overexpression of CREM observed following paroxetine exposure in this study may represent a compensatory or pathological response to testicular stress that ultimately interferes with normal spermatogenic progression.

Co-administration of ITREE with paroxetine resulted in a substantial reduction in CREM expression compared with the paroxetine-only group, although the levels remained higher than those of the control group. This finding suggests that ITREE possesses the capacity to partially restore the normal regulatory balance of spermatogenic transcription factors. The improvement observed may be attributed to the presence of bioactive phytochemicals in *I. trichantha*, which have been reported to exhibit antioxidant and cytoprotective activities capable of mitigating cellular damage induced by xenobiotics. Oxidative stress is widely recognized as a major mechanism through which pharmacological agents impair male reproductive function; therefore, the protective effect of ITREE on CREM expression may be related to its ability to reduce oxidative damage within the testicular microenvironment. The observation that ITREE improved CREM expression toward normal levels suggests a potential regulatory influence on the molecular pathways controlling spermiogenesis.

In comparison, treatment with tadalafil also resulted in a reduction in CREM expression relative to the paroxetine group, indicating partial restoration of transcriptional regulation. Tadalafil, a phosphodiesterase-5 inhibitor commonly used in the management of erectile dysfunction, has been reported to improve testicular perfusion and enhance reproductive function through improved blood flow and modulation of nitric oxide signaling pathways. Improved vascular supply to the testes may enhance the microenvironment required for germ cell development, thereby contributing to normalization of gene expression patterns.

Interestingly, administration of clomiphene in combination with paroxetine resulted in an even greater increase in CREM expression compared with the paroxetine-only group. Clomiphene is a selective estrogen receptor modulator that stimulates endogenous testosterone production by blocking estrogen-mediated negative feedback on the hypothalamic–pituitary–gonadal axis. While this mechanism may increase androgen levels and improve certain aspects of male reproductive function, the elevated CREM expression observed in this group suggests that clomiphene may not effectively correct the specific transcriptional imbalance induced by paroxetine. Instead, the increased gonadotropin stimulation may have further enhanced testicular transcriptional activity, thereby amplifying CREM expression.

Protamine proteins represent another essential component of spermatogenesis and are directly involved in the final stages of sperm chromatin condensation. During spermiogenesis, histones in the spermatid nucleus are progressively replaced by transition proteins and ultimately by protamines, leading to highly compacted chromatin that protects paternal DNA during fertilization. PRM I and PRM II are the two major protamines present in mammalian sperm, and their precise expression ratio is critical for maintaining proper chromatin structure and sperm function (Oliva, 2006; Jodar & Oliva, 2014).

In the present study, paroxetine administration resulted in significant increases in the expression of both PRM I and PRM II relative to the control group. This finding indicates that paroxetine may disrupt the regulatory pathways governing chromatin remodeling during spermiogenesis. Although protamines are essential for DNA condensation,

their expression must be tightly coordinated. Excessive or dysregulated expression can lead to abnormal chromatin packaging, which may compromise sperm morphology, motility, and fertilizing ability.

The PRM I/PRM II ratio is widely regarded as a sensitive biomarker of sperm chromatin integrity. In healthy individuals, this ratio remains relatively stable; however, alterations in the ratio have been associated with defective spermatogenesis and male infertility (Steger & Balhorn, 2018; Pardede *et al.*, 2020). In the current study, paroxetine increased the PRM I/PRM II ratio from 0.51 in the control group to 0.88 in the negative control group, indicating disruption of the normal balance between the two protamines. Such imbalance may impair chromatin condensation and increase susceptibility of sperm DNA to damage. Treatment with ITREE partially corrected this imbalance by reducing the PRM I/PRM II ratio to 0.76, suggesting an improvement in chromatin packaging dynamics. This observation supports the potential protective role of ITREE in maintaining the molecular processes required for proper sperm maturation. Similarly, clomiphene administration resulted in a moderate reduction of the ratio to 0.84, indicating partial normalization. In contrast, tadalafil treatment produced a pronounced increase in the PRM I/PRM II ratio (1.23), which represents a greater deviation from the normal value observed in the control group. This finding suggests that while tadalafil may improve certain physiological aspects of reproductive function, its influence on the molecular regulation of protamine expression may be limited or indirect.

Overall, the findings of this study demonstrate that paroxetine induces significant alterations in the expression of key spermatogenic genes, including CREM, PRM I, and PRM II, thereby disrupting the molecular mechanisms responsible for sperm chromatin remodeling. Co-administration of ITREE was able to partially restore normal gene expression patterns and improve the balance between protamine proteins, indicating a protective effect on spermatogenic processes. When compared with the conventional therapeutic agents evaluated in this study, ITREE demonstrated a comparable or potentially superior ability to modulate the molecular markers associated with sperm maturation.

These results suggest that *Icacina trichantha* root ethanolic extract may represent a promising natural therapeutic candidate for mitigating drug-induced reproductive toxicity. Nevertheless, further investigations involving larger experimental models, protein-level analyses, and fertility outcome studies are necessary to elucidate the precise molecular mechanisms through which ITREE exerts its protective effects on male reproductive function.

## Acknowledgements

The authors wish to acknowledge Poiema Analytical Laboratory and Education Consult for its immense contribution towards the success of this study.

## Declaration of Conflict of Interest

The authors declare no conflicts of interest

## References

- Agarwal, A., Baskaran, S., Parekh, N., Cho, C. L., Henkel, R., Vij, S., Arafa, M., Selvam, M. K. P., Shah, R. (2021). Male infertility. *The Lancet*, 397(10271), 319 – 333. [https://doi.org/10.1016/S0140-6736\(20\)32667-2](https://doi.org/10.1016/S0140-6736(20)32667-2)
- Aguwa, U. S., Okeke, S. N., Eze, C. E., Okoro, J., Obinwa, B. N., Obi, K. C., Ogbuokiri D. K., Okeke C., Onwuelingo, S., Okonkwo, D. Ovie, F., (2020). Evaluating methods of rat euthanasia on the liver and kidney of wistar rats: Cervical dislocation, chlorofoam inhalation, diethylether inhalation and formalin inhalation. *Letters in Health and Biological Science*. Available at <https://www.ommegaonline.org/article-details/Evaluating-Methods-of-Rat-Euthanasia-on-the-Liver-and-Kidney-of-Wistar-rats:-Cervical-dislocation,-chloroform-inhalation,-diethyl-ether-inhalation-and-formalin-inhalation./2736#Anchor-3>. Accessed 14<sup>th</sup> January, 2024.
- Bashiri, Z., Shokri, S., Zarei-Kheirabadi, M., Abolhasani, F. (2021). The role of protamine proteins in sperm chromatin structure and male infertility. *Andrologia*, 53(2), e13908. <https://doi.org/10.1111/and.13908>
- Behr, R., Weinbauer, G. F. (2001). CREM activator and repressor isoforms in spermatogenesis. *Molecular Reproduction and Development*, 58(2), 132–140.

- Blendy, J. A., Kaestner, K. H., Weinbauer, G. F., Nieschlag, E., Schütz, G. (1996). Severe impairment of spermatogenesis in mice lacking the CREM gene. *Nature*, 380, 162–165.
- Chioccarelli, T., Manfredola, F., Ferraro, B., Sellitto, C., Cobellis, G., Fasano, S. (2020). Histone-to-protamine transition and chromatin condensation in spermatogenesis. *Frontiers in Cell and Developmental Biology*, 8, 626.
- Corona, G., Rastrelli, G., Burri, A., Jannini, E. A., Maggi, M., Mannucci, E. (2020). The safety and efficacy of phosphodiesterase-5 inhibitors in male reproductive health. *Nature Reviews Urology*, 17(3), 131–146. <https://doi.org/10.1038/s41585-020-0270-1>
- Daniel, P. B., Rohrbach, L. Habener, J. F., (2000). Novel cyclic adenosine 3',5'-monophosphate (cAMP) response element modulator theta isoforms expressed by two newly identified cAMP-responsive promoters active in the testis. *Endocrinology* 141: 3923 - 3930.
- Elekofehinti, O. O., Lawal, A. O., Ejelonu, O. C. Molehin O. R. Famusiwa, C. D., (2020). Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of *Annona muricata* Annonaceae: in silico and in vivo studies. *Journal of Diabetes & Metabolic Disorders*, 19(1): 197 – 204.
- Elkelany, O. O., Kashef, M. T. (2023). Drug-induced male reproductive toxicity: Mechanisms and clinical implications. *Reproductive Toxicology*, 117, 108–118.
- Foulkes, N. S., Borrelli, E. Sassone-Corsi, P., (1991). CREM gene: use of alternative DNA binding domains generates multiple antagonists of cAMP-induced transcription. *Cell*, 64: 739 - 749.
- Foulkes, N. S., Mellstrom, B., Benusiglio, E. Sassone-Corsi, P., (1992). Developmental switch of CREM function during spermatogenesis: from antagonist to activator. *Nature*, 355: 80 - 84.
- Gellersen B., Kempf R. Telgmann R., (1997). Human endometrial stromal cells express novel isoforms of the transcriptional modulator CREM and up-regulate ICER in the course of decidualization. *Molecular Endocrinology* 11: 97 - 113.
- Jodar, M. Oliva, R., (2014). Protamine alterations in human spermatozoa. *Advances in Experimental Medicine and Biology*, 791: 83 - 102.
- Karami Hezarcheshmeh, F., Yaghmaei, P., Hayati Roodbari, N. Yari, K., (2024). Methylation Status of cAMP-responsive Element Modulator (CREM) Gene in Infertile Men and Its Association with Sperm Parameters. *Reproductive Sciences*, 31: 2001 – 2008.
- Kobayash, H., Sakurai, T., Miura, F., Imai, M., Mochiduki, K., Yanagisawa, E., Sakashita, A., Wakai, T., Suzuki, Y., Ito, T., Ishino, F. (2021). High-resolution DNA methylome analysis of primordial germ cells identifies gender-specific reprogramming. *Nature Communications*, 12, 1–14.
- Kumar, N., Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Sciences*, 8(4), 191–196. <https://doi.org/10.4103/0974-1208.170370>
- Krzastek, S. C., Sharma, D., Abdullah, N., Sultan, A. (2019). Non-testosterone management of male hypogonadism: An examination of clomiphene citrate and enclomiphene citrate. *Translational Andrology and Urology*, 8(Suppl 2), S185–S194.
- Molina C. A., Foulkes N. S., Lalli, E. Sassone-Corsi P., (1993). Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. *Cell*, 75: 875 - 886.
- Mosbah, R., Djerrou, Z., Mantovani, A., Abdennour, C. (2019). Paroxetine induced reproductive toxicity in male rats. *Environmental Science and Pollution Research*, 26, 31476–31486.
- Mosbah, R., Djerrou, Z., Mantovani, A., Abdennour, C. (2021). Histological and reproductive alterations induced by paroxetine in male rats. *Toxicology Reports*, 8, 120–128.
- Nantel, F., Monaco, L., Foulkes, N. S., Masquillier, D., LeMeur, M., Henriksen, K., Dierich, A., Parvinen, M., Sassone-Corsi, P. (1996). Spermiogenesis deficiency and germ-cell apoptosis in CREM-mutant mice. *Nature*, 380, 159–162.
- National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). The National Academies Press. Available at <https://doi.org/10.17226/12910>
- Nevels, R. M., Dehon, E. E., Alexander, K., Williams, J. M. (2016). Paroxetine—The antidepressant from hell? Probably not, but caution required. *Psychopharmacology Bulletin*, 46(1), 77–104.
- Nwankwo, I. U., Nwodo, O. F. C., Joshua, P. E. (2022). Pharmacological and phytochemical properties of *Icacina trichantha*: A review of its ethnomedicinal applications. *Journal of Ethnopharmacology*, 283, 114709.
- Oliva R., (2006). Protamines and male infertility. *Human Reproduction Update*, 12(4): 417 - 435.

- Olumegbon, L. T, Lawal, A. O, Oluyede, D. M, Adebimpe, M. O, Elekofehinti, O. O. Umar, H. I., (2022). Hesperetin protects against diesel exhaust particles induced cardiovascular oxidative stress and inflammation in Wistar rats. *Environmental Science and Pollution Research*, Available at <https://doi.org/10.1007/s11356-022-19494-3>
- Olumese, F. E., Onoagbe, I. O., Eze, G. I. Omoruyi, F. O., (2018). Subchronic toxicity study of ethanolic extract of *Uvaria chamae* root in rats. *Tropical Journal of Pharmaceutical Research*. 17(5): 831 – 836.
- Pardede, B. P., Rahardjo, D., Wantania, J. (2020). Protamine deficiency and male infertility. *Asian Pacific Journal of Reproduction*, 9(3), 101–106.
- Peri, A., Serio, M. (2000). The CREM gene in human spermatogenesis. *Molecular and Cellular Endocrinology*, 161(1–2), 19–25.
- Ren, X., Liu, Q., Li, C., Zhang, W., Zhang, X. (2021). Protamine genes and male infertility: New insights into chromatin remodeling during spermatogenesis. *Genes*, 12(10), 1505.
- Sánchez-Jasso, B. E., Rojas-Castaneda, J. C., García-Lopez, J. (2023). Molecular regulation of spermatogenesis and its role in male infertility. *Frontiers in Cell and Developmental Biology*, 11, 1182346.
- Schenker, J. G., Meirow, D. Schenker, E., (1992). Stress and human reproduction. *European Journal of Obstetrics and Gynecology and Reproductive Biology*, 45(1): 1 – 8.
- Steger, K. (1999). Transcriptional and translational regulation of gene expression in haploid spermatids. *Anatomy and Embryology*, 199, 471–487.
- Steger, K. Balhorn, R., (2018). Sperm nuclear protamines: A checkpoint to control sperm chromatin quality. *Anatomia, Histologia, Embryologia (Journal of Veterinary Medicine, Series C)*, 47: 273 - 279.
- Stehle, J. H., Foulkes, N. S., Molina, C. A., Simonneaux, V., Pevet, P. Sassone-Corsi, P., (1993). Adrenergic signals direct rhythmic expression of transcriptional repressor CREM in the pineal gland. *Nature*, 365: 314 - 320.
- Tanrikut, C., Feldman, A. S., Altemus, M., Paduch, D. A. Schlegel, P. N. (2010). Adverse effect of paroxetine on sperm. *Fertility and Sterility*, 94(3): 1021 – 1026.
- Yardimci, A., Ulker, N., Bulmus, O., Kaya, N., Colakoglu, N., Ozcan, M., Canpolat, S. Kelestimur, H., (2019). Effects of long-term paroxetine or bupropion treatment on puberty onset, reproductive and feeding parameters in adolescent male rats. *Andrologia*, 51 (6): e13268. Available at <https://doi.org/10.1111/and.13268>.