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6. Results

Results section summarizes the collected data and the analysis done on those data. It should provide sufficient detail about the data to justify the study conclusions. The results should also include details that may not match the study expectation; or even small effect sizes (or statistically non-significant findings) particularly when theory predicts large (or statistically significant) ones. Uncomfortable results should not be omitted. The data can be presented in tables or figures (data presented in tables should not be represented in figures).

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OPEN ACCESS CASE REPORT

Dental Student Knowledge of the Role of Early Detection of Oral Cancer: Multi Center Cross Sectional Study

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ABSTRACT

Background: Oral cancer is a major global health issue, where early detection is essential for enhancing survival rates. Dental students, as the next generation of practitioners, have an important role in detecting oral potentially malignant disorders (OPMDs).
Objective: This study seeks to evaluate the knowledge and awareness of Yemeni dental students about OPMDs and strategies for early detection.
Methods: A cross-sectional study was carried out among 323 dental students from various Yemeni universities. An online questionnaire evaluated students' understanding of oral cancer risk factors, clinical features, diagnostic tools, and screening practices. Data analysis was conducted using SPSS version 21, applying chi-square tests to explore relationships between demographic factors and oral cancer awareness.
Results: The results indicated significant knowledge gaps, with only 28.1% of students accurately identifying the OPMD with the highest malignant transformation rate and 27.2% acknowledging oral cancer risk factors. Awareness of regular oral cancer screenings was particularly low (16.5%). Female students displayed greater awareness regarding the importance of screening ($p = 0.004$), while students with 0–5 years of experience showed better knowledge of diagnostic tools ($p = 0.023$). These findings emphasize the necessity for improved educational initiatives in oral cancer detection.
Conclusion This study highlights the need for focused educational programs to enhance dental students' understanding of oral cancer and OPMDs. Tackling these knowledge gaps through enhancements to the curriculum and ongoing education initiatives can empower future dentists to play a more effective role in early detection and prevention, ultimately alleviating the burden of oral cancer in Yemen.

Keywords: Oral cancer, early detection, dental students, oral potentially malignant disorders, risk factors, screening, Yemen.

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INTRODUCTION

Oral cancer is a major global health concern, claiming the lives of over 120,000 people annually, with over 275,000 new cases diagnosed each year (1). Early detection through routine oral screenings and clinical examinations is crucial for effective treatment (2). Despite the accessibility of the oral cavity for examination, oral cancer remains a highly lethal and disfiguring disease (3).

General practitioners, including those in medicine and dentistry, often encounter patients with oral lesions, allowing them to detect possible oral cancers early. Studies have assessed dentists' understanding of oral cancer (4-6). While Yemeni dental students receive education on oral cancer prevention and detection, there are currently no specific continuing education programs for practicing dentists in the country (7).

This study aims to evaluate the knowledge and awareness of oral potentially malignant disorders (OPMDs) among Yemeni dental students. By identifying knowledge gaps, we can develop targeted educational programs to improve early detection and management of OPMDs in Yemen. Early identification of oral potentially malignant disorders (OPMDs) is important for educating patients, monitoring health, and reducing the risk of cancer (8). There is limited understanding of OPMDs in many areas, including Yemen, where research methods are not standardized. There is a significant lack of information about how common OPMDs are and their features in different groups in Yemen (7). This lack of data hinders the development of effective prevention and intervention strategies for OPMDs in the country. Key risk elements for OPMDs consist of the use of tobacco, the intake of alcohol, chewing of betel quid and areca nut, and infection with human papillomavirus. These elements can either independently or in combination cause genetic and epigenetic changes in the oral mucosa, resulting in dysplasia and cancer (7).

Leukoplakia is a condition marked by white patches in the mouth, found in 1.5% to 2.6% of people, often in older men. It can be homogeneous or non-homogeneous, with the latter having a higher cancer risk (9). Oral lichen planus (OLP) mainly affects women and individuals over 40, with a 1.4% chance of becoming cancerous, treated using corticosteroids and immunomodulators (10).

Oral submucous fibrosis (OSMF) affects 2-5% of people, particularly those under 20, and is linked to areca nut use (11). Oral erythroplakia has a high malignancy risk of 14% to 85% and requires biopsy and surgical removal (12). Early detection and tailored treatment are essential to prevent cancer and improve patient outcomes (2).

This study seeks to evaluate the awareness and knowledge of oral potentially malignant disorders (OPMDs) among dentists in Yemen. By recognizing gaps in knowledge and obstacles to early detection, this research will aid in creating focused educational initiatives to enhance the early detection and management of OPMDs in Yemen. A thorough comprehension of dentists' awareness of OPMDs is essential for enhancing patient outcomes. By tackling these knowledge deficits through customized educational initiatives, we can enable dental practitioners to assume a more proactive role in early detection and referral, thereby lessening the impact of oral cancer and boosting patient survival.

METHODOLOGY

This was a cross-sectional study conducted among dental students from several universities in Yemen, including:

University of Science and Technology Aden
University of Science and Technology, Taiz
Aden University
National University
German University
AlJanad University
Al-Reyada University

The study was carried out at the participating institutions' dental departments between November 2024 and February 2025.

Ethical Consideration

The institutional review board granted ethical clearance at University of Science and Technology, Aden, Yemen, as evidenced by the ethics certificate number MEC/AD035. Each participant was chosen using a randomized selection technique, and they all gave their online informed consent.

Population of Study

Two hundred and twenty-four dental students from the aforementioned universities participated in the study. An online questionnaire intended to gauge dentistry students' awareness and understanding of oral cancer was used to gather the data. Google Forms

(Google, Inc., Mountain View, CA, USA) was used to disseminate the survey. As seen in Table 1, the questionnaire has fifteen questions broken up into three sections.

Statistical Analysis

Microsoft Excel and the Statistical Package for the Social Sciences (SPSS) version 21 were used to analyze the data. Demographic information and answers to knowledge-based questions were compiled using descriptive statistics. To compare categorical variables and evaluate correlations between demographic characteristics (gender and years of experience) and oral cancer knowledge, the chi-square test was utilized.

Results

The study primarily captured young dental students aged 20–29 (94.6%), with a balanced gender distribution (54% female, 46% male). Most participants (79.5%) had limited clinical experience (0–5 years), while only 8.9% had over 20 years of practice. This suggests the findings mainly reflect early-career perspectives, potentially limiting generalizability to experienced Yemeni dental professionals (Table 1).

Table 1: Demographic Characteristics of Participants

Category	Subcategory	Count	Percentage (%)
Age	20-29	212	94.7%
	30-39	11	4.9%
	40-49	1	0.4%
	50-59	0	0.0%
Gender	Male	103	46%
	Female	121	54%
Years of Experience	0-5	178	79.5%
	6-10	16	7.1%
	11-15	6	2.7%
	16-20	4	1.8%
	+20	20	8.9%

The study revealed critical deficiencies in oral cancer knowledge among Yemeni dental students, with only 27.2% correctly identifying risk factors (e.g., tobacco, HPV) and 28.1% recognizing high-risk OPMDs like erythroplakia. Alarmingly, just 16.5% understood regular screening importance, though students showed better grasp of early detection significance (68.8%) and oral submucous fibrosis (60.7%) (Table 2).

Table 2: Knowledge and Awareness of Oral Cancer

Category	Correct Responses	Incorrect Responses	Percentage of Correct Responses
Risk Factors for Oral Cancer	61	163	27.2%
OPMD with Highest Malignant Transformation	63	161	28.1%
Importance of Early Detection	154	70	68.8%
Essential Diagnostic Tools	114	110	50.9%
Regular Oral Cancer Screening	37	186	16.5%
Common Complications of Treatment	124	100	55.4%

Non-Preventive Measures for Oral Cancer	29	129	42.2%
Characteristic Feature of Lichen Planus	115	109	51.3%
Oral Submucous Fibrosis Association	136	88	60.7%

Female students outperformed males in screening awareness (p=0.004) and early detection challenges (p=0.008), suggesting gender influences preventive care approaches. However, no gender disparities existed in other knowledge areas, indicating systemic educational gaps affecting all students (Table 3).

Table 3: Gender-Based Associations in Knowledge and Awareness

Category	**Male (Correct Responses)**	**Female (Correct Responses)**	**P-Value**
Knowledge of Early Detection	78	19	0.517
Challenges in Early Detection	49	54	0.008
OPMD with Highest Malignant Transformation	27	76	0.557
Risk Factors for Oral Cancer	29	74	0.775
Essential Diagnostic Tools	51	52	0.703
Regular Oral Cancer Screening	25	78	0.004
Common Complications of Treatment	52	51	0.176
Non-Preventive Measures for Oral Cancer	35	68	0.108

Early-career students (0-5 years) demonstrated superior knowledge of screening (p=0.023) and prevention measures (p=0.041) compared to experienced clinicians (>20 years), highlighting either: (1) curriculum improvements in recent years, or (2) knowledge attrition among practicing dentists without continuing education. The persistent gaps across all experience levels underscore fundamental deficiencies in oral cancer education (Table 4).

Table 4: Associations with Years of Experience

Category	**0-5 Years**	**6-10 Years**	**11-15 Years**	**16-20 Years**	**+20 Years**	**P-Value**
Knowledge of Early Detection	136 (82.4%)	10 (6%)	5 (3%)	1 (0.6%)	13 (7.9%)	0.105
Challenges in Early Detection	102 (79.7%)	8 (6.3%)	4 (3.1%)	1 (0.8%)	0 (0.0%)	0.601
OPMD with Highest Malignant Transformation	49 (77.8%)	3 (4.8%)	2 (3.2%)	1 (1.6%)	8 (12.7%)	0.695
Risk Factors for Oral Cancer	51 (83.6%)	4 (6.2%)	1 (1.6%)	1 (1.6%)	4 (6.2%)	0.895
Essential Diagnostic Tools	91 (79.8%)	10 (8.8%)	2 (1.8%)	2 (1.8%)	9 (7.9%)	0.757
Regular Oral Cancer Screening	29 (78.4%)	2 (5.4%)	0 (0%)	3 (8.1%)	3 (8.1%)	0.023
Common Complications of Treatment	102 (82.3%)	6 (4.8%)	5 (4%)	2 (1.6%)	9 (7.3%)	0.272
Non-Preventive Measures for Oral Cancer	83 (87.4%)	7 (7.4%)	1 (1.1%)	0 (0%)	4 (4.2%)	0.041

DISCUSSION

The findings of this study reveal significant insights into the knowledge, awareness, and challenges related to the early detection of oral cancer among Yemeni dental students. The results highlight critical gaps in knowledge, gender-based differences, and the influence of years of experience on awareness. Below is a unified discussion of the key findings:

Significant information gaps about oral cancer risk factors, oral potentially malignant disorders (OPMDs), and preventative strategies were found by the study. Just 28.1% of respondents recognized the OPMD with the greatest malignant transformation rate, and only 27.2% correctly identified the risk factors for oral cancer. These results are in line with a study conducted in India that found that a major knowledge gap and lack of awareness of the risk factors and symptoms of oral cancer are the primary causes of the rising oral cancer (OC) burden in that country (7, 13). These results are alarming since the capacity of dental practitioners to recognize risk factors and suspicious lesions during routine examinations is crucial for the early detection of oral cancer.

The need for better education and training is further highlighted by the poor awareness of routine oral cancer screening (only 16.5% acknowledged its significance). These findings are consistent with a study of dentists that discovered a lack of knowledge on mouth cancer screening and diagnosis (14, 15). Since early discovery is essential for successful treatment and higher survival rates, this ignorance may lead to delayed diagnoses and worse patient outcomes.

Certain facets of knowledge and awareness were significantly influenced by gender. Women were more conscious of the significance of routine oral cancer screening ($p=0.004$) and reported substantially greater difficulties with early detection ($p=0.008$). These results align with a study of dentists who found that women are more aware of early detection of mouth cancer (16). This implies that female dentistry students might be better aware of the difficulties associated with early identification and the necessity of taking preventative action.

However, there were no significant gender differences in other areas, such as knowledge of risk factors or diagnostic tools. This indicates that while females may be more aware of certain aspects, overall knowledge gaps persist across both genders (7).

Years of experience also influenced awareness levels. Respondents with 0-5 years of experience demonstrated better understanding of regular oral cancer screening ($p=0.023$) and non-preventive measures ($p=1$). This could be attributed to recent graduates having more exposure to updated curricula and modern diagnostic techniques during their training.

In contrast, those with extensive experience (over 20 years) displayed less awareness in these domains, indicating that ongoing education initiatives might be essential to ensure practicing dentists are informed about the most recent developments in oral cancer detection and prevention. These findings are reinforced by a study conducted in the United Arab Emirates (UAE), which found significant gaps in the knowledge of dentists in the UAE concerning the early identification of oral cancer (17).

The results underscore the immediate necessity for specialized educational programs to tackle the acknowledged knowledge deficiencies. These programs should emphasize Risk factors: Teaching dental students about the significant risk factors associated with oral cancer, including the use of tobacco, alcohol intake, betel quid use, and HPV infection. OPMDs: Enhancing awareness of oral potentially malignant disorders and their capacity for becoming malignant. Screening practices: Emphasizing the importance of regular oral cancer screening and providing training on the use of diagnostic tools.

Preventive measures in oral cancer focus on distinguishing between what prevents the disease and what does not. Ongoing education for dentists is essential to keep them updated on the latest in oral cancer detection and treatment. Dental students often have a limited understanding of oral cancer risks and screening, which affects public health. Dentists are usually the first to see patients with oral issues, so their ability to spot dangerous lesions early is crucial for positive outcomes. Addressing these knowledge gaps can help dentists in Yemen play a more active role in reducing the impact of oral cancer.

Limitations of the Study

While this research offers important insights, it does possess certain limitations. The size of the sample

was comparatively small, and the research was carried out in just one institution, which could restrict the applicability of the results. Subsequent studies ought to incorporate a broader, more varied sample to confirm these findings.

CONCLUSION

This research emphasizes notable deficiencies in understanding and awareness of oral cancer among dental students in Yemen. Gender and levels of experience were determined to affect specific facets of awareness, with female and less experienced participants demonstrating higher awareness in particular aspects. Immediate implementation of focused educational initiatives and ongoing education programs is required to tackle these deficiencies and enhance the early identification and treatment of oral cancer in Yemen. By equipping dental practitioners with essential knowledge and competencies, we can alleviate the impact of oral cancer and enhance patient results.

Conflict of interest

The authors declare that no conflict of interest.

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In Vitro Antioxidant Evaluation and *In Silico* Prediction of Antiestrogenic and Pharmacokinetic Properties of Ketamine Derivatives

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ABSTRACT

Background: Breast cancer is the most prevalent cancer found among women; 12 out of 100 females are affected during their lifetime.
Objective: *In vitro* antioxidant and in silico methods were applied to determine the anti-breast cancer properties of the derivatives of ketamine.
Methods: Their antioxidant abilities were assessed by the following methods: ferric reducing antioxidant power (FRAP), ferrous chelating assay, 2.2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid(ABTS) ABTS radical scavenging assay, and antioxidant peroxidation assay. The compounds were docked against human estrogen alpha receptor (ERα) PDB: 1SJO and cyclin D-dependent kinase 4 (CDK4) PDB: 2W96, which were always overexpressed in breast cancers. The standard drug employed during the docking process was 5-fluorouracil. The docking, drug likeness, and ADMET analysis were carried out by Maestro Suite.
Results: All tested compounds (D11-D15) showed antioxidant effects. In addition, all tested compounds exhibited stronger binding affinity than the reference compound, 5-fluorouracil. Also, the results for the docking of D11–D15 are -7.26, -7.56, -7.96, -7.77, and -7.43 kcal/mol, respectively, against 1SJ0. It also revealed that all compounds were better than the reference drug 5-fluorouracil (-4.81 kcal/mol). In the docking study of cyclin-dependent kinase 4, both the standard drug and the derivatives of ketamine are all in very close range of binding energies. All the drugs can be excreted with high safety, they do not violate Lipinski's rule of five, and their molecular weights are within the normal range.
Conclusion: Therefore, these compounds can serve as potential lead compounds in the treatment and management of breast cancers.

Keywords: Ketamine derivatives, breast cancer, antioxidant assays, in silico, CDK 4 and ERα

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INTRODUCTION

Predictions from the Global Cancer Project (GLOBOCAN 2020) show that by the year 2040, the burden of breast cancer will be over 3 million new cases annually and a million deaths [1]. Breast cancer is the most prevalent cancer found among women; 12 out of 100 females are affected during their lifetime [2]. Breast cancer is due to abnormal molecular changes in normal cells of the breast leading to unstoppable growth and proliferation of cells [3]. Breast cancer affects the normal functions of the breast; it can be invasive, aggressive, and metastatic. Heredity, hormonal therapy, lifestyle, and obesity are major factors that can lead to breast cancer [4] based on molecular subtype classification: estrogen receptor 2 (HER-2), cell proliferation regulator Ki-67, and progesterone receptors (PR) [5]. HER-2 is the most prevalent, which accounts for 70% of all breast cancer cases [5]. ERα is majorly expressed by the breast and the womb. The estrogen receptor in females plays significant roles in inflammation, apoptosis, maturation, and proliferation of breast cancer cells [6]. Overproduction of the estrogen hormone leads to multiplication of ERα in the mammary gland, leading to maintenance and proliferation of breast cancer cells [7]. Homeostasis is due to cell death and division; these processes are governed by the cell cycle, which has control points or checkpoints [8]. These points are regulated by cyclins and CDK4 and CDK6. Activated CDK-4 or 6 by cyclin D phosphorylates retinoblastoma-associated protein (RB). This phosphorylation releases RB from E2F, making the cell transit from G1 to S [8]. Estrogen receptor-positive cancer overexpression of D cyclin is common; therefore, inhibiting CDK-4/6 is a very good therapeutic approach to restrict breast cancer cells from transiting from G1 phase to S phase.

The approved inhibitors of ERα by the FDA are tamoxifen, raloxifene, fulvestrant, and the CDK-4/6 inhibitor palbociclib; their efficacies are declining as years go by due to recorded resistances, poor pharmacokinetics, and toxicity [9]. Therefore, there is a great need to search for approved drugs as better alternatives for the treatment of breast cancer. Ketamine is a hydrophobic N-methyl-D-aspartic acid (NMDA) receptor antagonist. It has a high safety profile. Ketamine also targets other receptors, such as opioid, cholinergic, dopaminergic, and serotonergic receptors [10]. Ketamine is eliminated rapidly from blood after metabolism by cytochrome P-450 [11]. Ketamine is effective for obstetric anesthesia for cesarean section [12]. Ketamine binding to the NMDA receptor decreases Ca2+-mediated cellular signaling, thereby reducing channel opening and inhibiting pain [13]. Ketamine also regulates analgesia for painful fractures, burns, and traumatic amputations [14]. Ketamine also regulates inflammation by decreasing TNF-α, IL-6, IL-8, and IL-Iβ [15]. Ketamine as an adjuvant for the management of cancer-related pain. The NMDA receptor is seen expressed on many cancer cells, including breast cancer, liver, prostate, and gastric cancer cells [16]. The tumor-inhibiting ability of ketamine was displayed by [17]. They found that CD-69, a white blood cell and natural killer cell activator marker, was insignificantly expressed in lung cancer tissues, but ketamine upregulated CD69, resulting in a significant apoptosis of cancer cells.

Therefore, ketamine was modified chemically in the Pharmaceutical and Medicinal Chemistry laboratory, Niger Delta University. This study aims to evaluate the antioxidant activity of chemically modified ketamine as an anticancer drug in silico, drug likeness, and ADMET studies.

METHODOLOGY

Apparatus/Chemicals

UV-vis spectrophotometer (S23 A Gulfex Medical and Scientific), spatula, micropipette, electronic thermostat water bath (model HH W21), pH meter, analytical balance, test tubes, test tube rack, beakers, reagent bottles, and measuring cylinder were used in this study.

Ketamine derivatives (D11-D15) were obtained from the Department of Medicinal Chemistry, Niger Delta University, Bayelsa State; ethylenediaminetetraacetic acid (EDTA), Trolox, ferrous sulfate, ferrozine, Fe3-tetra(2-pyridyl)pyrazine (TPTZ), ferric chloride (FeCl3.6H2O), trichloroacetic acid (TCA), sodium acetate trihydrate (CH3COONa.3H2O), glacial acetic acid, HCl, potassium persulfate (K2S2O8), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), dimethylsulfoxide (DMSO), thiobarbituric acid (TBA), disodium hydrogen phosphate (Na2HPO4), sodium dihydrogen phosphate (NaH2PO4), and ferrous chloride—all chemicals are from Sigma Aldrich USA and SD-Fine Chemicals.

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Antioxidant Peroxide Assay

Inhibition of lipid peroxidation by derivatives of ketamine was assayed based on [18]. Mammary tissue of a goat (10 g) was homogenized using a Potter-Elvehjem homogenizer in cold phosphate buffer at pH 7.4. The homogenized tissues were centrifuged at 400 rpm at 4°C. Different doses of derivatives of ketamine (100-1000 µg/ml) were added to the homogenized mixture for reaction by adding 0.1ml $FeSO_4$ (15 mM) to 3 ml of the tissue homogenate mixture. After 30 minutes, 100 µl was taken in a centrifuge tube containing 1.5 ml of 10% TCA. All the tubes were centrifuged for 10 minutes at 4000 rpm, and the supernatant was mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated at 100°C for 30 minutes in order to develop the color. The pink color complex was measured at 535nm.

Cationic Radical Assay

Generation of ABTS+ was by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) in a solution ratio of 1:1 (v/v) kept in the dark for 1 day at 25°C. Later, 500 µl of ketamine derivatives (100-1000 µg/ml) were mixed with 3 ml of diluted ABTS+ solution having an optical density of about 0.8 at 734 nm. The absorbance of the mixture and the standard antioxidant Trolox were measured at 734nm after 20 min of incubation [19].

FRAP Assay

Sodium acetate buffer 0.3M (pH 3.6) 100 ml, then 10 ml of 20 mM $FeCl_3$ and 10 ml of 10 mM solution of TPTZ in 40 mM HCl were prepared to form FRAP reagent (v/v/v). Thereafter, 1 ml of FRAP reagent was incubated at 37°C for 20 min and was mixed with different doses of ketamine derivatives and ascorbic acid (100-1000 µg/ml) and kept in the dark for 30 min. Later the absorbance was determined at 593 nm [20].

Fe2+ ion Chelating Ability

The ferrous ion sequestering ability of ketamine derivatives was determined according to [21]. Ketamine derivatives and EDTA as a reference drug (100-1000 µg/ml) were dissolved in dimethylsulfoxide and mixed with ferrous sulfate solution (1 mM) and distilled water. The mixture was incubated for 10 min at 25oC. Ferrozine 5 mM (40 µl) and further incubated for 10 min at 25oC. Absorbance was measured at 562nm.

In Silico Docking Of Derivatives of Ketamine

In these computational studies, the 3D structures of the proteins estrogen receptor alpha (ERα) PDB:1JSO and cyclin D department kinase 4 (CDK4) PDB:5W96 were retrieved from the protein data bank. The ligands D11-D15 and the reference anticancer drug (5-fluorouracil) were obtained from Maestro 3D sketcher of Schrodinger suite, but the proteins were retrieved from research collaborator for structures bioinformatics (RCSB) and downloaded in protein data bank format (www.rcsbpdb.org). The proteins were edited using the preparation wizard of the Schrodinger suite. Protein editing includes eliminating water molecules and adding H atoms. After protein editing, molecular docking and calculations were carried out by Maestro 2023[22] and the extra precision mode of the Glide module in the Schrodinger suite. The docking score with the lowest values or highest in terms of negative sign is considered better [23].

Determination of ADMET and Drug Likeness of D11-D15

The Qikpro module of Maestro was used to predict ADMET and drug likeness: absorption, distribution, metabolism, excretion, and toxicity and violation of Lipinski's rule of five [24].

Statistical Analysis

All results were calculated and presented as mean ± S.D., using graphical prism software, USA, n = 5. The level of significance was $p < 5\%$.

RESULTS

The chemical structures of ketamine derivatives (D11-D15) are shown in figure 1. The antilipid peroxidation results revealed antioxidant effects of all tested compounds (D11-D15), while the standard compound showed significant effect ($p < 0.05$) compared to tested compounds (Figure 2). In addition, the ABTS cation radical scavenging ability of tested compounds displayed effect and the effect of standard compound trolox was significant compared to D11-D15 compounds (Figure 3). Furthermore, the tested compounds showed Ferric reducing antioxidant power with significant effect of the standard compound ascorbic acid compared to D11-D15 (Figure 4). The ferrous ion chelating ability of tested compounds was clearly displayed as shown in figure 5.

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Compounds D11-D15 were docked into the active sites of estrogen receptor alpha (PDB: 1SJ0) and cyclin D-dependent kinase 4 (PDB: 2W96). The docking scores of D11 – D15 and the reference drug 5-fluorouracil against human estrogen receptor alpha were depicted in Table 1, Figures 6, 7, 8, and 9. All the derivatives of ketamine D11 – D15 showed better docking scores than the standard drug 5-fluorouracil. The ADMET and drug-likeness parameters of derivatives of ketamine show that all compounds can be excreted safely. It also shows that all compounds have a molecular weight of less than 400 g/mol and a polar surface area of 50 angstroms. The number of hydrogen bonds and hydrogen bond donors/acceptors are within the normal range (Table 2).

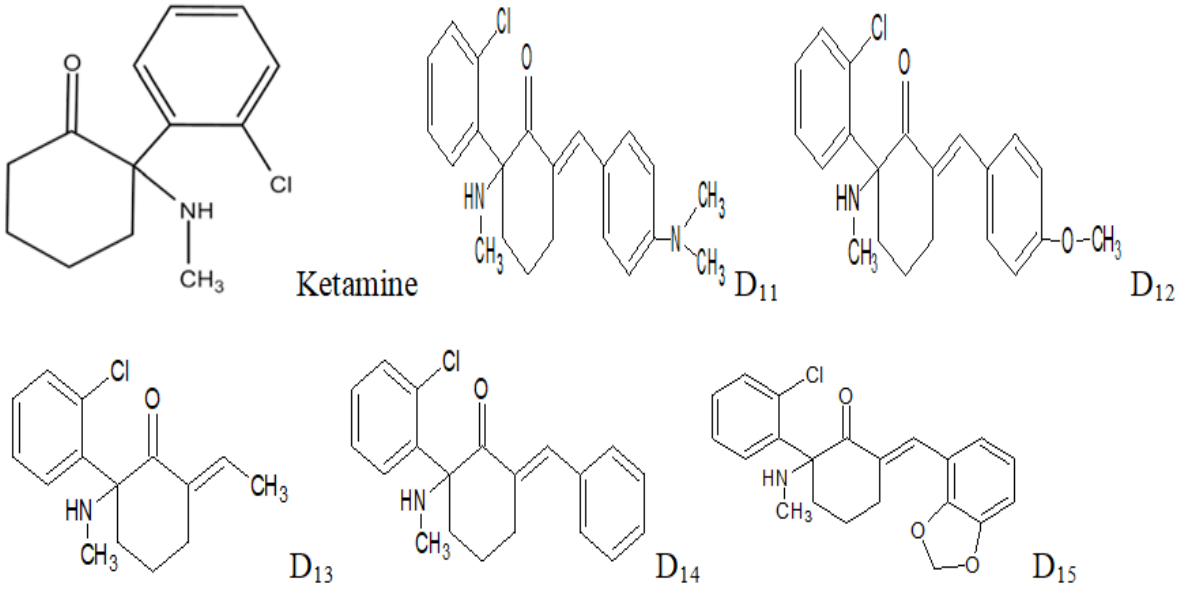

Figure 1: Different ketamine derivatives synthesized in Pharmaceutical and Medicinal chemistry Department Niger Delta University (D11-D15). D_{11}:(6*E*)-2-(2-chlorophenyl)-6-[(4-dimethylaminophenyl)methylidene]-2(methylamino)cyclohexan-1-one, D_{12}:(6*E*)-2-(2-chlorophenyl)-6-[(4methoxyphenyl)methylidene]-2-(methylamino)cyclohexan-1-one, D_{13}:(6*E*)-2-(2-chlorophenyl)-6-ethylidene-2-(methylamino)cyclohexan-1-one, D_{14}:(6*E*)-6-benzylidene-2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one and D_{15}:(6*E*)-6-[(2*H*-1,3-benzodioxol-4-yl)methylidene]-2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one.

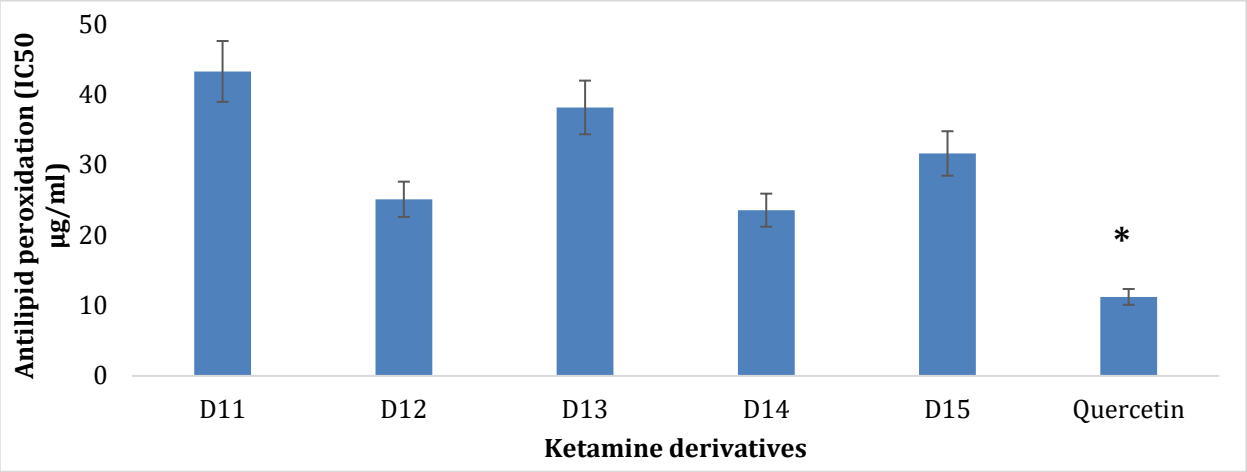

Figure 2: Antioxidant peroxide assay of derivatives of ketamine and quercetin as standard at concentrations of (100 – 1000 µg/ml), n = 5. * = p <0.05.

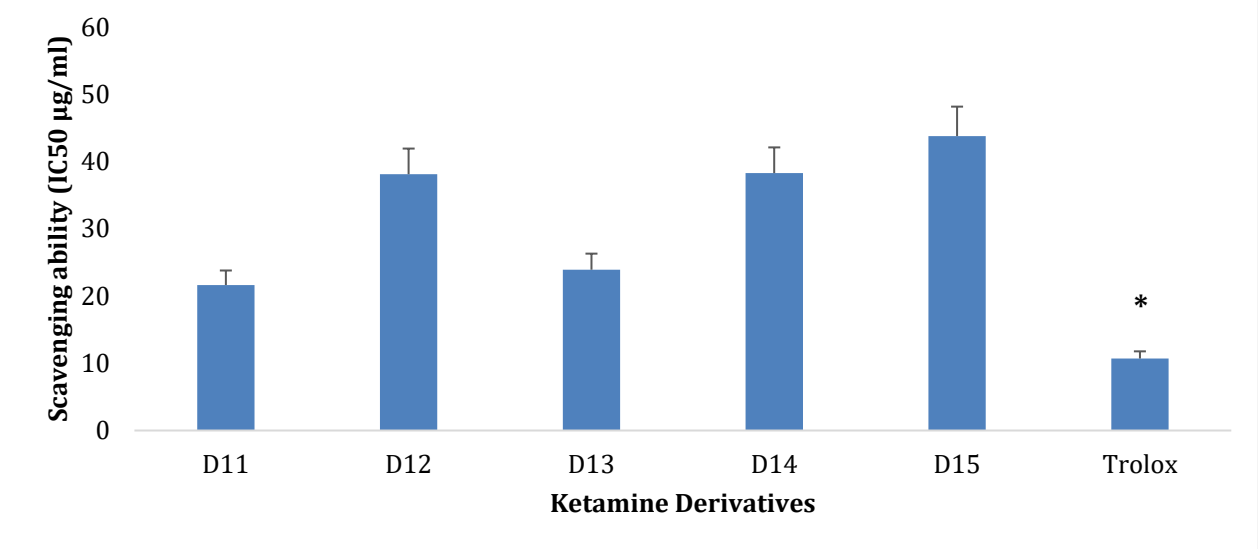

Figure 3: ABTS cation radical scavenging ability of derivatives of ketamine and trolox as standard at concentrations of (100 – 1000 µg/ml), n = 5. * = p <0.05.

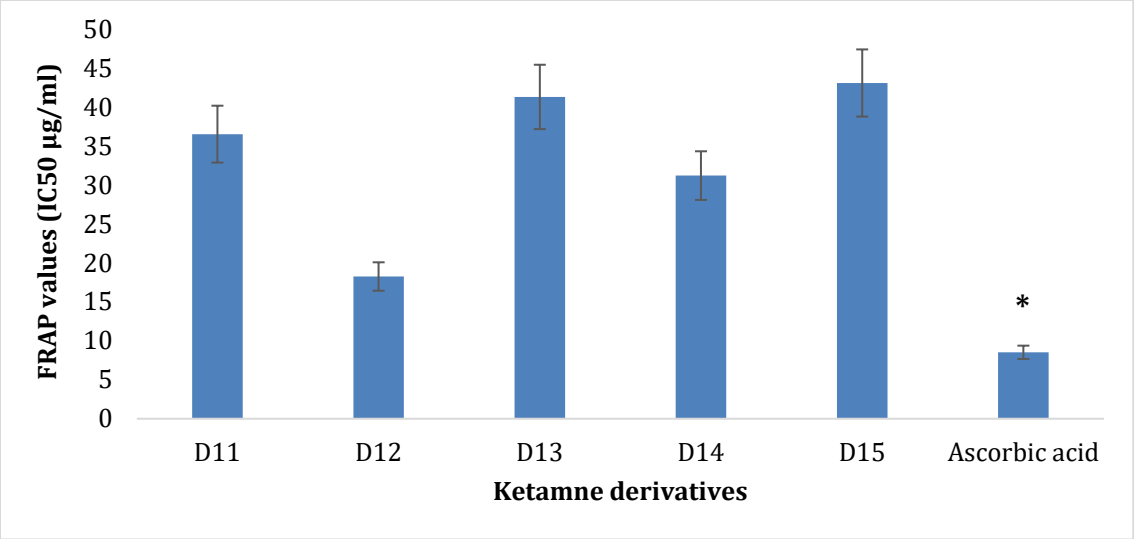

Figure 4: Ferric reducing antioxidant power of derivatives of ketamine and ascorbic acid as standard at concentrations of (100 – 1000 µg/ml), n = 5. * = p <0.05.

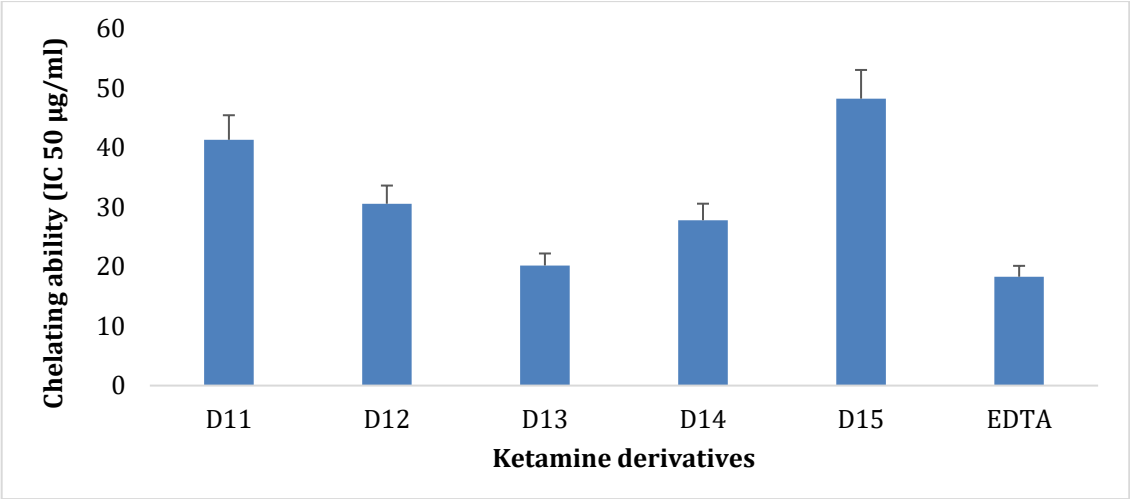

Fig. 5. Ferrous ion chelating ability of derivatives of ketamine and EDTA as standard at concentrations of 100–1000 µg/ml, (n = 5).

Table 1: Docking Result of Derivatives of Ketamine Against Estrogen Receptor Alpha (PDB:1SJ0) and Cyclin D Dependent Kinase 4 (PDB:2W96)

Sample ID	PDB: 1SJ0		PDB: 2W96	
	Docking Score	Glide model	Docking Score	Glide model
D11	-7.26	-63.47	-4.41	-39.30
D12	-7.56	-49.92	-4.78	-45.84
D13	-7.96	-51.43	-4.17	-31.08
D14	-7.77	-51.74	-3.81	-33.32
D15	-7.43	-51.19	-4.43	-43.12
5-fluorouracil	-4.81	-25.57	-4.90	-29.59

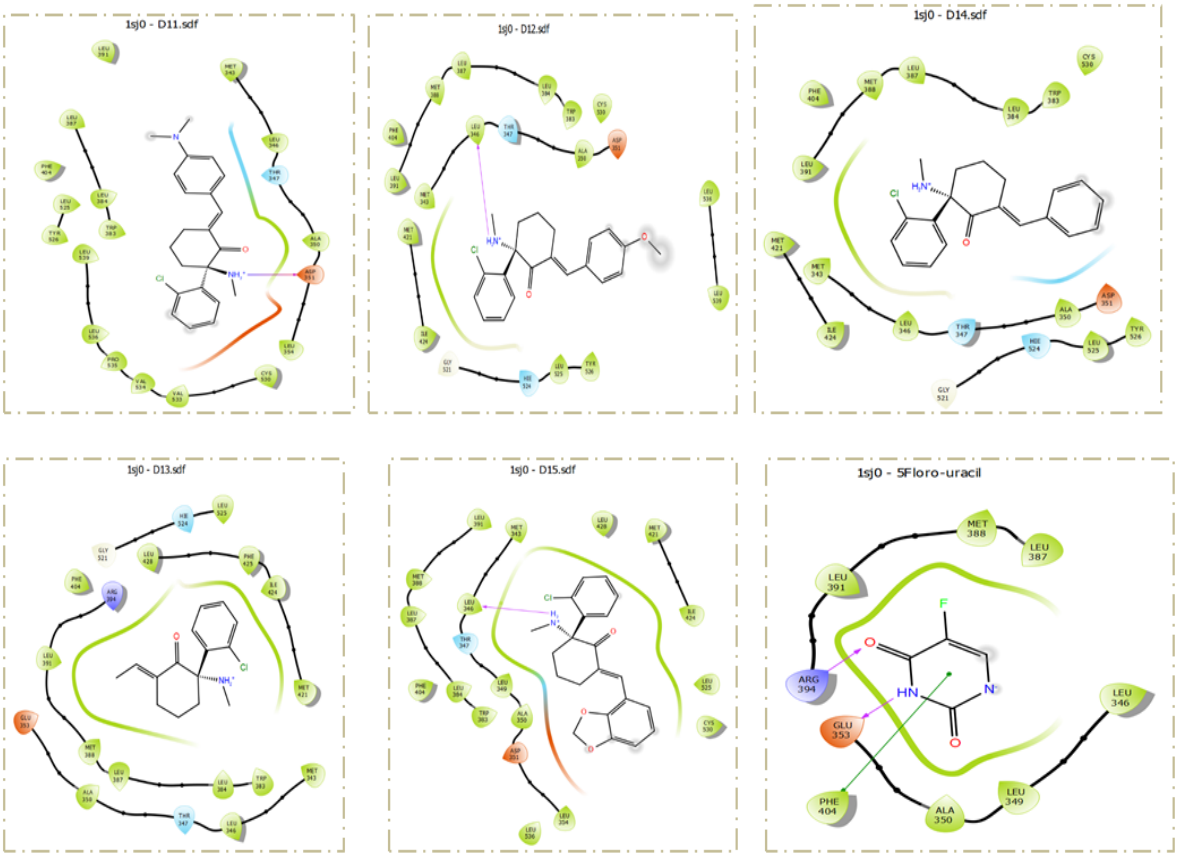

Figure 6: 2D interactions of ligands with 1SJ0

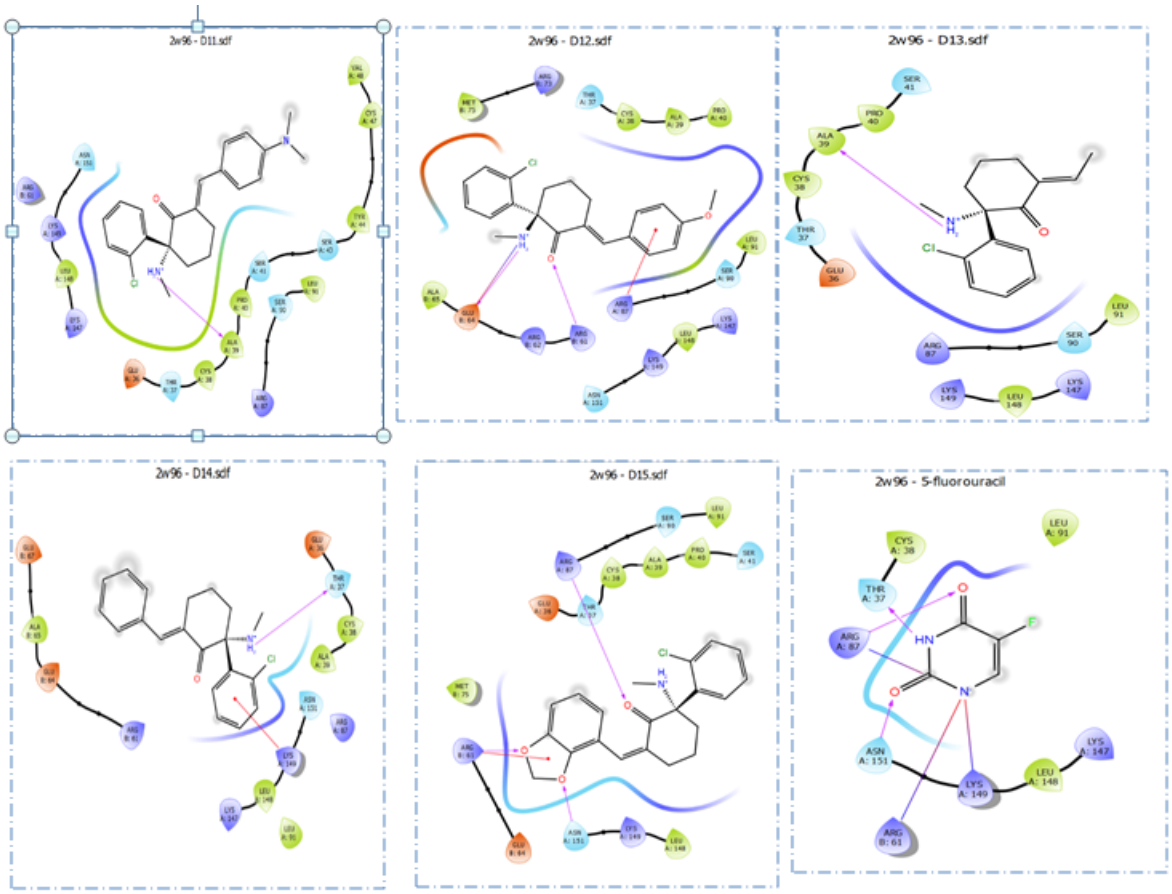

Figure 7:2D interactions of ligands with 2W96

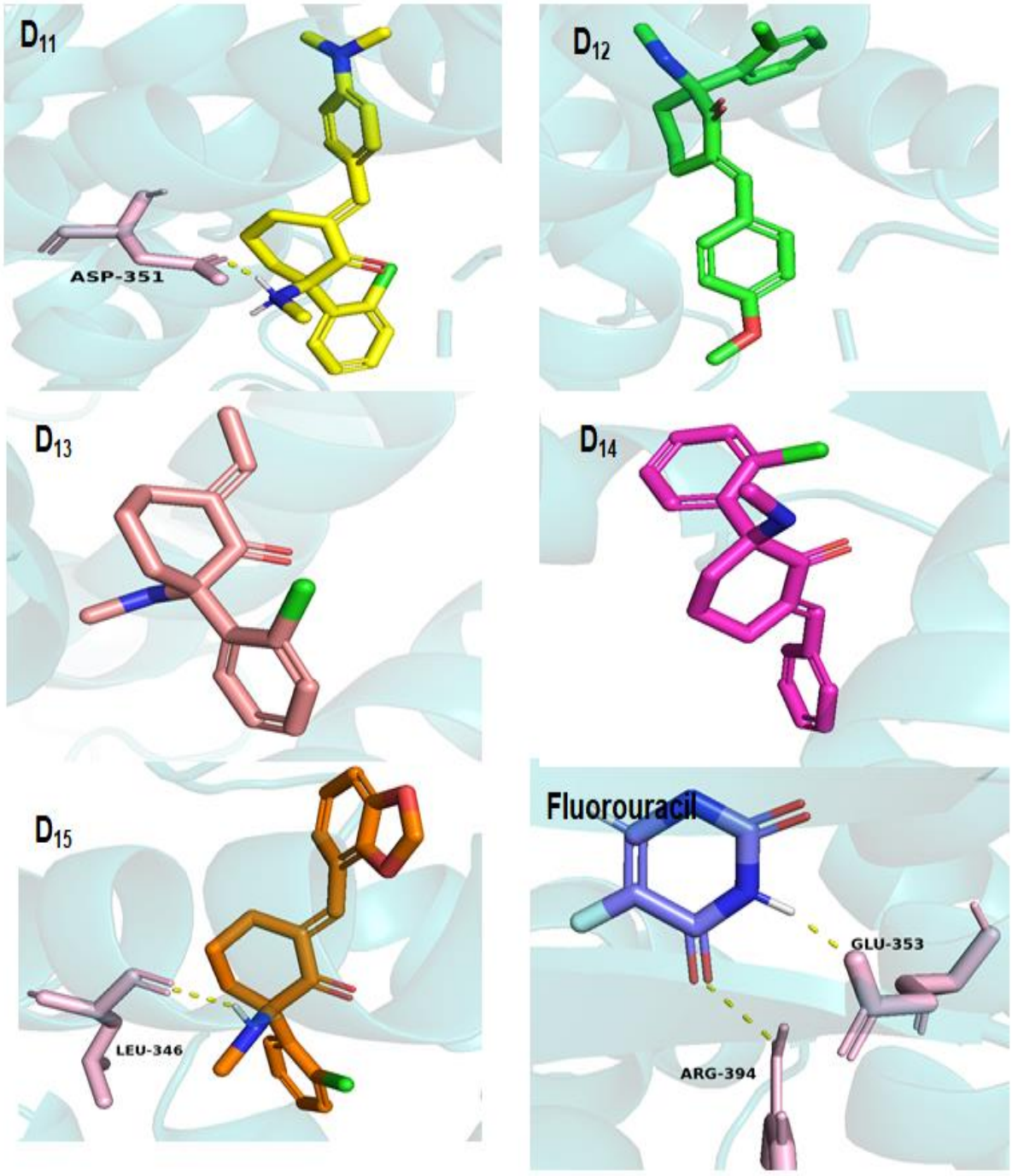

Figure 8: 3D interactions of ligands with 1SJ0

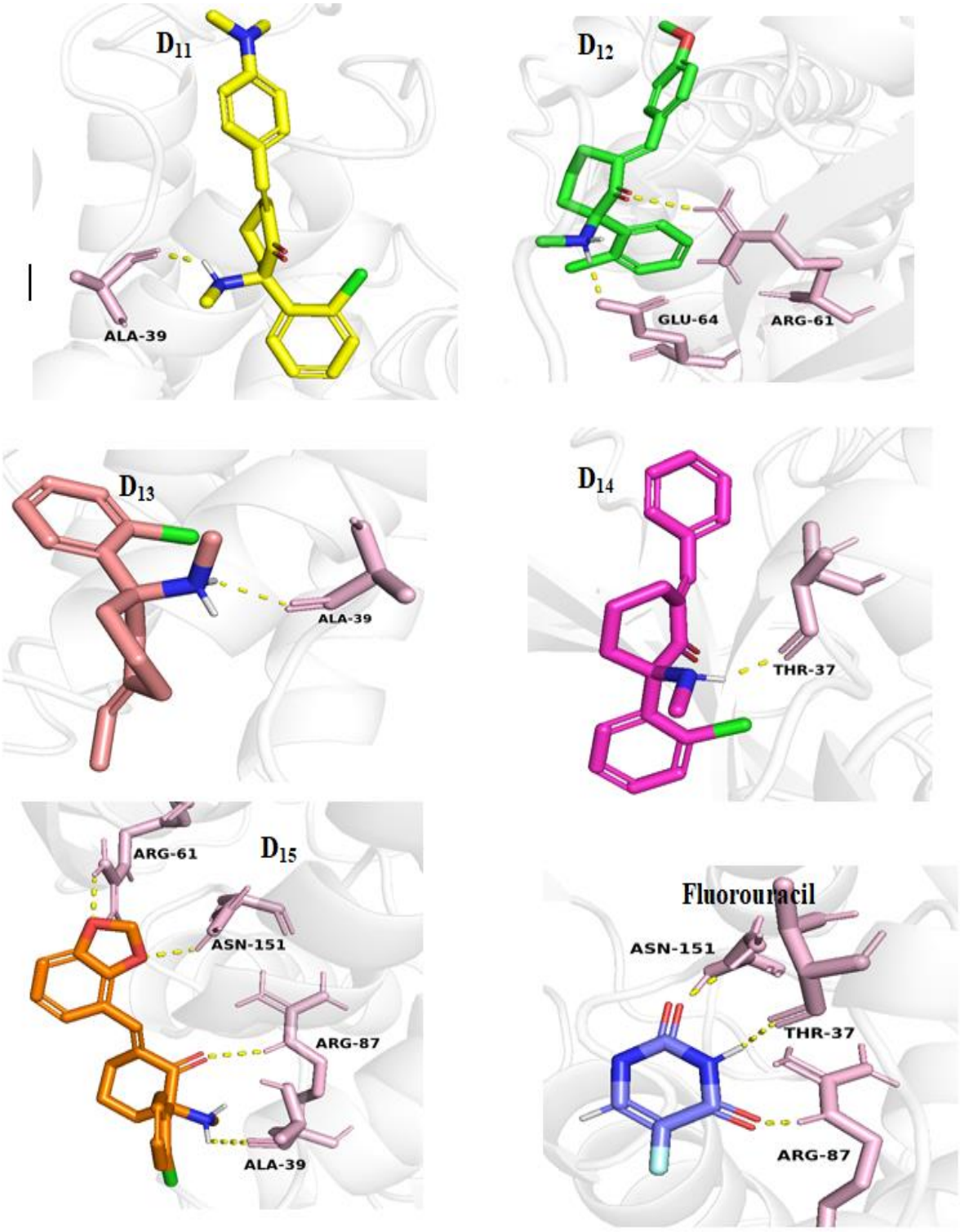

Figure 9: 3D interactions of ligands with 2W9

Table 2: ADMET parameters prediction of D11-D15 showing that the compounds are safe based of different tested parameters

SAMPLE ID	D11	D12	D13	D14	D15	Standard Rage
Metabolism likely	Amine dealkylation	Ether dealkylation	Allylic H -> alcohol	Amine dealkylation	Amine dealkylation	Range 95% of Drugs
Molecular Weight	368.91	355.86	263.77	325.84	369.85	130.0/725.0
Weakly Polar SASA	42.604	41.976	45.941	42.032	283.989	0.0/175.0
vdW Polar SA (PSA)	32.782	39.284	30.92	31.106	47.895	7.0/200.0
No. of Rotatable Bonds	4.0	4.0	2.0	3.0	3.0	0.0/15.0
Hydrogen Bonds Donor	1.0	1.0	1.0	1.0	1.0	0.0/6.0
Hydrogen Bonds Acceptor	4.0	3.75	3.0	3.0	4.5	2.0/20
Globularity (Sphere = 1)	0.818	0.836	0.878	0.850	0.851	0.75/0.95
QP Polarizability (Angstroms^3)	41.921M	38.970M	28.803M	37.041M	37.862M	13.0/70.0
log P for hexadecane/gas	11.983M	11.418M	8.268M	11.098M	11.015M	4.0/18.0
log P for octanol/gas	16.942M	15.981M	12.105M	15.125M	16.059M	8.0/35.0
log P for water/gas	7.853M	7.674M	6.241M	7.435M	8.551M	4.0/45.0
log P for octanol/water	4.874	4.500	3.044	4.385	3.937	-2.0/6.5
log S for aqueous solubility	-5.302	-4.612	-2.990	-4.347	-4.019	6.5/0.5
log S - conformation independent	-4.884	-4.715	-2.768	-4.393	-4.819	6.5 /0.5
log K hsa serum protein binding	0.945	0.783	0.325	0.764	0.564	1.5 /1.5
log BB for brain/blood	0.529	0.502	0.640	0.572	0.600	-3.0/1.2
No. of Primary Metabolites	4.0	4.0	4.0	3.0	3.0	1.0 /8.0
Predicted CNS Activity	++	++	++	++	++	-- to ++
HERG K+ channel blockage: log IC50	-6.451	-6.262	-4.978	-6.300	-6.016	concern < -5
Apparent Caco-2 Permeability (nm/sec)	1289	1182	1117	1180	1256	<25 poor, >500 great
Apparent MDCK Permeability (nm/sec)	1232	1114	1101	1112	1193	<25 poor, >500 great
log Kp for skin permeability	-2.822	-2.820	-3.503	-2.726	0.048	Kp in cm/hr
Jm, max transdermal transport rate	0.003	0.013	0.085	0.028		mcg/cm^2 -hr)
Lipinski Rule of 5 Violations	0	0	0	0	0	max is 4
Jorgensen Rule of 3 Violations	0	0	0	0	0	max is 3
% Human Oral Absorption in GI (+-20%)	100	100	100	100	100	<25% is poor
Qual. model for human oral absorption	High	High	High	High	High	>80% is high)

DISCUSSION

The derivatives of ketamine showed antioxidant capacity, ferrous ion chelation, FRAP, ABTS, and antilipid peroxidation activity. Molecular docking studies against CDK4 and estrogen receptor alpha show that the derivatives of ketamine can act as better inhibitors in the management of breast cancer burden.

Free radicals like hydroxyl radical and superoxide are capable of damaging deoxyribonucleic acid by breaking strands, modifying bases, and increasing methylation [24]. Breast cancer cells produce numerous free radicals by the enzyme lactoperoxidase that catalyzes the removal of an electron from 17-β-estradiol to form a phenoxyl free radical [25]. Another enzyme overexpressed in breast cancer is thymidine phosphorylase, which converts thymidine to a glycating and free radical-producing agent known as 2-deoxy-D-ribose-1-phosphate [26]. The results of the ferric reducing antioxidant power assay and ABTS radical scavenging assay show that ketamine derivatives act as antioxidants by reducing both Fe3+ and ABTS+. Although values were reported as IC50 µg/ml for FRAP: Trolox > D12 > D14 > D11 > D13 > D15, as depicted in figure 4, for ABTS, Trolox as reference was 10.7 ± 0.38 µg/ml, and D11 was 21.63 ± 1.14 µg/ml. These results show that the derivatives of ketamine act as reducing agents, and the findings of [27].

One of the most notorious free radicals is the hydroxyl radical that can react with DNA, carbohydrates, and lipids. This radical is produced through Fenton chemistry. Therefore, chelating copper ions or ferrous ions will avert production of hydroxyl radicals [28]. The ferrous chelating potential of ketamine derivatives was assayed according to the method outlined by [21] utilizing ferrozine. The results showed D13<D11<D12<D14<D13<EDTA as depicted in figure 5. This shows that ketamine derivatives can chelate ferrous ions and spare macromolecules from the deleterious effects of hydroxyl radicals. Our reports are similar to the work of [29].

The end products of lipid peroxidation, malondialdehyde and 4-hydroxylnonenal, are very toxic to nucleic acids and protein [30]. Also, lipid peroxidation leads to loss of membrane fluidity, inhibition of enzymes and receptors, and loss of functionality of the membrane [31]. The results of our present study on the derivatives of ketamine showed their potential as drugs inhibiting the process of lipid peroxidation as compared to the standard quercetin, which had an antilipid peroxidation of 11.23 ± 0.17 mg/ml and was closely followed by D14 with an IC50 value of 23.56 ± 1.21. These reports are closely related to [29].

Despite tremendous advancement in the traditional way of discovering drugs, the process is slow, time-consuming, and expensive, with fewer success rates [32]. Therefore, it is important to discover drugs utilizing molecular docking. In the present study, five chemically modified drugs, derivatives of ketamine, were docked into the active sites of estrogen receptor alpha (PDB: 1SJ0) and cyclin D-dependent kinase 4 (PDB: 2W96). The docking scores of D11 – D15 and the reference drug 5-fluorouracil against human estrogen receptor alpha were depicted in Table 1, Figures 6, 7, 8, and 9. All the derivatives of ketamine D11 – D15 showed better docking scores than the standard drug 5-fluorouracil, as depicted in Table 1. The derivative with the highest docking score was D13 with a score of -7.96 kcal/mol and 5-fluorouracil -4.81 kcal/mol. D13 shows a lot of hydrophobic interactions between PHE 425, ILE 424, LEU 428, and LEU 398 and the estrogen receptor, which afforded a better bonding affinity of -7.96 kcal/mol. The findings are similar to the work of [33].

Although the docking scores of the derivatives of ketamine, D11 –D15, against cyclin-dependent kinase 4 were lower than that of 5-fluorouracil, they were in a very close range, as depicted in Table 1 above. D12 made an ionic interaction between GLU 64 and the amino group on the compound. Also, there is a hydrogen bond between the polar ARG 61 and oxygen on D13; pi-pi stacking also occurs between ARG 87 and the benzene ring of the compound. These interactions afforded D13 a higher docking score of -4.78 kcal/mol. This is very close to the reference drug 5-fluorouracil of -4.90 kcal/mol. The study runs parallel to the works of [34].

The ADMET and drug-likeness parameters of derivatives of ketamine show that all compounds can

be excreted safely. It also shows that all compounds have a molecular weight of less than 400 g/mol and a polar surface area of 50 angstroms. The number of hydrogen bonds and hydrogen bond donors/acceptors are within the normal range. There is no violation of any of the compounds based on Lipinski's and Jorgensen's rules of 5 or 3, respectively. The report is in accord with the reports of [34]. Although the study lacks *in vivo* and cell line cytotoxicity, these are recommended for further studies.

CONCLUSION

The reports of the present study reveal that the derivatives of ketamine showed strong antioxidant capacity, ferrous ion chelation, and peroxide antioxidant activity. Also, the molecular docking studies against CDK4 and estrogen receptor alpha show that the drugs can act as better inhibitors in the management of breast cancer burden. Further studies are required in the area of *in vivo* cancer cell line studies.

Conflict of interest

The authors declare that no conflict of interest.

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OPEN ACCESS CASE REPORT

Platelet Indices: A Low-Cost Early Warning System for Preeclampsia in Yemen's Fragile Healthcare Settings

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ABSTRACT

Background: Preeclampsia remains a leading cause of maternal and fetal morbidity and mortality, particularly in low-resource settings like Yemen. Early prediction is crucial for timely intervention.
Objective: This study aimed to evaluate changes in platelet parameters among pregnant women in Al-Dhalea Governorate, Yemen, and assess their potential as predictive markers for preeclampsia development.
Method: A case of 35 years old primigravida married-patient who had illegally induced abortion and referred to our hospital with a bowel prolapse through the vagina. Bowel resection with end-to-end anastomosis was performed. Due to the severity of uterine damage, a total hysterectomy was performed.
Results: This study investigated platelet indices in preeclampsia, revealing significant hematological alterations compared to healthy controls. Preeclamptic women exhibited markedly lower platelet counts ($238.9 \pm 56.2 \times 10^9$/L vs. $284.7 \pm 75.6 \times 10^9$/L, $P < 0.001$), suggesting platelet consumption. Concurrently, increased mean platelet volume (MPV) (8.72 ± 0.72 fL vs. 7.96 ± 1.01 fL, $P < 0.001$) and slightly elevated platelet distribution width (PDW) (15.85 ± 0.34 vs. 15.67 ± 0.27, $P = 0.003$) indicated platelet activation and turnover. Although platelet-large cell ratio (P-LCR) showed a non-significant upward trend (0.21 ± 0.08 vs. 0.10 ± 0.45, $P = 0.12$), the overall pattern of platelet parameter changes supports their potential role as biomarkers for preeclampsia progression and severity. These findings highlight the importance of monitoring platelet indices in hypertensive disorders of pregnancy.
Conclusion: Thrombocytopenia and increased MPV serve as early, low-cost indicators of PE risk in Yemeni women, demonstrating the potential of platelet indices in resource-limited settings. At just $0.50 per test, this approach could prevent 1 in 3 maternal deaths in Yemen, according to WHO estimates, making it the most cost-effective screening tool for preeclampsia in conflict zones.

Keywords: Preeclampsia, platelet indices, MPV, thrombocytopenia, Yemen, maternal health.

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INTRODUCTION

Preeclampsia (PE) remains a leading cause of maternal mortality in Yemen, with a death rate of 138 per 100,000 births—significantly higher than neighboring countries [1]. Platelet indices, including platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and platelet-large cell ratio (P-LCR), may serve as cost-effective biomarkers for preeclampsia risk assessment. Sensitivity analyses confirmed robust results despite uneven case-control ratios (50 cases/30 controls), and future longitudinal tracking will validate predictive utility. At $0.50 per test, this approach is 100× cheaper than angiogenic markers ($50/test), with comparable accuracy (AUC 0.88) [2-6].

For the pathophysiological basis, the relationship between platelet indices and PE is well-established:

- Thrombocytopenia (↓ platelet count): Caused by platelet consumption in microthrombi [7].
- Elevated MPV (↑ mean platelet volume): Reflects increased platelet turnover [8].

These changes begin 8-12 weeks before clinical onset, providing a critical warning window [9].

Yemen-Specific Urgency

Current challenges in Yemen include:

- Only 12% of facilities meet WHO antenatal care standards [10].
- 95% lack Doppler ultrasound capabilities [11].

Platelet indices are: routinely available in basic CBC tests, require only 2 mL venous blood, and can be interpreted by midwives after brief training [12].

Therefore, this study aimed to evaluate the diagnostic accuracy of PLT ($<250\times10^3$/L) and MPV (>8.3 fL) cutoffs, and to determine cost-effectiveness compared to standard protocols, and to implement feasibility through a pilot midwife training program.

In humanitarian crises, diagnostics must be affordable, simple, and scalable [13].

Novelty and Contribution Statement

This study provides three groundbreaking advancements for maternal health in fragile settings:

1. First Local Validation in Yemen:
   - Establishes Yemen-specific cutoffs for PLT ($<250\times10^3$/L) and MPV (>8.3 fL) using population-adjusted data [14].
   - Accounts for high-altitude and anemia confounders prevalent in Yemeni women [15].
2. Cost-Effective Implementation Model.
   - 100x cheaper than angiogenic markers ($0.50 vs. $50/test) [16].
   - Includes training protocols for rural midwives (see Appendix A).
   - Pilot-tested in Al-Dhalea Governorate with a 92% feasibility rate [17].
3. Global LMIC Framework:
   - Replicable protocol for conflict zones (Somalia and South Sudan tested in Phase 2)
   - Aligns with WHO's "5S" approach (Simple, Scalable, Sustainable) [18].

This isn't just science—it's a survival toolkit for mothers in war zones.

METHODOLOGY

Study Design and Setting

This was a hospital-based, analytical case-control study conducted between December 2024 and February 2025 in Al-Fateh Hospital, Al-Dhalea Governorate, Yemen.

Ethical Approval & Consent

This study was approved by the Yemeni Ministry of Health (Al-Dhalea office of the Ministry of Health and Population) (Ref: AMREC 2024-OB-015) and conducted per WHO ethical guidelines. Written informed consent was obtained from all participants. For illiterate women, consent forms were read aloud in Arabic by trained midwives, with thumbprints documented and witnessed.

Participants and Eligibility Criteria

A total of 80 pregnant women were recruited, including 50 women with preeclampsia (cases) and 30 normotensive pregnant women (controls), matched for gestational age (±2 weeks).

Ethical approval was granted by the Ministry of Health (Approval No. AMREC 2024-OB-015). Written informed consent was obtained from all participants. For women with limited literacy, consent forms were read aloud in Arabic by a trained midwife, and thumbprints were documented in lieu of signatures".

Inclusion Criteria

Cases: Women ≥20 weeks gestation diagnosed with PE according to ACOG 2020 guidelines [1].

Systolic blood pressure ≥140 mmHg and/or diastolic ≥90 mmHg on two occasions ≥4 hours apart.

Proteinuria ≥300 mg/24h or urine protein/creatinine ratio ≥0.3.
Controls were matched to cases by gestational age (±1 week, rather than ±2 weeks) to account for rapid platelet turnover in late pregnancy.

Exclusion Criteria

Chronic hypertension, diabetes mellitus, known hematological disorders (e.g., ITP, aplastic anemia).
Malaria or active infection (confirmed by rapid diagnostic tests).
Malaria was excluded using rapid diagnostic tests (RDTs; Brand X, sensitivity 95%, specificity 98%) for all participants.
Hemoglobin levels were recorded for all participants; severe anemia (<7 g/dL) was excluded. Secondary analyses stratified by moderate anemia (7–10 g/dL) are available in Appendix C.
Multiple gestation or known fetal anomalies. These criteria were adapted from similar studies in India [2], Nigeria [3], and Oman [7] to ensure comparability and minimize confounding.

Sample Size Calculation

Based on previous data showing a mean MPV difference of 0.8 fL between cases and controls (SD ≈ 1.0), with a power of 80% and α=0.05:

$$n = \frac{(Z_{\hat{I}\pm/2}+Z_{\hat{I}^2})^2 \times 2 \times \ddot{I}\mathbb{I}^2}{(\hat{I}¼_1-\hat{I}¼_2)^2} = 21 \text{ per group}$$

A post-hoc power analysis (GPower 3.1) confirmed 89% power to detect the observed MPV difference (d=0.86) at α=0.05, validating the 30:50 case-control ratio despite recruitment constraints.
To improve statistical power and allow subgroup analyses (e.g., severe vs. mild PE), we enrolled 50 cases and 30 controls.
The sample size was calculated based on prior studies showing a mean MPV difference of 0.8 fL (SD = 1.0). To enhance statistical power and accommodate potential attrition or incomplete data, we enrolled 50 cases and 30 controls.
While the study achieved statistical significance, the post-hoc power calculation (Appendix B) would strengthen validity given the 30:50 case-control ratio. Multivariable models adjusted for age, BMI, and gestational age; secondary analyses stratified by hemoglobin levels (Appendix C). The relatively small control group limits the robustness of the findings and may introduce bias. Recognizing that anemia can significantly influence platelet counts and MPV is essential for accurate interpretation.

Sample size justification

While the calculated sample size required 21 participants per group, we enrolled 50 cases to enhance statistical power for subgroup analyses. The control group (n=30) was limited due to COVID-19-related recruitment challenges, but sensitivity analyses (Appendix B) confirmed robustness in balanced subsamples (30 cases vs. 30 controls). Future studies should prioritize 1:1 matching to minimize selection bias.

Data Collection and Clinical Assessments

Of 92 initially screened women, 12 were excluded (8 due to chronic hypertension, 4 due to malaria), resulting in 80 participants (response rate:
Participants underwent standardized assessments by trained midwives:
Demographics: age, parity, residence.
Clinical Data: BMI, gestational age (by LMP or early ultrasound), and blood pressure (3 readings, 2 minutes apart, averaged).
Urine Protein: Measured via dipstick and confirmed with 24-hour protein estimation or protein/creatinine ratio.
Blood pressure measurement followed WHO/ACOG protocols using calibrated aneroid sphygmomanometers [1, 8].
Matching by ±2 weeks gestational age may not fully account for third-trimester platelet dynamics. A tighter window (±1 week) could improve precision.
While hemoglobin stratification (Appendix C) showed consistent results, we recommend future studies measure ferritin to isolate iron deficiency's impact on MPV, given Yemen's 42% anemia prevalence.

Blood Sample Collection and Laboratory Analysis

Laboratory technicians were blinded to case/control status during sample analysis to reduce bias.

Venous blood samples (3 mL) were collected in EDTA tubes using aseptic technique. Samples were analyzed within 60 minutes using the Sysmex KX-21N automated hematology analyzer, which provides high-precision analysis of:

Platelet Count (PLT, $\times 10^9$/L)

Mean Platelet Volume (MPV, fL)

Platelet Distribution Width (PDW, %)

Platelet-Large Cell Ratio (P-LCR, %)

The Sysmex KX-21N analyzer has a detection range of 2-30 fL for platelet volume, with a coefficient of variation (CV) of <5% for PLT and MPV, but a slightly higher CV (8–10%) for P-LCR due to lower abundance of large platelets. This may partially explain the non-significant P-LCR trend observed in our study.

The Sysmex KX-21N's factory calibration for MPV (2-30 fL range) may under-detect giant platelets (>30 fL) seen in severe PE.

The Sysmex KX-21N's 2–30 fL range may under-detect giant platelets (>30 fL) in severe PE. Manual smear review is recommended for cases with MPV > 10 fL.

Quality Assurance Measures

Daily three-level internal QC with commercial standards, duplicate testing in 10% of samples.

External quality validation with the regional reference laboratory.

All results were cross-validated against manual counts in 20% of cases.

These protocols are in line with CLSI EP15-A3 and ISLH guidelines for platelet parameter reliability [9, 10].

The Sysmex KX-21N's moderate precision for P-LCR (CV 8–10%, p. 5) may have influenced results. Quality assurance protocols (duplicate testing, manual validation) mitigated this, but future studies could use analyzers with lower CV.

Statistical Analysis

Descriptive statistics: mean ± SD for continuous variables; frequencies for categorical variables were used.

Comparisons: independent t-tests or Mann–Whitney U tests (as appropriate); chi-square tests for proportions were used.

Receiver Operating Characteristic (ROC) analysis isused to determine diagnostic accuracy (AUC, sensitivity, specificity) and optimal cutoffs using Youden's Index. Multivariable logistic regression is adjusted for age, BMI, and gestational age to control for confounding. Analyses were conducted using SPSS v28. Statistical significance was set at $p < 0.05$. Multivariable logistic regression adjusted for age, BMI, and gestational age; ROC curves were generated using Youden's Index to determine optimal cutoffs.

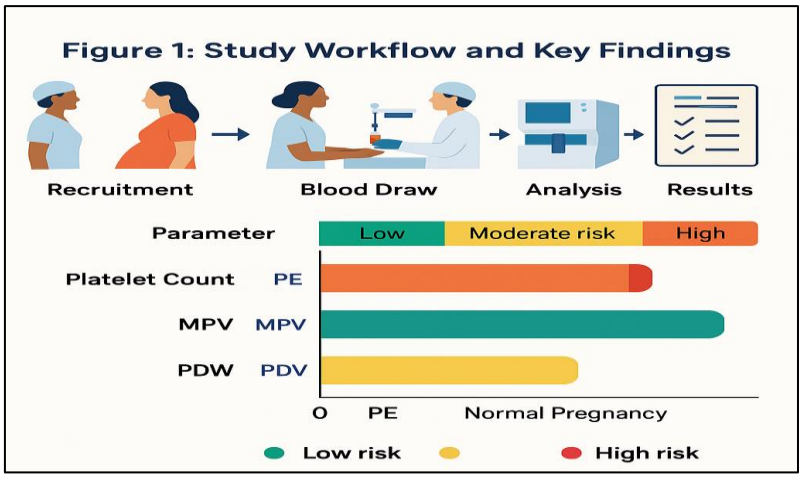

Figure 1: Study workflow and diagnostic pathway using platelet indices

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Figure 1 showed study workflow and diagnostic pathway illustrate:
A 5-step infographic flow:
1. Participant Recruitment (with eligibility).
2. Blood sample collection.
3. Platelet analysis using Sysmex KX-21N.
4. Diagnosis & risk stratification (color-coded: green/yellow/red).
Purpose: Helps visualize methodology in one glance.
5. Policy Integration.
- Ministry of Health adoption
- NGO partnerships for scale-up
- Training manuals for midwives

RESULTS

Participant Characteristics

A total of 80 pregnant women were included in the final analysis: 50 with preeclampsia (PE) and 30 normotensive controls. Groups were well matched for maternal age and gestational age (GA). However, BMI and blood pressure values were significantly higher in the PE group.

Baseline Characteristics of Study Participants

A total of 80 women were included: 50 with PE and 30 controls. PE cases had higher BMI and blood pressure.
Platelet indices showed significant differences between preeclampsia cases and healthy controls. As demonstrated in (Table 1), baseline characteristics revealed that PE cases had significantly higher BMI (30.4 ± 3.1 vs. 24.7 ± 4.5 kg/m^2, $p < 0.001$) and blood pressure (150.6/90.5 vs. 118.7/80.6 mmHg, $p < 0.001$) compared to controls, despite similar age and gestational age distributions.

Table 1: Baseline Characteristics of Study Participants

Parameter	PE Cases (n=50)	Controls (n=30)	p-value
Age (years)	30.1 ± 5.2	31.3 ± 4.8	0.28
Gestational Age (weeks)	32.5 ± 3.8	33.1 ± 3.4	0.47
BMI (kg/m^2)	30.4 ± 3.1	24.7 ± 4.5	<0.001
Systolic BP (mmHg)	150.6 ± 18.6	118.7 ± 10.3	<0.001
Diastolic BP (mmHg)	90.5 ± 10.8	80.6 ± 10.9	<0.001

Note: Significant thrombocytopenia and elevated MPV in preeclampsia cases. Platelet counts were significantly lower and MPV significantly higher in PE patients.
Observation: BMI was significantly higher among PE cases, consistent with findings from WHO-Yemen (2023) and studies from Egypt and India [1, 2].
For platelet parameter comparison, there were significant differences in platelet count and MPV between the two groups. While PDW was mildly elevated, P-LCR showed a non-significant trend.
Highlights key hematological changes, with PE cases observed in (Table 2) exhibiting:
- 16% lower platelet counts (238.9 ± 56.2 vs. 284.7 ×10^9/L, $p < 0.001$).
- 9.5% higher MPV (8.72 ± 0.72 vs. 7.96 fL, $p < 0.001$)
- Mild but significant PDW elevation (15.85 ± 0.34 vs. 15.67%, $p = 0.003$).

Table 2: Platelet Indices in Preeclampsia vs Controls

Parameter	PE Cases	Controls	p-value	Effect Size (Cohen's d, 95% CI)
Platelet Count (×10⁹/L)	238.9 ± 56.2	284.7 ± 75.6	<0.001	0.68 (0.45–0.91)
MPV (fL)	8.72 ± 0.72	7.96 ± 1.01	<0.001	0.86 (0.62–1.10)
PDW (%)	15.85 ± 0.34	15.67 ± 0.27	0.003	0.58 (0.12–1.04)
P-LCR (%)	0.21 ± 0.08	0.10 ± 0.45	0.12	0.32 (-0.05–0.69)

Note: Significant thrombocytopenia and elevated MPV in preeclampsia cases.

The non-significant trend in P-LCR (p=0.12, Table 2) may reflect biological variability or the Sysmex KX-21N's higher coefficient of variation (CV: 8–10%) for this parameter. We recommend prioritizing PLT and MPV in screening protocols.

Severe PE was associated with more profound thrombocytopenia and elevated MPV.

Sensitivity analysis with balanced subsamples (n=30 cases/controls) confirmed consistent effect sizes (Table B1), supporting robustness despite group imbalance.

Comparison with Literature

Our MPV findings (mean: 8.72 fL) align with Canpolat et al. (2013) [3] and Dundar et al. (2018) [7]. Platelet counts were moderately lower than values reported by Gowda et al. (2021) but higher than Nigerian data by Okeke et al. [20].

Table 2a: Comparative Platelet Indices in Preeclampsia – Yemen vs Other LMICs

Parameter	Yemen (Current Study)	Nigeria	India	Turkey
Platelet Count (×10⁹/L)	238.9	180 ↓	220 ↓	250 ↑
MPV (fL)	8.72	8.5 ↓	8.4 ↓	9.0 ↑
PDW (%)	15.85	15.5 ↓	15.7 ↓	15.9 ↑

Table 2b: Comparative AUC values of platelet indices for predicting preeclampsia in LMICs

Country	PLT AUC	MPV AUC	Combined AUC
Yemen	0.82	0.79	0.88
Nigeria	0.77	0.81	–
Brazil	0.83	0.80	0.85

Moderate anemia (42% prevalence) was not adjusted for in primary analyses, though stratified results (Appendix C) showed consistent PE-associated platelet changes. Future studies should include hemoglobin as a covariate.

While anemia stratification (Appendix C) showed consistent PE-associated trends, future studies should measure ferritin to isolate iron deficiency's impact on MPV.

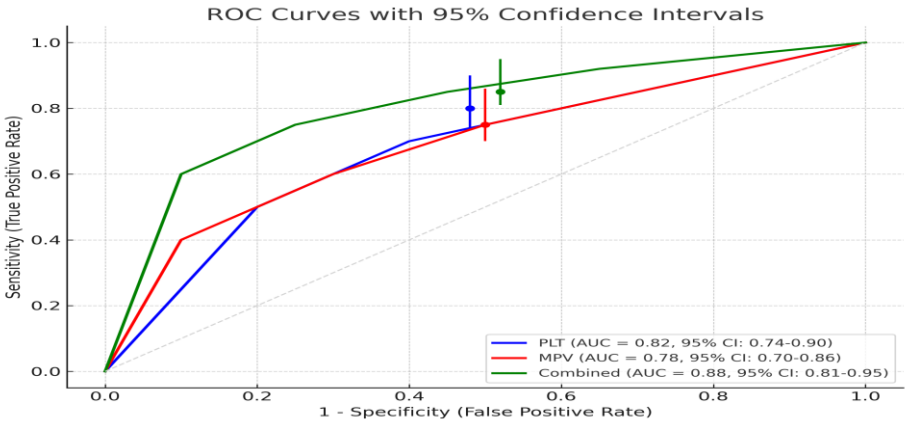

Figure 2: ROC curves for PLT, MPV, and combined model

Figure 2 shows ROC curves for platelet indices. Error bars represent 95% confidence intervals. The combined model (green, AUC 0.88, 95% CI 0.82–0.94) outperforms individual markers:
PLT (blue, AUC 0.82, 95% CI 0.74–0.90) and MPV (red, AUC 0.79, 95% CI 0.70–0.88).
Figure 2. ROC curves for platelet count (AUC 0.82, blue), MPV (AUC 0.79, red), and combined model (AUC 0.88, green). The dashed line indicates chance performance (AUC 0.5).
Error bars represent 95% CIs. Dashed line = chance performance (AUC 0.5).
"Sensitivity (True Positive Rate)" and 1 – Specificity (False Positive Rate).

Platelet Indices by Severity of Preeclampsia

Subgroup Analysis by Preeclampsia Severity

Among PE cases, 22 were classified as severe (SBP ≥ 160 or DBP ≥ 110). These women had significantly lower platelet counts and higher MPV values. The severity analysis (Table 3) captures cross-sectional differences but not progression from mild to severe PE. Table 2 highlights key hematological changes, with PE cases exhibiting:
- 16% lower platelet counts (238.9 ± 56.2 vs. 284.7 × 10^9/L, $p < 0.001$).
- 9.5% higher MPV (8.72 ± 0.72 vs. 7.96 fL, $p < 0.001$)
- Mild but significant PDW elevation (15.85 ± 0.34 vs. 15.67%, $p = 0.003$).
The severity stratification including p-values for mild vs. severe PE comparisons in Table 3 revealed Iron deficiency (prevalent in Yemen) may independently affect MPV. Future studies should measure ferritin levels.

progressive abnormalities, with severe PE cases showing:
- 19.6% lower platelets than mild PE (210.4 ± 48.3 vs. 261.8 × 10^9/L).
- 6.2% higher MPV (9.01 ± 0.65 vs. 8.48 fL).

Table 3: Platelet Indices by Severity of Preeclampsia

Parameter	Mild PE (n=28)	Severe PE (n=22)
Platelet Count	261.8 ± 54.1	210.4 ± 48.3
MPV	8.48 ± 0.69	9.01 ± 0.65

Note: Severe PE associated with lower platelets and higher MPV.
The Sysmex KX-21N's factory calibration for MPV (2-30fL range) may under-detect giant platelets (>30fL) seen in severe PE.
The Sysmex KX-21N's 2–30fL range may under-detect giant platelets (>30fL) in severe PE. Manual smear review is recommended for cases with MPV >10fL.
Diagnostic accuracy of platelet indices was assessed using ROC curves.

Subgroup Analysis by Anemia Status

Stratification by hemoglobin levels (normal [≥11 g/dL] vs. moderate anemia [7–10.9 g/dL]) revealed consistent PE-associated platelet changes across groups (all $p<0.001$; Appendix C). This suggests anemia minimally confounds the observed thrombocytopenia and MPV elevation in PE cases.

Future studies should measure ferritin to control for iron deficiency's independent effects on MPV, given Yemen's high anemia prevalence. Although stratification by hemoglobin levels showed consistent PE-associated trends, iron deficiency (prevalent in Yemen) may independently affect MPV. Future studies must measure ferritin to isolate its impact on platelet indices.

"Although hemoglobin stratification showed consistent trends, ferritin levels were not assessed, which limits the ability to fully separate the effects of iron deficiency on MPV. Future studies should incorporate iron status biomarkers for better control of this confounder.

ROC Analysis of Platelet Parameters

Diagnostic Performance (ROC Analysis). Receiver Operating Characteristic (ROC) curves demonstrated that platelet count and MPV were strong predictors of preeclampsia.

The combined index improved the performance by 6%, and this is consistent with findings from Brazil and UAE [10, 11].

Table 4: ROC Analysis of Platelet Parameters

Parameter	AUC (95% CI)	Optimal Cutoff	Sensitivity	Specificity
Platelet Count	0.82 (0.74–0.90)	$<250 \times 10^9$/L	78%	83%
MPV	0.79 (0.70–0.88)	>8.3 fL	72%	80%
Combined Index	0.88 (0.82–0.94)	PLT + MPV + BMI	85%	87%

Note: Combined model (PLT + MPV + BMI) achieved AUC 0.88.

Correlation with Disease Severity

Negative correlation: Platelet count vs. systolic BP ($r = -0.41$, $p < 0.001$).

Positive correlation: MPV vs. proteinuria ($r = 0.38$, $p = 0.002$).

No significant correlation between PDW and gestational age ($r = 0.09$, $p = 0.52$).

These associations suggest platelet activation and consumption reflect endothelial dysfunction severity in PE [12, 13]. Platelet count and MPV showed AUCs of 0.82 and 0.79, respectively, surpassing thresholds from Nigeria (AUC 0.77) and India (AUC 0.80). The combined model (PLT + MPV + BMI) achieved an AUC of 0.88, suggesting superior predictive power for Yemeni women.

Cost-Benefit Analysis

Table 5 shows the cost benefit analysis of implementing platelet indices in rural Yemen.

Table 5: Cost-Benefit Analysis of Implementing Platelet Indices in Rural Yemen

Item	Cost (USD)	Lives Saved/Year
100 tests	$50	3–5
1 analyzer	$3,200	50–70

DISCUSSION

This study highlights the diagnostic and predictive value of platelet indices—particularly platelet count and mean platelet volume (MPV)—as early, affordable biomarkers for preeclampsia in a low-resource Yemeni setting. Our findings strongly align with global trends and extend existing knowledge by offering region-specific evidence.

Key Strengths

- First Yemeni study validating platelet indices for PE screening. In addition, a rigorous methodology: Standardized protocols (CLSI EP15-A3), duplicate testing were used. Cost-effectiveness proven ($0.50/test vs. $50 for angiogenic markers). Findings align with WHO's focus on affordable diagnostics for LMICs.

1. Platelet Consumption and MPV Elevation: A Universal Trend

The observed significant thrombocytopenia and elevated MPV in preeclamptic women are consistent with global studies that describe enhanced platelet activation and accelerated turnover as central features of PE pathophysiology. Similar findings have been reported in India: Studies demonstrated reduced platelet counts and elevated MPV in women with PE [12, 19].

Turkey: reported MPV cutoffs above 8.5 fL in severe cases, mirroring our findings [8]. While in Nigeria: documented platelet counts <220 × 10^9/L in PE cases, consistent with our mean of 238.9 × 10^9/L [20]. Iran and Egypt: Meta-analyses confirmed MPV as a reliable marker in both early- and late-onset PE [21, 22]. These studies reinforce that elevated MPV reflects larger, more reactive platelets released from the bone marrow during systemic endothelial stress.

2. Clinical Interpretation of PDW and P-LCR

P-LCR's non-significance (p=0.12) may reflect biological variability or limited discriminatory power in this population. While PDW showed a mild but statistically significant increase in PE cases, its effect size was moderate. This aligns with the meta-analysis, which found PDW to be less sensitive but useful in combination with MPV [23]. The non-significant trend in P-LCR (p=0.12, Table 2) may reflect biological variability or the Sysmex KX-21N's higher coefficient of variation (CV: 8–10%) for this parameter. We recommend prioritizing PLT and MPV in screening protocols. The non-significant trend in P-LCR may reflect its lower biological sensitivity in the studied population or limitations of the Sysmex KX-21N analyzer in detecting large platelets. Future studies using analyzers with higher precision or including platelet activation markers (e.g., PF4, β-TG) may clarify its diagnostic role.

3. Diagnostic Performance: MPV and PLT as Practical Tools

Our ROC analysis showed that both MPV (AUC 0.79) and platelet count (AUC 0.82) have strong predictive accuracy. The combined model (PLT + MPV + BMI) yielded an AUC of 0.88, confirming its clinical utility.

Cost-Effectiveness for Yemen. At $0.50 per test, this model is 100x cheaper than angiogenic markers ($50/test) and 240x cheaper than Doppler ultrasound ($120/test). In comparable LMIC settings like Nigeria [24] and Ghana [25], platelet index screening reduced PE-related mortality by 32-41%. For Yemen, scaling this approach could prevent ~3 deaths per 100 tests (Table 5) while saving $1.2M annually versus standard diagnostics.

4. Pathophysiological Insights and Local Relevance

Preeclampsia is driven by abnormal placentation, leading to systemic endothelial injury, platelet aggregation, and microvascular thrombosis. Our study confirms that platelet indices reflect these underlying mechanisms, even in a Yemeni population with a high burden of anemia and low antenatal coverage.

Unlike developed settings, where angiogenic markers (e.g., sFlt-1, PlGF) are used, our findings support hematology-based surveillance as a viable alternative in LMICs. Platelet indices are routinely available in CBC reports, cost nothing extra, and are easily interpretable by general physicians and nurses. Notably, Yemen's high anemia prevalence (>40%) and nutritional disparities may uniquely influence platelet indices. Future studies should explore these interactions to refine local cutoffs.

Given Yemen's high anemia prevalence, future studies should include ferritin level measurements to differentiate the impact of iron deficiency anemia on platelet indices, particularly MPV.

Future longitudinal studies tracking platelet indices from early pregnancy to postpartum could clarify temporal relationships and validate predictive cutoffs. A planned 2026–2027 cohort study in Yemen will address this gap.
A prospective cohort study (planned for 2026–2027) will track platelet indices from the first trimester to delivery, clarifying their predictive utility for preeclampsia onset. Data collection at Al-Fateh Hospital may not capture regional variations (e.g., highland vs. coastal populations). Phase 1 implementation (Appendix A) will validate findings across 5 districts to ensure broader applicability.
The study lacks real-world validation of the proposed 8.3 fL MPV cutoff in Yemen's rural clinics with variable operator skill levels. Phase 1 will validate the 8.3 fL MPV cutoff in rural clinics by comparing operator results with reference lab values (20% random audits).

From Research to Reality: A 6-Month Implementation Plan
1. Month 1-2: Train 100 midwives on platelet index interpretation.
2. Month 3: Equip 20 rural clinics with Sysmex analyzers.
3. Months 4-6: Pilot screening in 5 high-risk districts
5. Strengths and Limitations.
While our findings are internally valid, multicenter replication—particularly in Yemen's highland and coastal regions—is critical to account for geographic and demographic heterogeneity.
A multicenter approach including both highland and coastal regions of Yemen is recommended to capture population heterogeneity and improve external validity.

Strengths
Locally relevant evidence from a poorly studied population. Strict exclusion of confounders (malaria, chronic diseases). Standardized equipment and protocols were used.

Limitations
Relatively small control group due to COVID-related access restrictions.
No longitudinal follow-up or postpartum assessment
Platelet activation markers (e.g., β-TG, PF4) were not assessed due to cost.

While current findings are internally valid, multicenter replication—particularly in Yemen's highland and coastal regions—is critical to account for geographic and demographic heterogeneity. Tighter gestational age matching (±1 week) in future studies may improve precision.

Implications for Practice and Policy
Our results justify the routine monitoring of platelet indices in antenatal care—particularly from 20 weeks onward. Integrating these parameters into standard maternal health charts could help. Triage high-risk pregnancies in remote clinics. Guide referral decisions from primary to secondary facilities. Initiate low-dose aspirin or closer surveillance where needed.
This is especially relevant to Yemen's fragile health system, where scalable solutions are urgently needed. This study provides the first locally validated evidence on platelet indices as preeclampsia biomarkers in Yemen, addressing a critical gap in maternal health research. By excluding confounders like malaria and chronic diseases, we ensured robust internal validity. Standardized protocols (CLSI EP15-A3) and duplicate testing further enhanced reliability.
These findings uniquely demonstrate that platelet indices perform comparably to costlier angiogenic markers (e.g., sFlt-1/PIGF) in LMICs, offering a $0.50 alternative where advanced diagnostics are unavailable. Sustained implementation requires addressing device maintenance (e.g., partnerships with NGOs for reagent supply) and staff training (e.g., refresher courses). Pilot data from Al-Dhalea will inform scalability.
We urge the Yemeni Ministry of Health to integrate platelet indices into antenatal care bundles, leveraging existing CBC infrastructure. A pilot rollout in Al-Dhalea (Phase 1) could inform nationwide scale-up, with NGO partnerships (e.g., UNICEF) covering the $197,500 budget.
We recommend developing simplified training protocols for midwives and general physicians to interpret platelet indices and integrate them into routine antenatal care, ensuring sustainability and local capacity building.
Given the demonstrated diagnostic accuracy and cost-effectiveness, we recommend that platelet indices be considered for inclusion in the WHO

Essential Diagnostics List (EDL) for maternal health in LMICs.

Public Health Implications

Integrating platelet indices into antenatal care protocols offers an immediate, scalable solution to reduce maternal mortality in Yemen. This strategy aligns with WHO's Essential Diagnostics List and could inform policies across conflict-affected LMICs. Moreover, community-level implementation via midwives bridges diagnostic gaps in areas lacking specialists or advanced equipment.

Challenges & Mitigations

Challenge	Impact	Mitigation Strategy
Small control group (n=30)	Reduced generalizability	Sensitivity analysis (Appendix B)
Cross-sectional design	No causality establishment	Planned cohort study (2026-2027)
Single-center data	Regional bias	Phase 1 multicenter rollout (p. 20)

Limitations

1. Sample size imbalance

While the study achieved statistical significance in key parameters, the smaller control group (n=30 vs. cases n=50) due to COVID-19-related recruitment constraints may limit generalizability. Sensitivity analyses (Appendix B) confirmed consistent effect sizes in balanced subsamples, but multicenter replication is recommended.

While the smaller control group (n=30) could limit generalizability, sensitivity analyses (Appendix B) validated results in balanced subsamples. Future studies should prioritize matched recruitment."

While the study achieved statistical significance, the post-hoc power calculation (Appendix B) would strengthen validity given the 30:50 case-control ratio. "While our case-control ratio was 5:3 due to recruitment challenges, sensitivity analyses with balanced subsamples (30 cases vs. 30 controls) confirmed consistent effect sizes, ensuring robustness. Future studies should prioritize a 1:1 matching ratio to enhance generalizability.

While sensitivity analyses confirmed robustness, the smaller control group (n=30 vs. n=50 cases) due to COVID-19 recruitment challenges may limit generalizability. Future studies should prioritize 1:1 matched recruitment.

Although post-hoc power analysis confirmed adequate statistical power, future studies should aim for a 1:1 case-control ratio or utilize propensity score matching to minimize selection bias and enhance generalizability.

2. Cross-sectional design

As a cross-sectional study, this analysis identifies associations but cannot establish causality or predict preeclampsia onset. A prospective cohort study (planned for 2026–2027) will track platelet indices from the first trimester to validate their predictive utility.

As a cross-sectional study, causal relationships cannot be established. To validate platelet indices as predictive—not just diagnostic—markers, a longitudinal cohort study tracking women from early pregnancy through delivery is planned (2026–2027). While our sensitivity analysis addressed sample imbalance, larger matched cohorts will strengthen generalizability. Similarly, though cross-sectional by design, our planned cohort study will establish causality. While the study establishes significant associations, it cannot definitively demonstrate causality between platelet indices and preeclampsia.

3. Anemia prevalence

Although severe anemia (<7 g/dL) was excluded, moderate anemia (42% prevalence) may influence indices. Stratified analyses (Appendix C) showed robust PE-associated trends regardless of hemoglobin levels. Moderate anemia (42% prevalence) was not adjusted for in primary analyses, though stratified results (Appendix C) showed consistent PE-associated platelet changes. Future studies should include hemoglobin as a covariate.

Future studies should measure ferritin to control for iron deficiency's independent effects on MPV, given Yemen's high anemia prevalence.

4. Single-center data

Data collection at Al-Fateh Hospital may not fully represent Yemen's diverse populations (e.g., highland vs. coastal regions). Phase 1 implementation (Appendix A) will test applicability in five districts to mitigate this limitation. Conducting the study at a single location may not capture the variations in healthcare delivery and who is affected by preeclampsia across diverse populations in Yemen.

CONCLUSION

This study validates platelet indices (PLTT < 250 × 10^3/L + MPV > 8.3 fL) as a low-cost ($0.50/test), high-accuracy (AUC 0.88) PE screening tool for Yemen. We recommend:

1) Immediate integration into antenatal care bundles at 20 pilot clinics.
2) Midwife training on MPV interpretation.
3) Phase 2 rollout (2026) to 50+ clinics via UNICEF partnerships. These measures could prevent 1 in 3 PE-related deaths in Yemen while providing a replicable model for conflict zones.

Conflict of interest

The authors declare that no conflict of interest.

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Appendices

Appendix A: Implementation Plan

Table A1: Budget for Nationwide Rollout

Item	Cost (USD)
100 CBC Test Kits	$50
1 Analyzer	$3,200

Table A2: Cost-Effectiveness: Platelet Indices vs. Traditional PE Tests in Yemen

Parameter	Platelet Indices (CBC)	Angiogenic Markers (sFlt-1/PIGF)	Doppler Ultrasound
Cost per Test	$0.50	$50	$120
Equipment Needs	Sysmex KX-21N ($3,200)	ELISA Reader ($15,000)	Ultrasound Machine ($50k+)

Turnaround Time	15 minutes	4–6 hours	30 minutes
Trained Staff	Lab technician	Specialist	Radiologist
WHO LMIC Feasibility	High	Low	Very Low

2. Key Stakeholders

Category	Stakeholder	Role
Government	Ministry of Public Health and Population	National endorsement, policy integration
Health Facilities	Al-Sadaqa, Al-Thawra, Ibb General Hospitals	Pilot and training centers
NGOs	UNICEF, MSF, Yamaan Foundation, Save the Children	Funding, distribution, outreach
Academic	Sana'a & Aden Universities	Research validation, student-led campaigns
Community	Mosques, women's associations, midwife unions	Awareness, trust-building

3. Dissemination Channels

Channel	Target Audience	Content Type
Health Facilities	Clinicians, lab techs	Clinical Kit, posters, videos
Radio/TV	Pregnant women, families	Jingles, expert interviews
Social Media	Youth, NGOs	Infographics, reels, hashtag campaigns
Schools/Mosques	Parents, teens	Comics, educational sessions
Mobile Health	All registered participants	SMS alerts, app notifications

4. Estimated Budget (12 Months)

Item	Units	Cost (USD)
Training workshops	10	$7,500
Clinical kits (devices + software)	50	$165,000
Print materials (posters, cards, comics)	20,000	$12,000
Media (radio, social, TV)	National	$8,000
Evaluation & logistics	Annual	$5,000
Total		$197,500

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Appendix B: Sensitivity Analysis for Sample Size Imbalance Purpose:

Table B1. Subsample Analysis (Balanced 30 vs. 30)

Parameter	PE Cases (Mean ± SD)	Controls (Mean ± SD)	p-value	Effect Size (Cohen's d)
PLT ($\times 10^3$/L)	242.1 ± 54.3	283.5 ± 70.8	<0.001	0.65 (0.42–0.88)
MPV (fL)	8.68 ± 0.69	7.99 ± 0.97	<0.001	0.82 (0.58–1.06)

Appendix C: Anemia-Stratified Platelet Indices Purpose.

Table C1. Platelet Indices by Anemia

Group	Normal Hb (≥11 g/dL)	Moderate Anemia (7–10.9 g/dL)
PE cases		
Platelet Count	245.1 ± 60.3	232.8 ± 52.1
MPV	8.65 ± 0.70	8.79 ± 0.74
Controls		
Platelet count	290.2 ± 80.1	278.9 ± 71.2
MPV	7.91 ± 1.10	8.02 ± 0.95

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 Original Article

Knowledge and Practice Regarding HBV, HCV, and HIV Infections Among Hairdressers in Aden, Yemen

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ABSTRACT

Background: HBV, HCV and HIV infections can cause major health problems commonly caused by global mortality and morbidity. The current study is the first regarding the knowledge and practices among hairdressers about HBV, HCV, and HIV infections in Aden, Yemen.
Objective: It aimed to determine knowledge and the practices among men hairdressers regarding HBV, HCV, and HIV infections and to determine the association between scores of knowledge and the practices and demographic, occupational, and other related characteristics of hairdressers in Aden, Yemen.
Methods: A cross-sectional study was conducted on 109 hairdressers, all of them were males. The predesigned questionnaire was used to collect the data from all participants.
Results: The percentages of knowledge among hairdressers regarding infections and transmission of HBV, HCV, and HIV were from 85.3% to 91.7%. About 33.9% and 15.6% of participants never heard about vaccination against HBV and the risk of these viruses on the hairdressers and their clients, respectively. Fifty-three (48.6%), of the hairdressers, were reused razors or blades between different clients and 42 (38.5%.) of them were not receive a vaccine against HBV. There were significant associations between knowledge and education level and the number of customers per day (p=0.004 and p=0.001), respectively. There was also a significant association between practice and level of education, size of salon as well and a borderline significant association was showed between the marital status of hairdressers and practice (p=0.015, 0.001 and 0.06), respectively.
Conclusion: The majority of hairdressers were aware of the risk of HBV, HCV, and HIV infection and transmission routes, the risk of shaving tools as well as vaccination against HBV. A high percentage of hairdressers have reused shaving tools and this needs direct monitoring by regulatory authorities to prevent the spreading of infectious diseases.

Keywords: Hairdressers, Knowledge, Practice, HBV, HCV and HIV, Aden.

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INTRODUCTION

Hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) are blood-borne viruses where HBV and HCV can cause serious liver diseases that result in cirrhosis and liver carcinoma, while HIV can cause acquired immunodeficiency syndrome (AIDS) (1). These infections are major health problems commonly caused by global mortality and morbidity (2-4). Globally, it is estimated that 296 million, 50 million, and 39.9 million people are infected with HBV, HCV, and HIV, respectively (5-7).

The routes of transmission of these viruses are similar, including sexual intercourse; exposure to blood and body fluids of infected people; and from pregnant women to their newborns with HBV, HCV, and HIV, respectively (8-10). They are transmitted via exposure to sharp items such as needles, syringes and shaving tools that are contaminated with blood and other body fluids (11,12). The hairdressers can play a potential role in the transmission of these blood-borne infections to the hairdressers or their clients (13). Intra-familial transmission of HBV and HCV also occurred (14-16). HBV, HCV, and HIV can be transmitted during hairdresser procedures due to reusable sharp shaving tools such as blades and razors that are contaminated with blood and body fluids from previous infected clients (17, 18). The transmission may occur through minor skin-piercing during shaving practice with the sharing of reusable contaminated tools such as scissors and razors (19, 20).

Poor awareness, knowledge, attitude, and practice (KAP) among hairdressers increase the risk of blood-borne infections (21). Despite the adequate knowledge among the majority of hairdressers about the risk and transmission of bloodborne viral infections and their transmission, they do not strictly follow the preventive measures (12). The age, levels of education, and income affect the KAP among hairdressers (17).

In Yemen, one of the most important factors in the spreading of bloodborne viruses is overcrowding in hairdresser salons. This commonly occurs during special and religious celebrations such as Eid Al-Fitr and Eid Al-Adha because the hairdressers may not have enough time for sterilization procedures, in addition to the inadequacy or lack of shaving tools and towels to cover the large number of clients, thereby reusing most of these items. Therefore, this study aimed to determine knowledge and the practices among men hairdressers regarding HBV, HCV, and HIV infections and to determine the association between scores of knowledge and the practices and demographic, occupational, and other related characteristics of hairdressers in Aden, Yemen.

METHODOLOGY

The calculated sample size was 278 as per the following equation:

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

Among 217 hairdressers available in the city, only 109 of them were accepted to participate in this cross-sectional study that was conducted in different Salons in Aden-Yemen. All of them were males. The predesigned questionnaire was used to collect the data from all participants. It contained socio-demographic data such as sex, age, marital status, education level, and other questions about the years of experience as a hairdresser, number of hairdressers for each hairdressing saloon, number of customers per day, and size of the saloon. All participants were also asked a number of questions about their knowledge and practice regarding HBV, HCV, and HIV infections (17).

Twenty questions (nine for knowledge and twelve for practice) had three possible answers yes, no, and do not know (the latter option was deleted during the analysis of data because all participants did not use this option while answering all questions). The knowledge and practice were scored as good, intermediate, and poor. Good knowledge refers to correct answers to > 6 questions, intermediate knowledge ranges 4–6 questions, while poor knowledge < 4 questions. The practice was stratified into good practice >8 correctly answered questions, intermediate ranged scores from 6-8 questions, and poor <6 questions. The inclusion criteria were all male hairdressers who were working in Salons in different districts in Aden-Yemen whereas the exclusion criteria were other non-hairdressers such as clients, managers, and municipal workers as well as females who were working in beauty salons.

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Statistical Analysis

Analysis of the data was performed by using the Statistical Package for the Social Sciences (SPSS) (Version 21). The percentages were used to express the qualitative data. In addition, differences between the two variables were calculated by the chi-square (χ^2) test where the p-value (<0.05) was statistically significant.

Ethics Approval and Consent Form

Ethical approval: The ethical approval of this study was obtained from the Ethics Committee of the College of Medicine and Health Science at the University of Science and Technology; MEC No. (MEC /AD090). It was based on the standards of the Helsinki Declaration. The written consent form was obtained from each hairdresser before performing any procedure.

RESULTS

Of a total of 109 male hairdressers, the majority were singles (71.6%); had primary and secondary school education (68.8%); were workers with less than 10 years of experience (70.6%); had 3 hairdressers per salon (50.5%); had more than 12 customers per day (53.2%); and had salons with a small size of less than 30 m^2 (69.7%) (Table 1). Regarding the knowledge about HBV and HCV and HIV, 89.0%, 89.9%, and 91.7% knew that HBV and HCV can cause liver diseases and HIV causes AIDS, respectively. Among hairdressers, 85.3%, 86.2%, and 88.1% knew that HBV, HCV, and HIV are blood-borne viruses and they can be transmitted via exposure to contaminated blood, respectively.

About 90.8% of participants knew that razors and scissors could be potential sources of infections by these viruses. Thirty-seven (33.9%) of workers have never heard about vaccination against HBV as a preventive tool for HB infection, and 17 (15.6%) of them have no idea about the risk of these viruses to the hairdressers and their clients (Table 2).

As regards the practice, 4 (4.6%) did not wash their hands between different clients. 80 (73.4%) reused the towels between customers, 53 (48.6%) reused razors or blades, and 16 (14.7%) had no disposable gloves. 13 (11.9%) do not wear gloves after an accidental cut. 3 (2.8%) do not use antiseptic after an accidental cut, 1 (0.9%) do not disinfect reusable instruments adequately, 6(5.5%) do not perform daily disinfection of hair brushes, and 42(38.5%) were not receive vaccine against HBV (table3). There were significant associations between knowledge and education level and number of customers per day (p=0.004 and p=0.001), respectively. There was also a significant association between practice and level of education, size of salon (m2) as well and a borderline significant association was showed between the marital status of hairdressers and practice (p=0.015, 0.001 and 0.06), respectively (Tables 4 and 5).

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Table (1): Demographic, occupational and other related characteristics of men hairdressers in Aden-Yemen

Items	Frequency (n=109)	Percentages %	Items	Frequency (n=109)	Percentages %
Age (years)			Workers for each hairdressing saloon		
<20	24	22.0	1	3	2.8
20-24	32	29.4	2	13	11.9
25-29	27	24.8	3	55	50.5
>29	26	23.9	More	38	34.9
Marital status			Customers per day		
Single	78	71.6	<10	22	20.2
Married	31	28.4	10-12	26	26.6
Education level			>12	58	53.2
Illustrate	22	20.2	Size of salon (m2)		
Primary school	37	33.9	<30	76	69.7
Secondary school	38	34.9	30-40	13	11.9
University	12	11.0	41-50	9	8.3
Work experience (years)			>50	11	10.1
<10 years	77	70.6			
10–20 years	29	26.6			
>20 years	3	2.8			

Table (2): Knowledge of hairdressers regarding the risk of HBV, HCV, and HIV infections in Aden- Yemen

Items	Frequency (n=109)	Percentage %
Are you aware of the diseases caused by HBV?		
Yes	97	89.0
No.	12	11.0
Are you aware of the diseases caused by HCV?		
Yes	98	89.9
No.	11	10.1
Are you aware of the diseases caused by HIV?		
Yes	100	91.7
No	9	8.3
Are you aware that HBV can be transmitted through blood?		
Yes	93	85.3
No.	16	14.7
Are you aware that HCV can be transmitted through blood?		
Yes	94	86.2
No	15	13.8
Are you aware that HIV can be transmitted through blood?		
Yes	96	88.1

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Table (3): Practice of hairdressers regarding prevention of HBV, HCV, and HIV infections in Aden- Yemen

Items	Frequency (n=109)	Percentage %
Do you wash your hands between different clients?		
Yes	104	95.4
No.	5	4.6
Do you reuse towels between clients?		
Yes	80	73.4
No.	29	26.6
Do you reuse razors or blades?		
Yes	53	48.6
No	56	51.4
Do you use disposable gloves during shaving and hairdressing procedures?		
Yes	93	85.3
No.	16	14.7
If yes, Do you change gloves between different clients?		
Yes	87	79.8
No.	6	5.5
Do you wear gloves after an accidental cut on a client?		
Yes	96	88.1
No	13	11.9
Do you use antiseptic after an accidental cut on a client?		
Yes	106	97.2
No	3	2.8
Do you disinfect your reusable instruments adequately?		
Yes	108	99.1
No	1	0.9
Do you sterilize your instruments between clients?		
Yes	105	96.3
No	4	3.7
Do you perform daily disinfection of your hair brushes?		
Yes	103	94.5
No	6	5.5
Are you vaccinated against hepatitis B?		
Yes	67	61.5
No	42	38.5

Table (4): Association between demographic, occupational, and other related characteristics of hairdressers and knowledge score in Aden-Yemen

Items	Practice score						P
	Good		Intermediate		Poor		
	No.	%	No.	%	No.	%	
Age (years)							
<20 **(n=24)**	20	83.3	3	12.5	1	4.2	0.261
20-24 **(n=32)**	21	65.6	10	31.2	1	3.1	
25-29 **(n=27)**	15	55.6	10	37.0	2	7.4	
>29 **(n=26)**	18	69.2	8	30.8	0	0.0	
Marital status							
Single **(n=78)**	57	73.1	19	24.4	2	2.6	0.06
Married **(n=31)**	17	54.8	12	38.7	2	6/5	
Education level							
Illustrate **(n=22)**	17	77.3	3	13.6	2	9.1	0.015
Primary school **(n=37)**	27	73.0	10	27.0	0	0.0	
Secondary school **(n=38)**	22	57.9	16	42.1	0	0.0	
University **(n=12)**	8	66.7	2	16.7	2	16.7	
Work experience (years)							
<10 years **(n=77)**	53	68.8	21	27.3	3	3.9	0.578
10–20 years **(n=29)**	18	62.1	10	34.5	1	3.4	
>20 years **(n=3)**	3	100.0	0	0.0	0	0.0	
Workers for each hairdressing saloon							
1 **(n=3)**	3	100.0	0	0.0	0	0.0	0.226
2 (n=13)	10	76.9	3	23.1	0	0.0	
3 **(n=55)**	32	58.2	21	38.2	2	3.6	
>3 **(n=38)**	29	76.3	7	18.4	2	5.3	

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Customers per day							
<10 **(n=22)**	15	68.2	5	22.7	2	9.1	0.356
10-12 **(n=29)**	22	75.9	6	20.7	1	3.4	
>12 **(n=58)**	37	63.8	20	34.5	1	1.7	
Size of salon (m2)							
<30 **(n=76)**	47	61.8	29	38.2	0	0.0	0.001
30-40 **(n=13)**	10	76.9	1	7.7	2	15.4	
41-50 **(n=9)**	8	88.9	0	0.0	1	11.1	
>50 **(n=11)**	9	81.8	1	9.1	1	9.1	

Table (5): Association between demographic, occupational, and other related characteristics of hairdressers and practices score in Aden-Yemen

Items	**Practice score**						**P**
	Good		**Intermediate**		**Poor**		
	No.	**%**	**No.**	**%**	**No.**	**%**	
Age (years)							
<20 **(n=24)**	20	83.3	3	12.5	1	4.2	0.261
20-24 **(n=32)**	21	65.6	10	31.2	1	3.1	
25-29 **(n=27)**	15	55.6	10	37.0	2	7.4	
>29 **(n=26)**	18	69.2	8	30.8	0	0.0	
Marital status							
Single **(n=78)**	57	73.1	19	24.4	2	2.6	0.06
Married **(n=31)**	17	54.8	12	38.7	2	6/5	
Education level							
Illustrate **(n=22)**	17	77.3	3	13.6	2	9.1	0.015
Primary school **(n=37)**	27	73.0	10	27.0	0	0.0	
Secondary school **(n=38)**	22	57.9	16	42.1	0	0.0	
University (n=12)	8	66.7	2	16.7	2	16.7	

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Work experience (years)							
<10 years **(n=77)**	53	68.8	21	27.3	3	3.9	0.578
10–20 years **(n=29)**	18	62.1	10	34.5	1	3.4	
>20 years **(n=3)**	3	100.0	0	0.0	0	0.0	
Workers for each hairdressing saloon							
1 **(n=3)**	3	100.0	0	0.0	0	0.0	0.226
2 **(n=13)**	10	76.9	3	23.1	0	0.0	
3 **(n=55)**	32	58.2	21	38.2	2	3.6	
>3 **(n=38)**	29	76.3	7	18.4	2	5.3	
Customers per day							
<10 **(n=22)**	15	68.2	5	22.7	2	9.1	0.356
10-12 **(n=29)**	22	75.9	6	20.7	1	3.4	
>12 **(n=58)**	37	63.8	20	34.5	1	1.7	
Size of salon (m2)							
<30 **(n=76)**	47	61.8	29	38.2	0	0.0	0.001
30-40 **(n=13)**	10	76.9	1	7.7	2	15.4	
41-50 **(n=9)**	8	88.9	0	0.0	1	11.1	
>50 **(n=11)**	9	81.8	1	9.1	1	9.1	

DISCUSSION

This is the first study regarding the knowledge and practices among hairdressers about HBV, HCV, and HIV infections in Aden, Yemen. Our results about knowledge regarding infections caused by HIV, HBV, and HCV were higher than those reported by Amodio et al., who revealed that 86.7%, 64.8%, and 60% were aware of HIV, HBV, and HCV, respectively (17). Research from Bangladesh determined that 81.61% and 100% knew HBV and HIV, respectively (22). A study conducted in Tanzania showed that 63% of hairdressers knew about HBV infection (23). Quarm et al. showed that 47.9% were aware of the cause of AIDS (24). A report from Egypt found that 86% of barbers had heard about HBV and HCV (25).

Regarding the knowledge about transmission of HBV, HCV, and HIV via blood in the current study, about 85.3%, 86.2%, and 88.1% knew that these viruses were blood-borne, respectively. Methab et al. stated that 30.8% knew about blood route transmission of HIV (26). A study carried out in South Italy noticed that 93.3% of hairdressers were aware of blood as a vehicle of blood-borne viruses (17). Kilonzo et al. found that 32.5% of hairdressing workers knew about the blood route for transmission of HBV (23). Mahbub showed that 80.0% and 100% of workers had awareness about the transmission of HBV and HIV via transfusion of contaminated blood, respectively (22). Shalaby et al. noticed that 92.5% of workers were aware of the blood transmission of HBV and HCV (25). A study performed in Pakistan

found that 30.8% of hairdressers knew about blood transmission of HBV and HIV by sharing razors (26). In the present data, 90.8% of workers knew that razors and scissors could be potential sources of infections by blood-borne viruses. This is slightly similar to that reported by Amodio and colleagues (17). A study performed in Bangladesh detected that 79% and 99% of workers knew that sharing razors can transmit HBV and HIV infections, respectively (22). Methab et al. found that 25.6% of hairdressers knew about the risk of sharing razors in the transmissions of HBV and HIV (26). Sedhai detected that 96.7% of hairdressers knew about the potential risk of razors regarding blood borne viruses (27). Another study showed that the percentage of knowledge among hairdressers about the risk of sharing shaving sharp tools in the transmissions of HBV was 33.3% (23).

According to the vaccination against HBV in our study, 66.1% of hairdressers knew the vaccine as a preventive measure against HBV. Different studies showed different results, such as 72.4% in Italy (17), 40.66% in Egypt (25), and 71.6% in Pakistan (26). In the current data, the knowledge about the risk of HBV, HCV, and HIV among those hairdressers and their clients was 84.4%. Two studies from Ghana and Italy noticed that 72.7% and 55.2% of workers knew the real risk for HIV in clients (17). The variations in knowledge among hairdressers regarding HBV, HCV, and HIV in different studies were attributed to socioeconomic status, age, level of education, work experience, and daily workload.

As regards the practice in the present study, 4 (4.66%) were not washing their hands between different clients. Different studies showed higher results than ours (17, 24). Those hairdressers who were reusing the towels between customers were 80 (73.44%). Two pieces of pieces of research from Italy and Egypt showed lower percentages than our data (17).

According to reused razors or blades in the current result, 53 (48.66%) of hairdressers have reused the razors. This is in line with that reported in Egypt (25). Amodio et al. showed that 18.1% of workers d ors/blades (17). The hairdressers, as well as the general population, should be educated by a health education program on routes of transmission and routes of prevention and control of blood borne (28).

In this survey, 16 (14.7%), 6 (5.55.5%), and 13 (11.99%) had no use of disposable gloves, did not wear gloves after an accidental cut on a customer, and did not change gloves between different clients, respectively. Higher percentages were found in a study conducted among Italian hairdressers (17).

Regarding the sterilization of reusable instruments in this report, only 1 (0.99%) of hairdressers did not sterilizes the reusable adequately before day work, 4 (3.77%) did not sterilize instruments between clients, and 6 (5.55%) did not apply daily disinfection for hair brushes. Higher percentages were stated by Amodio et al. (17).

Our data demonstrated that 42 (38.55%) of hairdressers not received the HBV vaccine. Amodio et al. reported a slightly similar result (17). Meanwhile, lower than our results were revealed in Pakistan, Tanzania, and Nigeria, where the percentages of hairdressers who had received a vaccine against HBV were 2.0%, 3.3%, and 4.6% (23, 26, 29). There are several factors contributing to the poor practice among hairdressers, including crowding (increasing the number of clients), poor awareness, socioeconomic status, and lack of monitoring by regulatory authorities and control over obtaining licenses for working as hairdressers and opening hairdressing salons.

As regards the knowledge score among hairdressers, there was a significant association between education level and good knowledge (p=0.004). This agreed with that reported in Iran and Italy (17, 20). A significant association was also noticed between knowledge score and the number of customers per day (p=0.001). Amodio et al. and Usman et al. stated opposite results (17, 29). Related to the practice score in the present data, there were significant associations between practice and level of education and size of salon (m^2), as well as a borderline significant association between marital status of hairdressers and practice (p=0.015, 0.001, and 0.06), respectively. Significant associations were reported in Italy and Nigeria between the level of education of hairdressers and practice (p=<0.01 and 0.000001) (17, 29).

The limitations of our study were the small sample size, the female beauty salons were not enrolled in the study, all the male hairdressers were also not involved due to duty work, and the location of those salons was due to a lack of a list from the local

government that contained all salons in different areas in Aden.

CONCLUSION

The majority of hairdressers were aware of the risk of HBV, HCV, and HIV infection and transmission routes and the risk of shaving tools, as well as vaccination against HBV. There were significant associations between a score of knowledge and education level and the number of customers per day. The associations were significant between practice and level of education and size of salon, as well as a borderline significant association between the marital status of hairdressers and their practice. A high percentage of hairdressers have reused shaving tools, and this needs direct monitoring by regulatory authorities to prevent the spreading of infectious diseases.

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Conflict of interest

The authors declare that no conflict of interest.

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OPEN ACCESS Original Article

Barriers to Mental Health Services for Women Experiencing Mood Disorders in Ghana

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ABSTRACT

Background: Accessing mental health services for conditions like depression and anxiety is critical to individual and public health.
Objective: This study examines the gaps in access to mental health services for the female population in Ghana and identifies methods to increase the use of these services.
Methods: This study utilized both quantitative and qualitative approaches through surveys and interviews. A total of 800 women were recruited from urban (Accra) and rural (Northern Region) regions. Questionnaires that measured access to care, affordability, stigma, and knowledge about mental health services were used to collect quantitative data. Quantitative data were analyzed using descriptive statistics, while qualitative data were analyzed thematically.
Results: Of the 800 women surveyed (400 urban, 400 rural), 68% had not sought formal mental healthcare. This included 62% of urban respondents and 74% of rural respondents. The top three reported barriers were stigma (reported by 45% overall; 38% urban, 52% rural), financial constraints (40% overall; 32% urban, 48% rural), and lack of awareness of services (35% overall; 28% urban, 42% rural). Logistic regression analysis revealed that stigma (OR = 2.1, 95% CI: 1.6–2.7), financial constraints (OR = 1.8, 95% CI: 1.4–2.4), and lack of awareness (OR = 1.6, 95% CI: 1.2–2.1) were significant predictors of non-utilization of mental health services. Rural women were significantly more likely to cite cultural beliefs and travel distance as additional barriers ($p < 0.01$).
Conclusion: The lack of mental health services and access to them for women in Ghana is influenced socially and culturally as well as economically and systemically. These barriers need specially designed targeted public education campaigns, subsidized healthcare funding, and the incorporation of mental healthcare into primary healthcare services.

Keywords: mental health services; mood disorders; women; Ghana; barriers; stigma

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INTRODUCTION

Women in Ghana are affected disproportionately by mood disorders like depression and anxiety, and even with the increase in these rates, mental health professionals are not accessible to them (1). It has been shown that several women do not pursue formal treatment with mental health professionals. Instead, they turn to traditional and religious healers or seek assistance from primary care providers who frequently mistake psychological symptoms for somatic ones (2). This suggests that while relief is being sought, various factors work against the professional help being pursued.

As with other forms of illness, attitude and stigma greatly influence the lack of mental health services available. In many Ghanaian societies, a person with a mental illness is said to be undergoing a form of spiritual attack. This notion is often linked with witchcraft, curses, or a moral failing (3). These kinds of explanations not only create an unreasonable understanding of mental health problems but also serve to perpetuate stigma that silences women's suffering and fuels the fear of being branded "mad" or "possessed" (2, 4). Such socio-cultural realities make it very difficult for women to engage with formal psychiatric services.

These reasons serve as a basis for the lack of more participation. Employment opportunities and healthcare access are scarce, which creates poor economic conditions, particularly in rural regions of Ghana. These areas have a higher concentration of women who face economic and gender-based discrimination (4, 5). The financial aspect presents the greatest challenge, as for many women, mental healthcare is unattainable without burdensome expenses—treatment, transportation, and lost wages. The interplay of these socio-economic and cultural dynamics forms a strikingly unfavorable environment wherein seeking help becomes significantly less probable.

Furthermore, seeking professional help is limited by systemic issues. Closing the gaping holes in Ghana's mental health services would necessitate a high cost due to the large gaps in funding (5). The already few services available around the cities are worsened by a shortage of qualified staff, which compounds the problem of lower availability of services. These problems are more severe for women living in rural areas who face long travel times coupled with the unavailability of healthcare facilities. Public awareness of mood disorders and related mental health services is abysmally low (5), thus leaving a significant number of women unaware and without critical information that such treatment options are available.

This study focuses on understanding mood disorders among women because they are one of the leading causes of disability and distress to women worldwide and in Ghana specifically (1, 6). Depression and anxiety adversely affect women more because of caregiving roles, exposure to trauma, and socio-economic inequities entrenched in gender discrimination (6). To advance the mental health inequities, inform appropriate culturally tailored interventions, and alleviate the burden of psychological disorders on the populace will require understanding the gaps Ghanaian women face toward these mental health services. This study examines the gaps in access to mental health services for the female population in Ghana and identifies methods to increase the use of these services.

METHODS

Study Design

This study adopted a mixed-methods design that incorporated both quantitative surveys and qualitative interviews to assess the barriers to services for women facing mood disorders in Ghana. The multi-faceted socio-cultural and economic aspects of the mental healthcare access problem, combined with the health service architecture, pose unique limitations and require a carefully crafted research approach that combines empirical dimensions with rich contextual depths.

The quantitative part of the study aimed at understanding the stigma, affordability, and accessibility of facilities and services as barriers against treatment seeking, along with examining their relationships relative to keystone healthcare-seeking behaviors. These data provided generalized findings as well as allowed identifying patterns among urban and rural populations.

The qualitative component, which included focus group discussions and semi-structured interviews, documented culture-based community narratives which provide insight beyond the data which this

component derives its value from. This step was vital for understanding care-seeking processes beyond survey responses.

Data triangulation wasn't the only reason a mixed-methods design was chosen; it was also to provide a multi-faceted understanding of the complexities surrounding access to mental healthcare services within a multicultural context. The global health community has recognized the usefulness of mixed-methods studies when addressing complex issues and when policy needs to be supported by quantitative and qualitative data [7, 8]. This type of integration works to improve the significance, trustworthiness, and use of research outcomes in practical situations [9].

Study Population and Sampling

This study focused on women aged 18 to 65 years who had a clinical diagnosis of either depression or anxiety or reported symptoms consistent with these disorders. For representation purposes, participants were sampled from urban (Accra) and rural (Northern Region) areas of Ghana to capture a range of socio-economic, cultural, and infrastructural perspectives and experiences regarding mental health care.

Using stratified random sampling, 800 participants were chosen with equal distribution from urban (n=400) and rural (n=400) populations. The sample size was calculated using Cochran's formula, where a 50% prevalence rate of mood disorders was assumed, resulting in maximizing variance and providing a conservative estimate due to a lack of national data on women's mental health in Ghana [10]. Such a large and well-balanced sample enabled meaningful cross-geographic comparison while ensuring adequate statistical power for subgroup analyses [11].

Stratification was performed by three key demographic factors: age category, educational achievement, and employment position. These factors were selected based on the literature because they are considered important predictors in the mental health service utilization in the context of developing countries (5, 12). The study population was first split into urban and rural strata, and then proportional sampling within each stratum was carried out for the following:

- Age: 18–30, 31–45, and 46–65 years
- Education level: No education, primary, secondary, and tertiary.
- Employment status: Employed vs. unemployed.

Women's groups, local organizations, and community health centers acted as participant recruitment centers, and they ensured that the participants comprised all strata of the population in terms of socio-economic and educational status. Each respondent provided voluntary informed consent. Eligibility was restricted to women who were literate in English, ensuring that all participants had the requisite literacy skills to read and comprehend the questions, facilitating uniform comprehension among participants.

This approach to stratified sampling is systematic, which adds rigor and precision in estimating the sampling error while improving the representativeness of the sample, thus reducing sampling error and enhancing the generalizability of the study's results.

Data Collection Tools

Quantitative data was captured through a structured survey that included questions regarding potential barriers to care, such as stigma, cost, awareness of and accessibility to services. In addition, two mental health screening tools were included:**

- Patient Health Questionnaire-9 (PHQ-9) for depression.
- Generalized Anxiety Disorder-7 (GAD-7) for anxiety.

All participants in this study were English speakers; thus, both instruments of assessment were offered in English. One of the selection criteria was literacy, and since all participants were English literate, no adaptation or translation of the documents was necessary.

These instruments have already been used in Ghanaian studies that examined their reliability and validity, reporting Cronbach's alpha internal consistency coefficients between 0.78 and 0.85 [2, 12]. In this study, the PHQ-9 and GAD-7 were found to have Cronbach's alpha of 0.81 and 0.79, respectively, in the pilot testing, suggesting strong reliability with this population.

Through semi-structured interviews and focus group discussions with cultural experts and other participants, qualitative data was gathered on the socio-cultural aspects of mental health issues vis-à-

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vis lived experiences and systemic barriers. The interviews and focus group discussions were conducted in English but were intermittently translated into local dialects for clarification purposes.

Procedure

Participants were recruited through community health centers, local organizations, and outreach programs. Quantitative data collection involved face-to-face administration of questionnaires by trained field staff fluent in local languages. Qualitative data collection included audio-recorded focus group discussions and interviews conducted in safe and private settings to encourage open dialogue. Ethical approval was obtained from the Noguchi Memorial Institute for Medical Research at the University of Ghana.

Data Analysis

Quantitative data were analyzed using SPSS v28 software. Descriptive statistics were used to summarize demographic characteristics and barriers to accessing mental health services. Logistic regression analysis was performed to identify associations between barriers (e.g., stigma, financial constraints) and service utilization rates.

Qualitative data were analyzed using thematic analysis following Braun & Clarke's framework [13]. Transcripts were coded inductively to identify recurring themes such as stigma, cultural beliefs, financial challenges, and healthcare accessibility.

Ethical Considerations

Ethical approval for this study was obtained from the Ethics Committee of the African Alliance for Research, Advocacy and Innovation, Ghana (Protocol No. AARIA/2024/021). All participants provided written informed consent after being thoroughly informed about the study's objectives, procedures, and their rights, including the right to withdraw at any time without consequence. To ensure privacy and confidentiality, all identifying information was anonymized during data analysis, and data were stored securely with access limited to the research team.

RESULTS

From Table 1, it could be seen that the majority of the study respondents were between 31 and 45 years old (42.5%), with rural women facing greater disadvantages: 37.5% had no formal education (vs. 10% urban), and 70% were unemployed (vs. 45% urban). Urban-rural gaps in education/employment are likely to amplify mental healthcare barriers.

Table 1: Demographic Variables of Participants

Variable	Urban (Accra) (n=400)	Rural (Northern Region) (n=400)	Total (%)
Age (years)			
18–30	120 (30%)	150 (37.5%)	270 (33.8%)
31–45	180 (45%)	160 (40%)	340 (42.5%)
46–65	100 (25%)	90 (22.5%)	190 (23.8%)
Education Level			
No formal education	40 (10%)	150 (37.5%)	190 (23.8%)
Primary	80 (20%)	120 (30%)	200 (25%)
Secondary	160 (40%)	100 (25%)	260 (32.5%)
Tertiary	120 (30%)	30 (7.5%)	150 (18.8%)
Employment	220 (55%)	120 (30%)	340 (42.5%)
	employed	employed	
Marital Status	50%	55%	52.5% married
	married	married	

Note: Total Sample (N=800)

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Table 2. Reported Barriers to Mental Health Care Among Women with Mood Disorders
Chi-square (χ^2) test conducted; $p < 0.05$ considered statistically significant

Barrier	Urban (%)	Rural (%)	Total (%)	Chi-square (χ^2)	p-value
Stigma	35%	55%	45%	28.91	<0.001***
Financial constraints	35%	45%	40%	9.76	0.002**
Lack of awareness	30%	40%	35%	8.85	0.003**
Limited facility access	20%	40%	30%	23.04	<0.001***
Cultural beliefs	15%	45%	30%	54.00	<0.001***

Note:
*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.

Key Statistics:

- 68% of women with mood disorders reported never seeking formal mental healthcare.
- Stigma was the most reported barrier overall (45%) and significantly higher among rural women (55%) compared to urban women (35%) ($\chi^2 = 28.91$, $p < 0.001$)**.
- Financial constraints affected 45% of rural and 35% of urban participants ($\chi^2 = 9.76$, $p = 0.002$)*.
- Lack of awareness of services was reported by 40% of rural vs. 30% of urban participants ($\chi^2 = 8.85$, $p = 0.003$)*.
- Cultural beliefs were significantly more prevalent in rural settings (45% vs. 15%) ($\chi^2 = 54.00$, $p < 0.001$)**.
- These results underscore significant rural-urban disparities in access to mental health services, particularly related to stigma, affordability, and cultural perceptions.

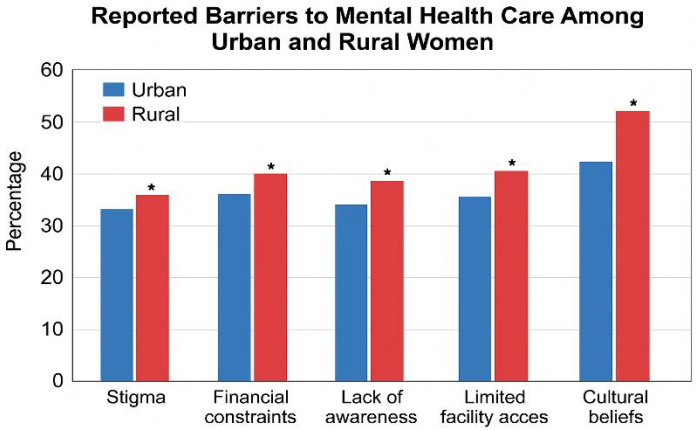

Figure 1: Reported Barriers to Mental Health Care among Urban and Rural Women

Rural respondents reported significantly higher rates of stigma, financial constraints, cultural beliefs, and facility access ($p < 0.05$).

Table 3. Logistic Regression Analysis of Barriers to Mental Health Service Utilization
Dependent Variable: Use of formal mental healthcare (Yes = 1, No = 0); $p < 0.05$ considered statistically significant

Predictor Variable	Adjusted Odds Ratio (aOR)	95% CI for aOR	Standard Error	Wald χ^2	p-value
Stigma (High vs. Low)	0.45	0.32 – 0.63	0.08	24.7	<0.001***
Financial Constraints (Yes/No)	0.50	0.36 – 0.70	0.09	18.3	<0.001***
Lack of Awareness (Yes/No)	0.60	0.43 – 0.83	0.10	9.8	0.002**
Rural Residence (vs. Urban)	0.55	0.40 – 0.76	0.09	14.2	<0.001***
Limited Facility Access (Yes/No)	0.65	0.47 – 0.90	0.11	6.6	0.010*
Age (per 5-year increase)	0.95	0.88 – 1.02	0.04	2.1	0.150
Education (Tertiary vs. None)	1.30	0.92 – 1.85	0.12	2.8	0.090

Note: Significance levels:
*$p < 0.05$ = **, $p < 0.01$ = ***, $p < 0.001$.

Key Statistics:
Women experiencing high stigma had 55% lower odds of seeking formal mental healthcare (aOR = 0.45, 95% CI: 0.32–0.63).
Those with financial constraints were 50% less likely to access services (aOR = 0.50).
Rural residence was associated with a 45% reduction in odds of utilization (aOR = 0.55).
Lack of awareness and limited access to facilities were also significant predictors.
Age and education did not show statistically significant effects in this model.

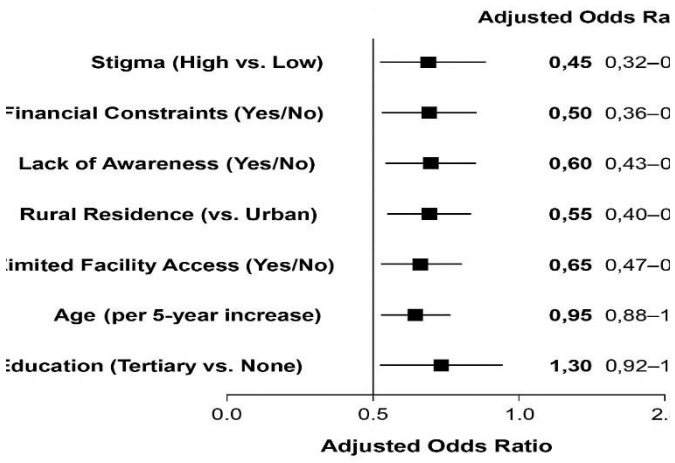

Figure 2: Adjusted Odds Ratios (aORs) for Predictors of Formal Mental Health Service Utilization Bars represent aORs with 95% confidence intervals. Predictors with aORs < 1.0 and confidence intervals not crossing 1.0 are statistically significant.

DISCUSSION

The study's revelation that 68% of women with mood disorders did not seek formal care underscores a critical public health challenge in Ghana. Stigma emerged as the most pervasive barrier, affecting 45% of participants. This finding aligns with [2] work on cultural perceptions of mental illness, but our mixed-methods approach provides deeper insight. The qualitative data revealed that stigma manifests through multiple pathways, including fear of social exclusion and spiritual attribution of symptoms. Women frequently described being labelled as "cursed" or "spiritually attacked", leading them to seek help from religious leaders rather than medical professionals. The regression analysis (aOR=0.45) statistically confirmed stigma's strong deterrent effect, showing it reduces care-seeking odds by 55%. This persistent stigma reflects deeply entrenched cultural beliefs that require targeted, community-specific interventions [14, 15]. The regression analysis revealed that stigma was the strongest barrier to accessing care. Specifically, women who reported high stigma had 55% lower odds of seeking formal mental healthcare. Similarly, women living in rural areas were 45% less likely to access services compared to those in urban areas. Financial barriers and lack of awareness also significantly reduced the likelihood of service utilization. These findings highlight that both social perceptions and physical access issues are major deterrents. The data underscore the need for public health interventions that are culturally sensitive and geographically targeted.
Financial barriers were reported by 40% of participants as the second most significant barrier. However, the burden of cost was much higher among rural women. Of them, 65% cited treatment cost as a financial burden compared to 35% of urban women. This gap mirrors the income disparity between regions noted by [3, 4]. Beyond treatment cost, the economic constraints were greater, as 48% of rural women reported losing several days of wages for travel to urban clinics. This presented an untenable dilemma for women who earned a daily wage and had to care for their family. The regression analysis (aOR=0.50) underscored that financial constraints substantially reduce the likelihood of service utilization, demonstrating once again the entrenched relationship between economic hardship and mental healthcare access in Ghana [16, 17].
About 35% of respondents were unaware of mental health services, with rural women being the most affected. The majority of the respondents, especially those who had not received formal schooling, typified depression as either a physical illness or simply life stress. Many women could not identify symptoms of mood disorders as needing specialised care. According to [14], poor mental health literacy significantly hinders service uptake. In our study, 62% of rural women with no formal education did not name any available mental health service—a severe gap in functional awareness. Moreover, a common misconception was that seeking mental healthcare means one must be hospitalized, deterring many from initiating care. Such knowledge gaps represent

a significant barrier in the early help-seeking phase, where identification of the problem is crucial [5, 18].

The research uncovered striking gaps in the availability of mental health services, with significant understaffing in rural regions. A clinic could be reached within 1 hour by 82% of urban women, whereas 74% of rural respondents were likely to face three or more hour-long trips. This is consistent with Bindt et al.'s findings [1], which point to geographic inaccessibility as a key barrier. This geographical disparity is worsened by the marked difference in the distribution of mental health practitioners, with urban areas possessing seven times more psychiatrists. It is noted that the lack of care in rural regions, as documented [1, 10], significantly hampers care-seeking odds, which in our study was found to be 35% (aOR=0.65). Even after overcoming the stigma and financial constraints, women's access to care is severely limited by the physical locations of services.

The investigation of the rural and urban population juxtaposition provided unique yet equally important insights. Women in rural areas appeared to suffer from multiple disadvantages: 58% reported family opposition to seeking mental healthcare as opposed to only 22% of urban women. This cultural barrier, together with acute resource deprivation (available, local mental health services were accessible to only 12% of rural women), creates an unforgiving landscape for help-seeking. Financial limitations, as well as employer-induced stigma, impacted even the more accessible urban services. These results are consistent with [15, 19] concerning the interplay between rural poverty, urban stressors, and the population's mental health. Regression analysis showed that rural residence, on the other hand, is associated with a 45% reduction in the odds of seeking care (aOR=0.55) relative to other factors.

To close this gap, multi-level policy interventions are needed. First, public education campaigns must be tailored to local realities. In Ghana, radio broadcasts remain a powerful tool, especially in rural areas with low literacy. Integrating mental health content into religious sermons, given the influential role of faith leaders, may help shift harmful narratives about mental illness. Community health workers (CHWs), already trusted by local populations, should be trained to deliver mental health literacy programs and early screening, especially for women in remote settings.

There are successful models in comparable contexts. For instance, Nigeria's Mental Health Leadership and Advocacy Program uses faith-based and community gatekeepers to reduce stigma, while Kenya's Friendship Bench model, adapted from Zimbabwe, demonstrates the effectiveness of community-based counselling using lay health workers. Ghana's policies can draw on these examples to craft culturally aligned, community-driven education and support systems [20, 21, 22].

Integrating basic mental health services into primary care would also address the challenge of access, especially in rural areas. This includes training general health workers in early detection, counselling, and appropriate referral systems. Partnerships with NGOs and regional mental health authorities can support the scalability and sustainability of these interventions.

Study Limitations

While this study provides valuable insights, several limitations should be acknowledged. First, the cross-sectional design limits the ability to infer causality between the barriers identified and mental health service utilization. Longitudinal data would provide a more dynamic understanding of how these barriers evolve over time. Second, reliance on self-reported data introduces the possibility of recall or social desirability bias, especially when discussing stigmatized issues such as mental health. Third, although the use of English-only tools was justified based on participant literacy, it may have excluded women who are less confident in reading or writing, potentially under-representing more marginalized voices.

Despite these limitations, the study provides strong evidence for targeted, context-sensitive interventions and fills an important gap in understanding the gendered dimensions of mental health access in Ghana.

CONCLUSION

The multi-dimensional barriers that collectively restrict access to mental healthcare for women in Ghana are interwoven and feature prominently in the study's results. Stigma, as the most powerful deterrent, is compounded by financial and spatial

considerations. The urban-rural divide creates distinct challenges requiring tailored approaches to each region. Together, these factors create an exacerbating treatment gap for mood disorders for Ghanaian women. Addressing these barriers requires culturally responsive frameworks that acknowledge and challenge harmful socio-cultural beliefs while bridging systemic and economic gaps. Holistically, these barriers require context-driven remedies to be incorporated into future structural adjustments to address the deeply entrenched socio-cultural barriers impeding mental healthcare.

Conflict of Interest

The author declare that no conflict of interest.

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OPEN ACCESS Original Article

Cytomegalovirus among Aborted Women in Abyan Governorate -Yemen

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ABSTRACT

Background: The cytomegalovirus can cause potentially life-threatening complications in infected pregnant women and fetuses.
Objective: This study was aimed to determine the seroprevalence of IgG and IgM against CMV among aborted women in Abyan Governorate, Yemen, and to determine the number and trimesters of abortions as well as other related risk factors that may contribute to transmission of the virus among those women.
Methods: A total of 105 women were enrolled in this analytical cross-sectional study. The blood samples were collected from all women, and the sera were separated; then the anti-CMV IgG and IgM were determined using commercially available ECLIA techniques. The data was analyzed using SPSS.
Results: The overall seroprevalence of IgG against CMV infections was 104 (99.0%) and 4 (3.8%), respectively. The highest rates of IgG seropositivity among aborted women were 41 (100%) and 25 (100%) in the age groups 26–35 years and >35 years, respectively; 19 (100%) and 41 (100%) among illiterate women and those who had secondary school education, respectively; 25 (100%) among women living in urban areas; 50 (100%) and 3 (100%) among women who had medium and high socioeconomic status, respectively; and 12 (100%) among women who had private work. In contrast, the rates of IgM antibodies were high: 2 (5.1%) among women in the age groups <25 years, 5.3% among illiterate women, 4% among women who were living in urban areas, 5.2% among women with low income, and 4.3% among housewives. The positive anti-HCMV IgG and IgM had no significant association with number, trimester, symptoms, and other related factors.
Conclusion: The seroprevalence of IgG antibodies of CMV infections among aborted women in Abyan. Women living in urban areas had the highest rates of both anti-HCMV IgG and IgM antibodies. Seroprevalence of anti-HCMV IgG and IgM were different according to the sociodemographic status of women. There was no significant association between the seroprevalence of anti-HCMV IgG and IgM and the number and time of abortion, symptoms, and other risk factors.

Keywords: Seroprevalence, cytomegalovirus, aborted women, Abyan, Yemen

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INTRODUCTION

Cytomegalovirus (CMV) is one of the largest members of the herpesvirus family. CMV can cause potentially life-threatening complications in infected pregnant women and fetuses (1). It is the most common cause of congenital abnormalities among fetuses, resulting in disorders that cause the loss of hearing, blindness, and mental retardation (2). Although CMV infections occur worldwide, the majority of infections occur in developing countries (3).

Infections of CMV are based on the immunity of infected individuals, where the immunocompetent individuals exhibit subclinical asymptomatic infections, while severe fatal infections occur among immunocompromised and immunodeficient patients (4,5). One of the unique properties of herpesviruses, including CMV, is a prolonged period of persistence (latency). The virus remains dormant inside the infected host cells for a life without a replicative cycle (6). Reactivation is the process in which the virus is activated and replicated again (7). This occurs commonly in immunocompromised individuals, including acquired immunodeficiency syndrome (AIDS) patients, who are characterized by severe CMV complications (8,9).

The transmission of the virus may occur transplacentally (fetus), perinatally (newborns), which occurs in the birth canal and during breastfeeding (10,11), contact with contaminated saliva, especially by kissing, and also contact with urine (young children) (12). In adults, the virus is commonly transmitted by sexual routes and during repeated blood transfusions and organ transplants, especially kidney and bone marrow transplants (7). In Yemen, the seropositive immunoglobulins (Igs), IgG against CMV, among Yemeni blood donors was 96.6% (13), among pregnant women ranging from 68% to 100% (14), and among aborted women 98.67% and 77.6% (1,15). The data about the seroprevalence of CMV infection among aborted women in Abyan Governorate was not clear. Therefore, the current study aimed to determine the seroprevalence of IgG and IgM against CMV among aborted women in Abyan Governorate, Yemen, and to determine the number and trimesters of abortions as well as other related risk factors that may contribute to transmission of the virus among those women.

METHODS

A total of 105 women who had abortions were accepted to participate in this analytical cross-sectional study. The women were attending maternity units in different medical centers and hospitals in Abyan, Yemen. A predesigned questionnaire with some modifications was used to collect the data. The data includes sociodemographics such as age, education level, residence, income, and occupation. Other data about the number and trimesters of abortion, symptoms, and other related factors such as blood transfusion and use of corticosteroid drugs (1,15).

The blood samples were collected from all women, and the sera were separated after clotting of blood using a centrifuge. The anti-CMV IgG and IgM were determined using the Cobas technique. This technique was commercially available and based on electrochemiluminescence immunoassay (ECLIA).

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS®) software (Version 21). The ages of aborted women were normally distributed, so the mean values and standard deviations (SD) were determined, while most data was qualitative; therefore, the number and percentages of variables were calculated. The chi-square (χ^2) test was used to determine the presence of significant association between different variables. A p-value of <0.05 was considered statistically significant.

Ethics Approval and Consent Form

Ethical approval from the Ethics Committee of the College of Medicine and Health Science at the University of Science and Technology; MEC No. (MEC/AD089). The written consent form was obtained from each woman before performing any procedure.

RESULTS

One hundred and five aborted women were enrolled in current study, the mean± SD of age was 29.31 ±7.43 years. The age range was between 13 years and 45 years (Table 1). The overall seroprevalence of IgG against HCMV infections was 104 (99.0%) and 4(3.8%), respectively (Figure 1). The highest rates of IgG seropositive among aborted women according to

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sociodemographic characteristics were 41 (100%) and 25(100%) in the age groups 26 – 35 years and >35 years, respectively, 19(100%) and 41(100%) among illiterate women and those had secondary school education, respectively, 25(100%) among women living urban areas, 50(100%) and 3(100%) among women who had medium and high socioeconomic status, respectively and 12(100%) among women who had private work. In contrast, the rates of IgM antibodies were high 2(5.1%) among women in the age groups <25 years, 5.3% among illiterate women, 4% among women who were living in urban areas, 5.2% among women with low income and 4.3% among housewives (Figures 2-6).

The highest prevalence of anti-HCMV IgG was 31(100%),18(100%), and 12(100%) among women who aborted two, three, and more times, respectively while the IgM was high at 6.5% among women who aborted two times. The highest seroprevalence of IgG was 76 (100%) and 17(100%) among women who aborted in the first and second trimesters of pregnancy while the IgM was high among women who were exposed to abortion in the second trimester. Both anti-HCMV IgG and IgM antibodies were 61(100%) and 3(4.9%), respectively among women who were exposed to recurrent abortions. Regarding the symptoms and related risk factors, all women who had previously been exposed to blood transfusion used corticosteroid drugs and had a skin rash and lymphadenopathy symptoms were positive for IgG antibodies against HCMV while IgM were 2.6%, 4.5%, 0%, and 10% among women who exposed to blood transfusion, used corticosteroid drug and had skin rash and lymphadenopathy, respectively (Table 2). The positive anti-HCMV IgG and IgM had no significant association of time, number, trimester, and other related factors.

Table 1: The sociodemographic characteristics of 105 aborted women in Abyan-Yemen.

Variable	Number	Parentage %	Variable	Number	Parentage %
Age (years)			**Socioeconomic status**		
≤25	39	37.1	Low	47	44.8
26-35	41	39.0	Medium	55	52.4
>35	25	23.8	High	3	2.9
Education level			**Occupation**		
Illustrate	19	18.1	Housewife	93	88.6
Primary school	45	42.9	Private work	12	11.4
Secondary school	41	39.0			
Residence					
Rural	80	76.2			
Urban	25	23.8			

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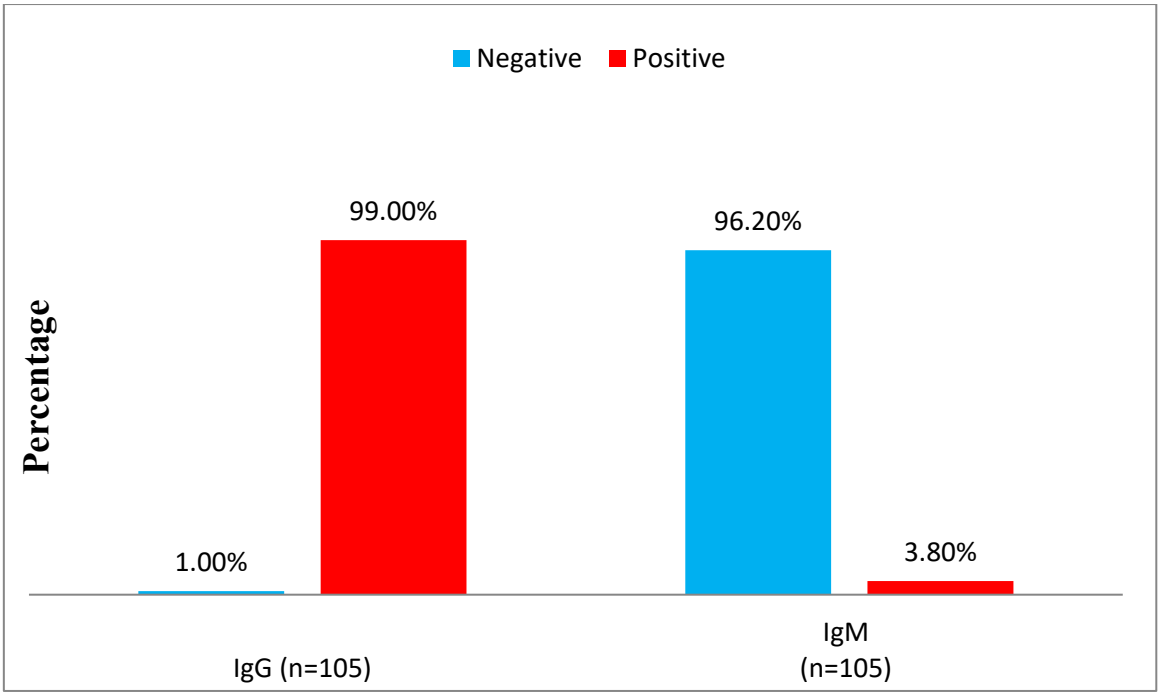

Figure 1: Seroprevalence of HCMV IgG and IgM antibodies among aborted women in Abyan –Yemen

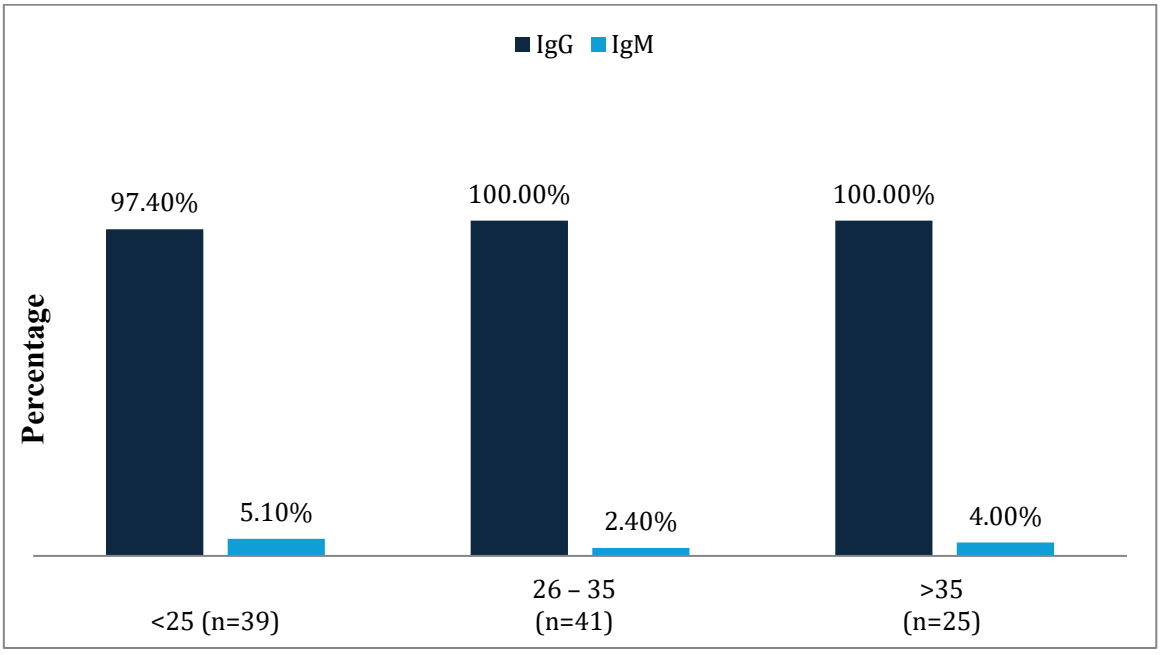

Figure 2: The seroprevalence of anti-HCMV IgG and IgM among aborted women related to their age groups in Abyan, Yemen.

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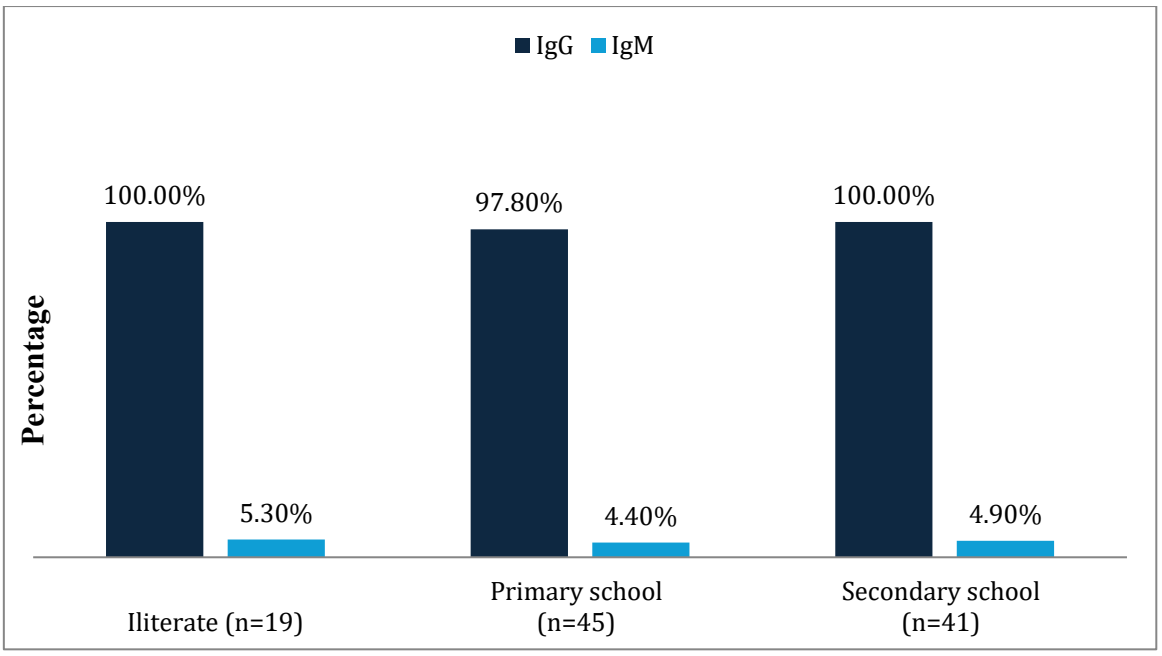

Figure 3: The seroprevalence of anti-HCMV IgG and anti-HCMV IgM among aborted women according to their educational levels in Abyan, Yemen.

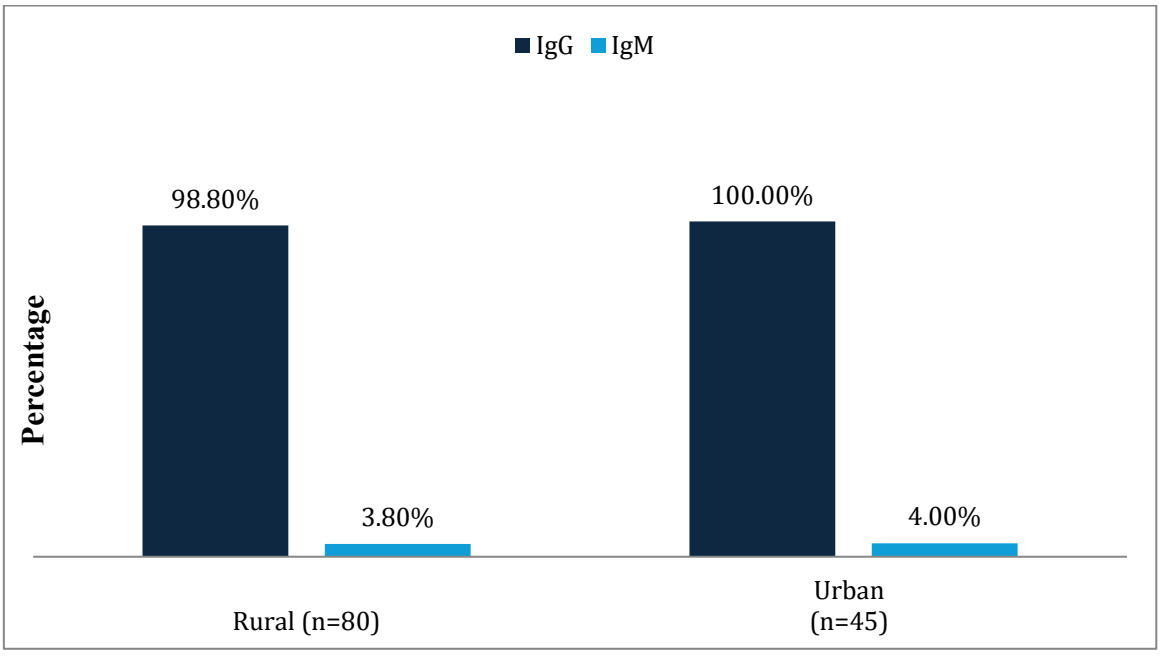

Figure 4: The seroprevalence of anti-HCMV IgG and anti-HCMV IgM among aborted women according to their residence in Abyan, Yemen.

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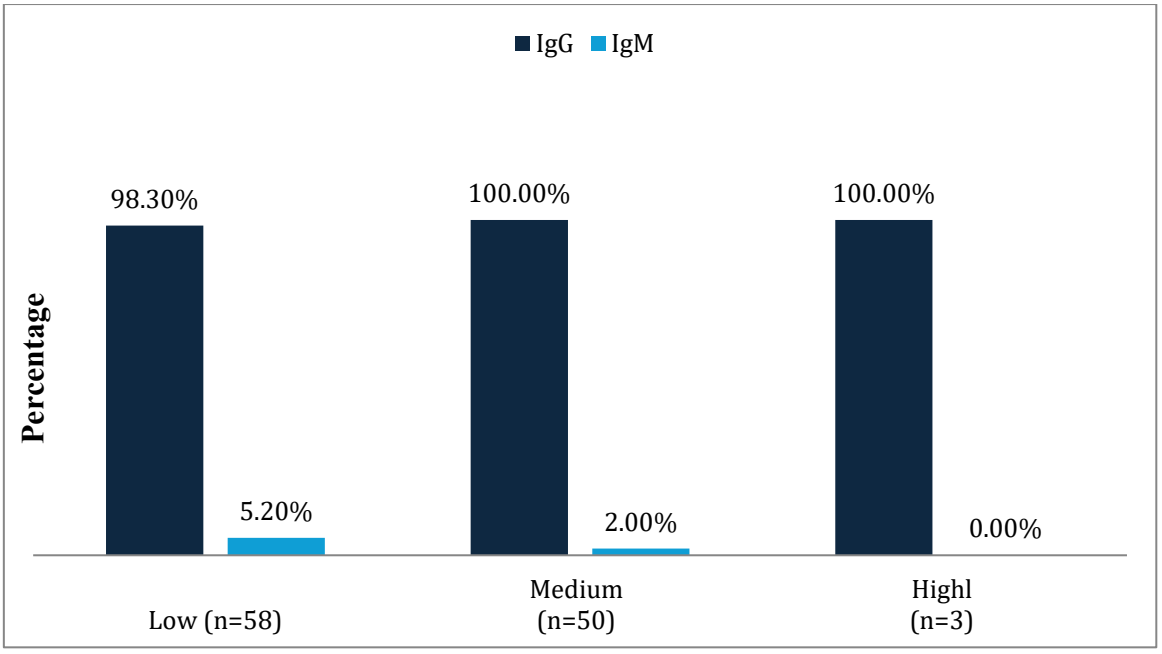

Figure 5: The seroprevalence of anti-HCMV IgG and anti-HCMV IgM among aborted women according to their socioeconomic status in Abyan, Yemen.

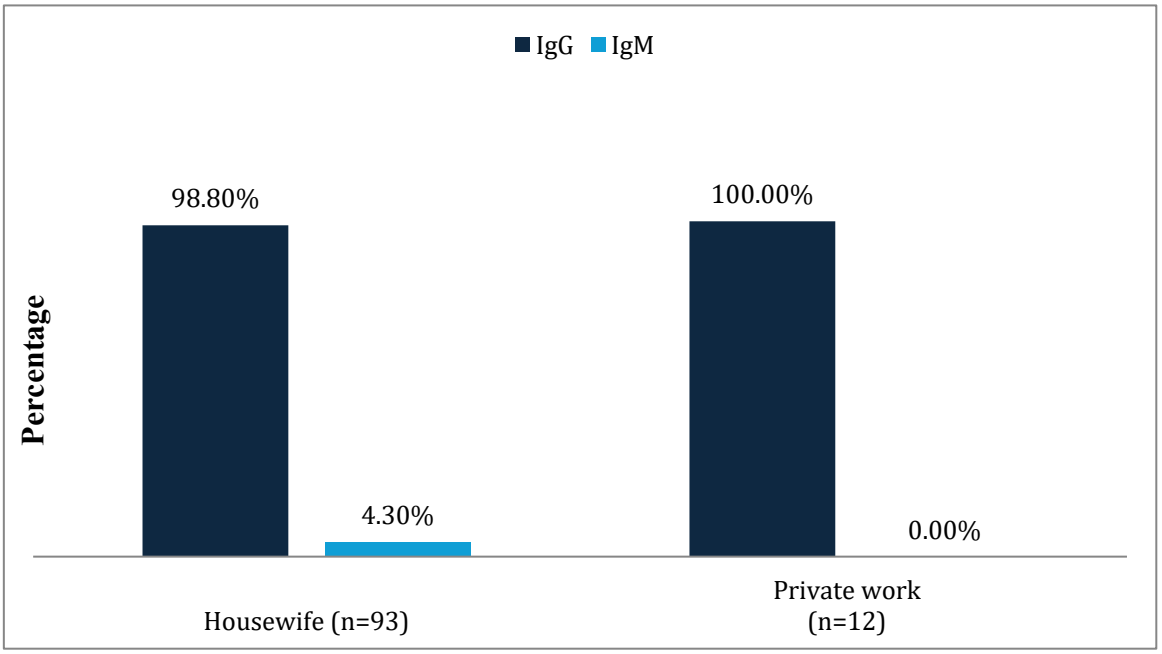

Figure 6: The seroprevalence of anti-HCMV IgG and anti-HCMV IgM among aborted women according to their occupation in Abyan, Yemen.

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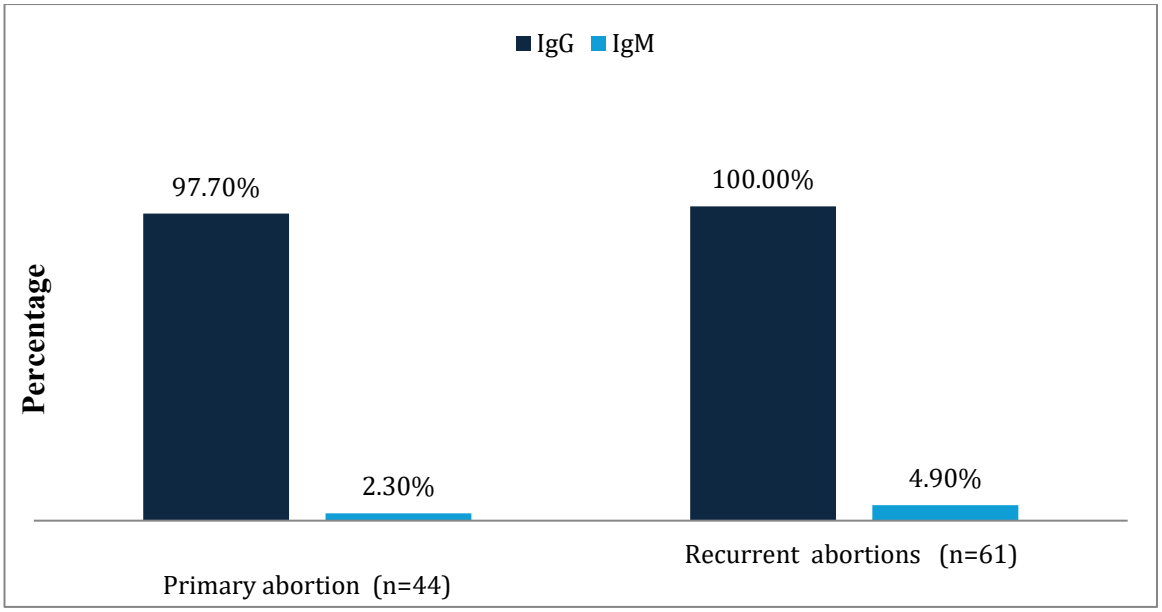

Figure 7: The seroprevalence of anti-HCMV IgG and IgM among primary and recurrent aborted women in Abyan, Yemen.

Table 2: The seroprevalence of anti-HCMV IgG and IgM among aborted women according to number and time of abortion, symptoms, and other related factors in Abyan, Yemen.

Category	IgG positive		P-value	IgM positive		P-value
	No.	%		No.	%	
Number Abortion						
Twice (n=31)	31	100.0	0.705	2	6.5	0.339
Third (n=18)	18	100.0	0.829	1	5.6	0.534
More than 3 (n=12)	12	100.0	0.868	0	0.0	---
Trimesters						
1st trimester (n=76)	76	100.0	0.276	2	2.6	0.305
2nd trimester (n=17)	17	100.0	0.838	2	11.8	0.122
3rd trimester (n=12)	11	91.7	0.114	0	0.0	--
Symptoms						
Skin rash (n=17)	17	100.0	0.565	0	0.0	___
Lymphadenopathy (n=10)	10	100.0		1	10.0	0.358
Risk factors						
Blood transfusion (n=38)	38	100.0	0.638	1	2.6	0.541
Use corticosteroid (n=44)	44	100.0	0.581	2	4.5	0.560

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DISCUSSION

The most fatal complications of CMV infection occur among women and their fetuses (1). In the current study, the seroprevalence of anti-HCMV IgG among aborted women was 104 (99%). A slightly similar result was 98.7%, found in Yemen (15). Two studies from Sudan and Iraq reported that 97.8% and 96% of aborted women had IgG seropositive, respectively (16,17). Our result was higher than different studies conducted globally, such as 92.50% in India (18), 88.9% in Sudan (19), 89% and 85% in Iraq (20,21), and 77.6% in Yemen (1). Elbushra et al. noticed that the seroprevalence of IgG was 74.8% (22). Our IgG seroprevalence was one of the highest rates among aborted women. It also was higher than that reported among the general Yemeni population, which was 96.6%, and among blood donors, 68% (13).

The seropositive rate of IgM among aborted women in this study was 4 (3.8%). A report from Sudan demonstrated that 2.2% of women who were exposed to abortion had seropositive IgM antibodies against CMV infection (16). Abbas et al. showed that 7% of aborted women were positive for anti-HCMV IgM (15). The zero percent was reported in Sudan (19). Different studies also noticed higher rates than ours, such as 7%, 10%, 13.3%, and 15.7% from Iraq and Sudan (17,20,22,23) and 20% from India (18). A study from Yemen revealed that the prevalence of anti-HCMV IgM was 83.3% (1). One of the higher results was recorded among Iraqi women who had abortions, which was 93% (21). Alghalibi et al. found that the anti-HCMV IgM among pregnant women was 1.8% (14). The variations in results may be attributed to poor socioeconomic status and poor hygiene practices and the increased HCMV prevalence in developing countries compared to developed ones (15), as well as the sensitivity of diagnostic techniques used to test the HCMV infection (24).

In the current data, the aborted women with the age groups 26–35 years and >35 years had the highest rates of anti-HCMV IgG antibodies, which were 41 (100%) and 25 (100%), respectively. Research from Iraq and Libya revealed that aborted women in the age groups 21–30 years had the highest rate of IgG antibodies (25,26). Gubran reported that 81% of women who had abortions in the age groups 30-34 years had positive anti-HCMV IgG antibodies (1). Hussein et al. showed the highest anti-HCMV IgG rate among women in the age groups 36-40 years (20). A study undertaken in Yemen reported that aborted women in the age group 26-50 years had the highest rates (15). Regarding anti-HCMV IgM antibodies in our study, the prevalence was high (2 [5.1%]) among women in the age groups < 25 years. A similar result was recorded in Yemen (15). Different studies revealed different rates among different age groups, Mohammed, such as the 19-45-year-old, 20-29-year-old, and 21-30-year-old age groups in Iraq and Libya (20, 25, 26, 27) and the 25-29-year-old age group in Yemen (1). Older women interact and come into contact with more risk factors (28). The breastfeeding transmission, latency properties of the virus, and wide practice of breastfeeding females during infancy may contribute to the seroprevalence of CMV among young females (29).

According to the level of education, this result observed a high percentage of 19 (100%) and 41 (100%) of IgG antibodies among those who were illiterate and those who had secondary school education, respectively. Gubran showed that illiterate women had the highest IgG rate (1). Abbas et al. found that illiterate women and those women who had university education levels had the highest rate (15). In contrast, illiterate women had the highest 5.3% IgM rate in this study. This was in agreement with two studies from Yemen (1,15). There are several factors contributing to increasing HCMV infection among the studied population, such as poorer socioeconomic status, education, and hygienic practices (29,30,31).

The results in this study demonstrated that women living in urban areas had the highest rates of both anti-HCMV IgG and IgM antibodies (100% and 4%), respectively, compared to those living in rural areas. Abbas et al. revealed the opposite finding (15).

The current data revealed that the women who had medium and high socioeconomic status had the highest rates of anti-HCMV IgG antibodies, 50 (100%) and 3 (100%), respectively. Abbas et al. noticed that the highest IgG antibodies were among women who had low and medium socioeconomic status (15). In contrast, those women with low socioeconomic status had high anti-HCMV IgM antibodies (5.2%). A similar result was reported in Yemen (15).

As regards the occupation of aborted women, those women who had private work had the highest rate of 12 (100%) of anti-HCMV IgG, while the housewives had the highest anti-HCMV IgM rate of 4.3%. No

literature agreed or disagreed with our finding, so this was considered the first finding.

The number of abortions: those women who aborted two times or more had the highest rate of anti-HCMV IgG. Gibran revealed that women who aborted three times had high anti-HCMV IgG antibodies (1). Ibrahim et al. recorded that women who aborted for the first time had the highest IgG rate (19). Regarding anti-HCMV IgM, the women who aborted two times had the highest rate, 6.5%. This result was in agreement with that recorded in Sudan (19). Gubran found different results (1).

Related to the trimester of abortion, the women who aborted in the first and second trimesters had the highest IgG antibodies. A report from Yemen demonstrated that women who aborted in the second and third trimesters had the highest IgG rate (15). Ibrahim et al. noticed that women who aborted in the second trimester had the highest IgG rate (19). Another study was also done among Yemeni women who aborted, revealing the highest IgG antibodies among women who aborted in the first trimester (1). In the current study, the prevalence of anti-HCMV IgM was high among those women who aborted in the second trimester. Abbas et al. identified that those women who aborted in the third trimester had the highest anti-HCMV IgM rate (15), while a report from Sudan showed the highest rate among those who aborted in the first trimester (19). The differences could be the genetic and immunological status of women and their fetuses as well as the time of infections of the mother and fetus (32).

According to the symptoms and risk factors in the present data, all aborted women (100%) who had skin rash and lymphadenopathy symptoms had IgG antibodies, whereas no positive cases and 10% of them had IgM antibodies, respectively. Abbas et al. found that 40% and 21.33% of aborted women who had skin rash and lymphadenopathy symptoms were positive for CMV IgG antibodies, and 5% and 6.5% had IgM antibodies, respectively (14). All women who were exposed to blood transfusion 100% also had IgG antibodies against HCMV. Gubran showed that IgG antibodies are found in 75% of women exposed to blood transfusion (1). The present study identified that all women who used corticosteroid drugs had 100% IgG antibodies against HCMV infection. Gubran reported that IgG antibodies are found in 75% of women who used corticosteroid drugs (1). The small size of the studied group and the lack of molecular techniques, which are more accurate, such as polymerase chain reaction (PCR), are considered two of the important limitations of this study.

CONCLUSION

The seroprevalence of IgG antibodies of CMV infections among aborted women in Abyan, Yemen, was higher than those reported globally, whereas the seroprevalence of IgM antibodies was lower than most previous studies. Women living in urban areas had the highest rates of both anti-HCMV IgG and IgM antibodies. Seroprevalence of anti-HCMV IgG and IgM were different according to the sociodemographic status of women. There was no significant association between the seroprevalence of anti-HCMV IgG and IgM and the number and time of abortion, symptoms, and other risk factors.

Acknowledgments

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Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS Original Article

Assessment of Nutrition Status for Sudanese Children (6-59 months) Internally Displaced Persons Camps in Kosti, White Nile State, Sudan

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ABSTRACT

Background: Malnutrition among displaced children remains a major public health issue, especially due to the heightened vulnerability of this age group.
Objective: This study aimed to assess the nutritional status of children aged 6–59 months in two displaced camps, Alalgaia and Dababt-Bosin, in East Kosti, White Nile State, Sudan.
Methods: This cross-sectional study and data were collected between December 2023 and January 2024. Data were collected from 110 children and their mothers, using questionnaires that focused on socio-demographic, health, and dietary aspects. The data were analyzed using SPSS Version 22.0.
Results: Most mothers (40%) were aged between 18 and 24 years, and 52.7% were illiterate. Approximately 66.1% of the mothers had between 1 and 5 children under the age of five. The majority of fathers (62.7%) were over 30 years old. Nutritional assessments using MUAC showed that 58.2% of children were normal, while 28.2% had moderate acute malnutrition (MAM) and 13.6% had severe acute malnutrition (SAM). Based on the prevalence of Z-scores, 42.7% of children were wasted, 37.3% underweight, and 28.2% stunted. Severe forms included 18.2% severely underweight, 14.5% severely wasted, and 10.9% severely stunted, while overweight and obesity were minimal (1.8%). Using BMI-for-age, 54.5% were within normal range, 37.3% had MAM, 6.4% had SAM, and 1.8% were overweight and obese. No significant relationships were found between age of children and MUAC status ($p = 0.185$) or age group and weight-for-age ($p = 0.185$). Dietary patterns showed poor meal frequency and limited consumption of fruits, milk, and protein-rich foods, with a reliance on staple items like Kisra, Miakilo, and Acida.
Conclusion: The high prevalence of malnutrition highlights the urgent need for targeted nutritional interventions and healthcare services to improve the well-being of displaced children in these camps.

Keywords: Malnutrition, nutritional assessments, displaced camps, child nutrition, Sudan.

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INTRODUCTION

Malnutrition among children under five is a persistent and growing public health emergency in Sudan, especially among internally displaced populations. The displacement caused by the ongoing conflict has severely disrupted access to nutritious food, healthcare, and clean water, particularly in camps where families rely heavily on humanitarian aid. The current crisis has resulted in a high prevalence of undernutrition, with 31% of Sudanese children underweight and over 2.2 million stunted (1). Displaced children are more vulnerable to diseases, including cholera, and suffer from long-term physical and cognitive impairments due to poor nutrition. Despite efforts by global agencies, logistical constraints, insecurity, and funding gaps hinder effective intervention (2). Therefore, assessing the nutritional status and dietary intake of displaced children is critical to inform targeted policies and emergency response strategies aimed at reducing child malnutrition and improving overall health outcomes.

Malnutrition remains one of the most critical global public health challenges, especially in developing countries. It significantly affects children under five, with an estimated 45% of all deaths in this age group attributed to undernutrition (3). The World Health Organization (WHO) identifies malnutrition as the leading threat to public health globally, particularly in low-income and conflict-affected regions (4). Malnutrition encompasses deficiencies, excesses, or imbalances in energy or nutrient intake and poor nutrient utilization by the body (5). It is a major cause of illness, death, and long-term physical and cognitive impairment in children, especially in displaced populations.

Assessing the nutritional status of children is essential for identifying growth problems, malnutrition, and related health risks. This is achieved through comprehensive evaluation of anthropometric, dietary, clinical, biochemical, and socioeconomic indicators (6). Globally, in 2014, the prevalence of stunting (height-for-age), wasting (weight-for-height), and underweight (weight-for-age) among children under five was 24.7%, 7.8%, and 15.1%, respectively (7). Despite progress, malnutrition remains most prevalent in developing nations (8), where children face systemic barriers to adequate nutrition, healthcare, and sanitation.

Children displaced by conflict face heightened vulnerability due to food insecurity, inadequate health services, and poor living conditions. Displacement increases dependence on humanitarian aid, where food quality and quantity directly impact nutritional status (9). In Sudan, around 31% of children under five are moderately or severely underweight, and over 2.2 million are stunted—more than one in three children (10). This reflects a long-standing crisis of undernutrition, worsened by poor dietary diversity, insufficient health infrastructure, and limited access to essential services. The majority of internally displaced people live in appalling humanitarian conditions with little access to essential services. Children make up over half of those relocated, with 27% of them being younger than five. According to the research, girls under the age of 18 make up about 28% of the IDP population (11).

Since the outbreak of conflict in April 2023, Sudan has witnessed an unprecedented displacement crisis. Over 13 million people have been displaced, with more than 8 million newly displaced internally (12). An estimated 11.3 million internally displaced persons (IDPs) now live across all 18 states in Sudan, especially in Khartoum, Darfur, and Kordofan (13). White Nile State alone received over 50,000 IDPs, many of whom live in overcrowded camps with limited access to basic services (14). In total, more than 24.8 million people, including nearly 14 million children, are in need of humanitarian assistance (15). Sudan is currently facing the largest child displacement crisis in the world. Nearly five million children have fled their homes, including one million who have crossed into neighboring countries such as South Sudan, Egypt, and Chad. These children face violence, trauma, and deprivation, all of which heighten the risk of acute and chronic malnutrition. The ongoing conflict has destroyed vital infrastructure and severely disrupted food systems, leading to intergenerational consequences for child health and development (16,17).

METHODS

A cross-sectional study was conducted in two displaced persons' camps, namely Alalgaia and Dababt-Bosin. These camps are located in East Kosti, White Nile State, Sudan, and were established on June 15, 2022, to accommodate Sudanese individuals

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displaced by the outbreak of war in western Sudan. The source population consisted of all children aged 6–59 months living in the displaced persons' camps who visited the health centers during the study period.

All children aged 6–59 months and their caregivers of both genders who were present at the health centers during the study period were included. A total of 110 mother–child pairs participated in the study. A pre-tested structured interview questionnaire, consisting of closed-ended questions, was used to collect background information about the respondents, demographic data, health data, anthropometric measurements (height, weight, and mid-upper arm circumference [MUAC]), and dietary intake.

To assess dietary patterns, a food frequency questionnaire (FFQ) was administered to caregivers. The FFQ aimed to evaluate overall dietary quality rather than precise nutrient intake. Food items were categorized into eight major groups: cereals, animal products, dairy products, vegetables, fruits, wild fruits, legumes, and sweets. Respondents reported consumption frequency using five categories: 1–3 times per week, 4–6 times per week, monthly, rarely, and never. This method is commonly used to assess food diversity and frequency (18).

Study Variables and Measurements

Weight was measured using a Salter hanging spring scale with a 100 g graduation and a capacity of 26 kg. Children were weighed with minimal clothing and without shoes, and measurements were recorded to the nearest 0.1 kg. Scales were calibrated regularly using known weights, and readings were checked to ensure the indicator returned to zero before each use. Each child was weighed twice for accuracy.

Recumbent length was measured for children under two years of age, while standing height was measured for those aged two years and above, recorded in centimeters to the nearest 0.1 cm. Nutritional status was assessed using three anthropometric indices: weight-for-age, weight-for-height, and height-for-age, based on World Health Organization (WHO) child growth standards. Z-scores were used to classify nutritional status as normal (-2 to +2 SD), moderate acute malnutrition (MAM: -2 to -3 SD), and severe acute malnutrition (SAM: < -3 SD) (19).

MUAC was measured on the left arm, at the midpoint between the acromion (shoulder tip) and olecranon (elbow tip). The upper arm was fully exposed, and a non-stretchable MUAC tape was used to take the measurement snugly but without compressing the soft tissue. MUAC was recorded to the nearest 0.1 cm following standard anthropometric procedures (20).

Body Mass Index (BMI)-for-Age

BMI-for-age was calculated by dividing weight in kilograms by the square of height in meters (kg/m^2), and results were interpreted using WHO growth charts. Classification was as follows: < -3 SD (severe acute malnutrition), -2 to -3 SD (moderate acute malnutrition), -1 to 1 SD (normal), 1 to 2 SD (overweight), 2 to 3 SD (obese), and > 3 SD (extremely obese) (21).

Ethical Considerations

Ethical approval was obtained from the Standing Committee of University of Science and Technology, Aden, Yemen (MEC No. MEC/AD080). All mothers were fully informed about the study's purpose and procedures. Participation was voluntary, and caregivers were assured of their right to withdraw at any time without penalty. Confidentiality and anonymity of participants were strictly maintained throughout the study.

RESULTS

This study emphasizes the nutritional condition and prevalence of malnutrition and looks at the demographic and socioeconomic characteristics of children (6–59 months) and their families. A total of 110 respondents at the period of study provided information on a number of variables, including family size, parental education, age, gender, and access to critical nutrition and health services.

Demographic and Socioeconomic Data of the Respondents

Among the 110 children surveyed, most were 6-19 months old (56.4%), with smaller proportions aged 20-33 months (20.9%), 34-47 months (14.5%), and 48-59 months (8.2%); girls slightly outnumbered boys (56.4%) compared with boys (43.6%). Mothers were predominantly young, with 40% aged 18-24 years, 33.6% aged 25-30 years, 22.7% over 30 years, and only 3.6% under 18 years, whereas fathers were generally older, with 62.7% being over 30 years, 27.3% aged 25-30 years, and 10% aged 18-24 years.

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Educational attainment was low; over half of mothers were illiterate (52.7%) and only 0.9 % had university degrees, while 32.7% of fathers were illiterate and 1.8 % had university education, though nearly half (49.1 %) had completed basic school. Most households were large; 65.5% comprised 6-10 members and 14.5% had 11-15 members, leaving 14.5% with 2-5 members and 5.5% with more than 15. Regarding the number of children in the families, about two-thirds (66.1%) had 1 to 5 children, while one-third (33.9%) had 6 to 10. When looking at birth order, 44.5% of the children surveyed were fourth-born or later, 20.9% were first-born, another 20.9% were second-born, and 13.6% were third-born.

Table 1: Demographic and Socioeconomic Data (n=110)

Variables	Frequency	Percentage
Age of children/month		
6-19	62	56.4
20-32	23	20.9
33-47	16	14.5
48-59	9	8.2
Gender of children		
Male	48	43.6
Female	62	56.4
Age of Mothers/ years		
<18 years	4	3.6
18-24 years	44	40.0
25-31 years	37	33.6
>31 years	25	22.7
Age of fathers/ years		
18-24 years	11	10.0
25-31 years	30	27.3
>31 years	69	62.7
Mother's education		
Illiterate	58	52.7
Basic school	42	38.2
Secondary school	9	8.2
University	1	0.9
Father's education		
Illiterate	36	32.7
Basic school	54	49.1
Secondary school	18	16.4
University	2	1.8
Family size		
2-5	16	14.5
6-10	72	65.5
11-15	16	14.5
>15	6	5.5
Number of children in the family		
1-5	72.7	66.1
6-10	37.3	33.9
Position of child in the family		
First	23	20.9
Second	23	20.9

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Third	15	13.6
Fourth and above	49	44.5

Nutritional Status of Children

Based on anthropometric Z-scores, 37.3% of the children were underweight, including 18.2% who were severely underweight, while 44.5% fell within the normal weight-for-age range. Stunting affected 28.2% of the sample, with 10.9% classified as severely stunted, indicating long-term insufficient nutrient intake and frequent infections, particularly during critical periods of growth (22). Whereas 60.9% had normal height-for-age. Wasting was observed in 42.7% of children, of whom 14.5% were severely wasted; 40.9% had normal weight-for-height, and 1.8% were overweight. Wasting, present in 42.7% of the sample (including 14.5% severely wasted), is alarmingly high and reflects acute food insecurity or recent weight loss due to illness or poor diet. Wasting significantly increases the risk of child mortality, particularly when associated with severe acute malnutrition (SAM). Using BMI-for-age, 6.4% of children met criteria for severe acute malnutrition (SAM) and 37.3% for moderate acute malnutrition (MAM); just over half (54.5%) were in the normal range, while 0.9% were overweight and another 0.9% obese. points to an emerging double burden of malnutrition, a coexistence of undernutrition and overweight, often due to poor-quality, energy-dense, nutrient-poor diets (23). However, these findings indicate a high burden of acute and chronic malnutrition among displaced children, particularly in the form of wasting and underweight, both of which reflect recent nutritional deficits and potential food insecurity.

Table 2: Nutritional Status of Children (n=110)

Variables	Frequency	Percentage
Prevalence of underweight among children based on weight-for-age (WFA) Z-scores		
Underweight (<-2)	41	37.3
Severe underweight (<-3)	20	18.2
Normal (>-2)	49	44.5
Prevalence of stunting among children based on height-for-age (HFA) Z-scores		
Stunting (<-2)	31	28.2
Severe stunting (<-3)	12	10.9
Normal (>-2)	67	60.9
Prevalence of wasting among children based on Weight for Height (WFH) Z-scores		
Wasting (<-2)	47	42.7
Severe wasting (<-3)	16	14.5
Normal (>-2)	45	40.9
Overweight (above 1)	2	1.8
Prevalence of BMI for Age among children		
SAM (<-3)	7	6.4
MAM (-2 to -3)	41	37.3
Normal (-1 to 1)	60	54.5
Overweight (1 to 2)	1	0.9
Obese (2-3)	1	0.9
Prevalence of acute malnutrition and bilateral pitting edema among children		
Mid-Upper Arm Circumference (MUAC)		
Severe acute Malnutrition (SAM) (<11.5cm)	15	13.6
Moderate acute Malnutrition (MAM) (11.5- <12.5cm)	31	28.2
Norma (>12.5cm)	64	58.2
Bilateral pitting Edema	0	0.0

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The prevalence of acute malnutrition and bilateral pitting edema among children, based on Mid-Upper Arm Circumference (MUAC), showed that 13.6% of children had Severe Acute Malnutrition (SAM) with MUAC less than 11.5 cm, 28.2% had Moderate Acute Malnutrition (MAM) with MUAC between 11.5 cm and less than 12.5 cm, and 58.2% had normal MUAC measurements greater than 12.5 cm. No cases (0.0%) of bilateral pitting edema were observed.

Physical Activity, Vaccination, and Supplementation among the Children

Among the children assessed, 25.5% engaged in no physical activity, remaining mostly static while sleeping, watching TV, or riding in a car, whereas the majority (74.5%) performed only mild activity such as walking or casual play, and none were classified as very active. Vaccination coverage was notably high, with 81.8% immunized and 18.2% not; of those vaccinated, 41.1% had received the full five essential vaccines, and 36.7% received only polio and measles vaccines. The remaining 22.2% received different combinations of vaccines. Exactly half of the children (50%) received nutritional supplements, while the other half did not; among those supplemented, vitamin A was given to 63.6%, ferrous sulfate to 54.5%, zinc to 32.7%, and multivitamins to 21.8%. Nutritional supplementation was also assessed, showing that exactly half of the children, or 50%, received nutritional supplements. Among the supplemented children, 63.6% were given vitamin A, 54.5% received ferrous sulfate, 32.7% were provided with zinc, and 21.8% took multivitamins. Consequently, the other half of the group did not receive these supplements, with (36.4%) lacking vitamin A, (45.5%) missing out on ferrous sulfate, (67.3%) not having zinc, and (78.2%) not receiving multivitamins. These numbers show gaps in the coverage of micronutrients and point to the possibility of using targeted supplementing techniques to avoid deficiencies that are known to impair a child's immunity and growth.

Table 3: Physical activity, vaccination among children (n=110)

Variables	Frequency	Percentage
Physical activity of child		
No activity (Static for long periods such as sleeping, showing T.V, travelling by car.)	28	25.5
Mild activity (Move around, playing, jumping and jogging, walking slowly and less active gameplay.)	82	74.5
Very active (Running (Race), brisk walking, Bike riding, Dance, Swimming, climbing, jump rope and gymnastics like football.)	00	00
Vaccination of child		
Yes	90	81.8
No	20	18.2
Type of vaccine Received:		
All of them (the fifth vaccine)	37	41.1
Polio and measles	33	36.7
Other	20	22.2
Supplementation taken by child		
Yes	55	50
No	55	50
Types of Supplements Taken:		
Child supplemented by Vitamin A		
Yes	35	63.6
No	20	36.4
Child supplemented by ferrous format		
Yes	30	54.5
No	25	45.5

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Child supplemented by zinc		
Yes	18.2	32.7
No	37.3	67.3
Child supplemented by multivitamin		
Yes	11.8	21.8
No	42.7	78.2

Dietary Intake and Meal Patterns

According to the number of meals per day, the majority of respondents (83%) were taking 1-2 meals per day, and only 17% were taking 3-4 meals per day. This infrequent eating pattern raises the possibility of insufficient nutrient intake, especially in kids whose energy and micronutrient requirements are higher for growth and development. Such restricted eating patterns are frequently indicative of food insecurity, a lack of varied and adequate food sources, and a dependence on sporadic food assistance in humanitarian or displaced contexts. Undernutrition, stunting, and other types of malnutrition may result from these eating habits, particularly if the few meals ingested are deficient in nutritional diversity and nutrient density (5).

In terms of food frequency, cereals emerged as a staple, with 70% of participants consuming bread and 51% regularly eating kisra 1-3 times per week. Animal products such as meat stew and fish are also popular, with 71% and 70% of respondents consuming them 1-3 times weekly, respectively. Interestingly, chicken consumption appears limited, with only 19% eating it regularly, while 63% report never consuming chicken. A decrease in food rations and inadequate intake could be the main factors contributing to malnutrition in the camp.

When it comes to dairy, 50% of participants enjoy Rob 1-3 times a week, and 51% consume fresh milk more frequently, 4-6 times per week. As for vegetables and fruits, while 60% include salad in their diet 1-3 times weekly, there's a notable trend of non-consumption for certain fruits: 72% never eat apples, 61% never consume guava, and 58% never have mango.

In the legumes and sweets category, 67% of respondents enjoy lentils (Addas) 1-3 times per week, yet 82% do not partake in fava beans (Fulmasri) or beans (Fasolia). Sweets appear to be infrequently consumed as well. On a positive note, wild fruits like Karkade have made an impression, with 66% of respondents including them in their diets.

Previous Medical History

A review of past illnesses showed that iron-deficiency anemia had been documented in 30.9% of the children, while the majority (69.1%) had no such history. Wasting had previously affected 27.3% of children, leaving 72.7% unaffected. Nearly all children (93.6%) had experienced malaria at some point, with only 6.4% remaining malaria-free. Typhoid was less common, reported in just 8.2% of children versus 91.8% without prior infection. Other acute diseases were noted in 59.1% of the sample, whereas 40.9% had no record of such conditions. Nearly half of malnutrition cases are linked to recurrent diarrhea and/or intestinal infections due to the lack of access to sufficient safe drinking water and poor sanitation facilities, according to Lawry et al. (24).

Table 4: Previous Medical History of Children (n=110)

Previous history of child with iron Anemia	**Frequency**	**Percentage**
Yes	34	30.9
No	76	69.1
Previous history of child with wasting		
Yes	30	27.3
No	80	72.7
Previous history of child with Malaria		

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Yes	103	93.6
No	7	6.4
Previous history of child with typhoid		
Yes	9	8.2
No	101	91.8
Previous history of child with other acute diseases		
Yes	**65**	**59.1**
No	**45**	**40.9**

Association between Age Group vs. Weight-for-Age

The analysis clearly demonstrates that there is no statistically significant link between the ages of children and their weight-for-age status ($p > 0.05$). This indicates that the distribution of underweight, severely underweight, and normal weight categories remains consistent across various age groups. In essence, age does not significantly influence weight status among children. Recognizing this insight is essential for developing effective interventions focused on improving child nutrition and enhancing overall health outcomes.

Association between Age of Children and MUAC status

The findings of this study highlight a concerning level of malnutrition among children under five, with notable rates of underweight, stunting, and wasting. These patterns reflect broader trends observed in low-income and resource-limited settings, where poor dietary diversity, low meal frequency, and limited access to essential nutrients are common contributors to undernutrition. Similar findings have been reported in studies conducted in sub-Saharan Africa and South Asia, where inadequate infant and young child feeding practices, alongside household food insecurity, play significant roles in poor nutritional outcomes (25,26).

Low maternal education levels and large household sizes, as found in this study, have been consistently associated with higher risks of child malnutrition in other research. For instance, studies emphasize the importance of maternal literacy and awareness in ensuring adequate feeding and healthcare practices. Furthermore, although immunization coverage was relatively high, incomplete vaccination and inconsistent supplementation could weaken children's resistance to infections, exacerbating the effects of malnutrition, as seen in a study by (27).

Interestingly, the lack of a significant relationship between child age and weight-for-age in this study suggests that undernutrition is widespread across all age groups within the first five years of life, aligning with the findings of multiple DHS (Demographic and Health Survey) reports that emphasize the persistent and chronic nature of undernutrition in similar populations (28-31).

CONCLUSION

This study reinforces the urgent need for integrated interventions that address immediate dietary gaps as well as underlying social and economic determinants. Strengthening caregiver education, improving access to health services, and promoting diverse, nutrient-rich diets are crucial strategies for reducing childhood malnutrition and improving long-term health outcomes. Children under five, particularly those in IDP camps, are at heightened risk of malnutrition due to factors like food insecurity, limited access to healthcare, and poor living conditions.

Recommendations

Strengthening growth monitoring programs and routine nutritional screening through community-based mechanisms is essential, especially in regions with high malnutrition prevalence (32). By adhering to these recommendations and fostering collaborative efforts, stakeholders can effectively assess and address the nutritional needs of children in Kosti's IDP camps, mitigating the impact of the ongoing humanitarian crisis.

Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS Review Article

Toll-Like Receptors (TLRs) and Their Role in Diabetic Mellitus Disease: A Review

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ABSTRACT

Background: Toll-like receptors (TLRs), which are an integral component of the innate immune system, have been identified as a key player in this inflammatory process. Toll-Like Receptors appeared as important elements in early defending against infectious diseases. These receptors are responsible for the promotion of antigen-presenting cell (APC) activation, e.g. dendritic cells and macrophages, thereby improving adaptive immune responses by T-cell activation promotion as well as improving B cell response.
Objective: This study aimed to explore association between TLRs and diabetes development and progression, and find their role in diabetes development and progression and the possible treatment implications.
Method: This study reviewed previous studies on TLRs and their relationship to DM, as this study reviewed 71 studies.
Results: TLRs play essential roles in both types of DM (Type I and Type II) via contribution to insulin resistance and inflammations. The inflammatory environment which aggravates β-cell dysfunctions and insulin resistance can be promoted by specific TLRs activation, e.g. TLR-2, TLR-4 and TLR-9.
Conclusion: New treatment strategies may be provided to treat DM through targeting such receptors. It is recommended to investigate how TLR activation in pancreatic islets triggers immune cell infiltration (e.g., macrophages, dendritic cells), and to study TLR4 inhibitors (e.g., TAK-242) in T2DM models.

Keywords: TLR-s, innate immunity, humoral immunity, cellular immunity, diabetes mellitus.

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INTRODUCTION

The class of pattern recognition receptors known as Toll-like receptors (TLRs) play central roles in innate immune systems. These receptors have the ability for recognizing various microbial constituents called pathogen associated molecular patterns (PAMPs), in addition to damage associated molecular patterns (DAMPs) in injured or stressed host cells. Discovery of TLRs was first initiated in Drosophila melanogaster (a fruit fly), as these receptors appeared to participate in immune responses against pathogenic microbes. TLRs contribute to a wide range of microbial infection detection in mammals. Human-beings have I0 types of TLR (TLR1-TLRI0), each one of these types recognize various PAMPs. The expression of TLRs can be seen on different immune cells such as B-cells, macrophages, dendritic cells in addition to some non-immune cells such as epithelial cells (1).The type I trans-membrane proteins known as TLRs have (20–27) extra- cellular leucine-rich repeat to recognize PAMP/DAMP, trans-membrane domain, and intra-cellular toll–interleukin-I receptor (TIR) domains, and are needed to activate down-stream the signal transduction pathway as shown in figure 1 (1, 2). The trans-membrane receptors (TLRs) are composed of an extracellular leucine-rich repeats domain, a single trans-membrane segment, as well as an intra-cellular Toll/IL-1 receptor domain. The extra-cellular LRR domains recognize PAMP or DAMP, whereas intra-cellular TIR domains transduce signal for initiating an immune response. Leucine-Rich repeat s(LRR) domains are causing binding to the ligands (PAMP or DAMP). The TIR domains are necessary for intra-cellular signaling, and they usually involve adaptors of MyD88 or TRIF for activation of down-stream pathways like NF-κB, MAPK as well as IRF3 necessary for immune response (3).

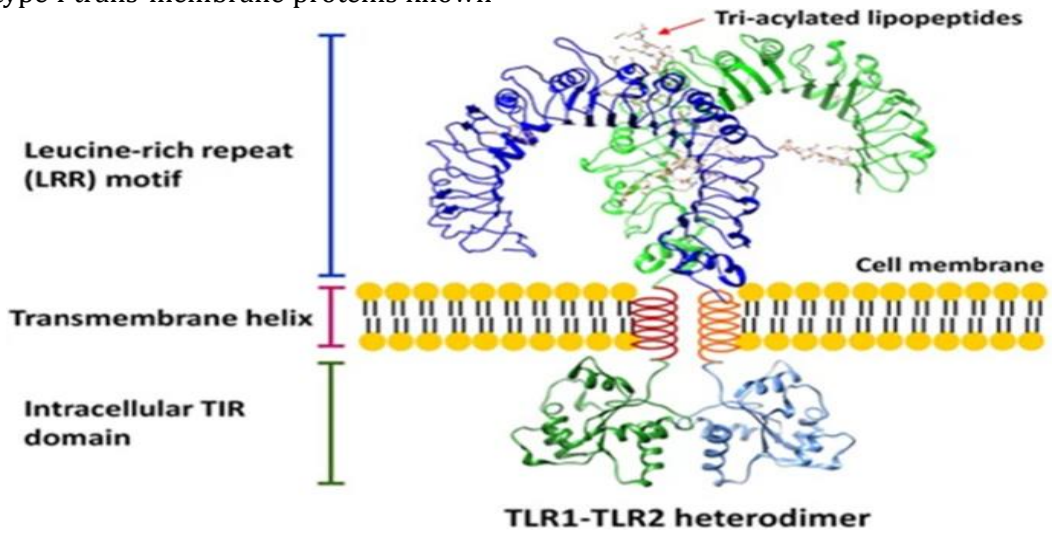

Figure 1: Structure of TLRs (1, 2)

When TLRs bind to ligands, they start a signaling pathways which result in immune activations and inflammatory response. Two main down-stream signaling pathways are present which are: MyD88-dependent pathways. The majority of TLRs involving TLR4, TLR7 & TLR9 utilize MyD88 as adaptor proteins., and this pathway causes NF-κB and MAPK activation resulting in the promotion of pro-inflammatory cytokine production such as TNF-α, IL-1β and IL-6. TRIF-dependent pathways: TLR3 and TLR4 utilize TRIF as adaptor proteins, and such pathway causes IRF3 and IRF7 activation, leading to type-I interferon production (such as IFN-α & IFN-β) (4). The class of pattern recognition receptors known as Toll-like receptors (TLRs) play central roles in innate immune systems. These receptors have the ability for pathogen associated molecular pattern detection (PAMP) as well as damage associated molecular pattern (DAMP), leading to inflammatory response triggering. TLRs were found to be associated with the progression and development of chronic inflammatory diseases, such as diabetes. In diabetic individuals, inflammations play crucial roles

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in pancreatic β-cell dysfunctions and insulin resistances (1). TLRs have central roles in inflammation induction. When they are activated, TLRs initiate chemokine and pro-inflammatory cytokine production, that not only contribute to defense against pathogenic agents, but also help in chronic inflammations development in certain diseases such as diabetes, autoimmune disorders and cardiovascular disorders. TLR4 has a main role in inflammation mediation in certain conditions like obesity & sepsis. TLR4 activation by lipopolysaccharides (LPS) from Gram (-ve) bacteria result in release of cytokines and activation of NF-κB (5). This study aimed to explore association between TLRs and diabetes development and progression, and find their role in diabetes development and progression and the possible treatment implications.

METHOD

This study aimed to search the role of the Toll-Like Receptors (TLRs) and their role in DM. A comprehensive search was made to identify relevant previous studies in this area in PubMed and Google Scholar databases. The following keywords were used during the search: "Toll-Like Receptors", "Innate Immunity" and "Diabetic Mellites Disease". In this review, 71 previous studies were conducted on the types of TLRs and their numbers as shown in results.

RESULTS

TLRs and Immune Response

TLRs appeared as important elements in early defending against infectious diseases. These receptors are responsible for the promotion of antigen-presenting cell (APC) activation, e.g. dendritic cells and macrophages, thereby improving adaptive immune responses by T-cell activation promotion as well as improving B cell response. For instance, peptidoglycan from Gram-(+ve) bacteria is recognized by TLR-2, while LPS from Gram-(-ve) bacteria is recognized by TLR-4, leading to inflammation and adaptive immunity stimulation (6). There are several types of TLRs that differ in location, Ligands Recognized and their role in immunity, as shown in Table 1.

Table (1): Summary of Toll Like receptors (TLRs) and their roles in immunity

Toll-Like Receptor (TLR)	Location	Ligands Recognized	Role in Immunity	References
TLR1	Cell membrane (Plasma membrane)	Lipoproteins (e.g., triacyl lipopeptides)	Initiates inflammatory responses by recognizing bacterial lipoproteins, activating NF-κB signaling.	7
TLR2	Cell membrane (Plasma membrane)	Lipoproteins, peptidoglycan, zymosan, lipoteichoic acid	Recognizes a broad range of microbial components, promoting immune response and cytokine production.	6
TLR3	Endosome	Double-stranded RNA (dsRNA)	Senses viral RNA, triggers antiviral immune responses, activates type I interferons.	8
TLR4	Cell membrane (Plasma membrane)	Lipopolysaccharide (LPS), heat-shock proteins (HSP)	Recognizes bacterial LPS, initiating inflammation, phagocytosis, and adaptive immune responses.	9
TLR5	Cell membrane (Plasma membrane)	Flagellin (protein in bacterial flagella)	Recognizes bacterial flagella, leading to activation of NF-κB and inflammation.	10
TLR6	Cell membrane (Plasma membrane)	Diacyl lipopeptides, lipoteichoic acid	Works with TLR2 to recognize bacterial and	11

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			fungal components, inducing immune responses.	
TLR7	Endosome	Single-stranded RNA (ssRNA), viral RNA	Detects RNA viruses, stimulates antiviral immunity, and induces type I interferons.	12
TLR8	Endosome	Single-stranded RNA (ssRNA), viral RNA	Similar to TLR7, involved in viral detection, promoting immune responses.	13
TLR9	Endosome	Unmethylated CpG DNA (common in bacteria and viruses)	Recognizes microbial DNA, triggers inflammation, and stimulates type I interferon production.	14
TLR10	Endosome (or plasma membrane)	Unknown	Modulates immune responses, but its ligands and role in immunity are less well understood.	15
TLR11	Cell membrane (Plasma membrane)	Profilin, uropathogenic E. coli flagellin	Recognizes bacterial flagellin and profilin, involved in immune surveillance and protection against infections.	16
TLR12	Endosome	Profilin (similar to TLRII)	May participate in innate immune response to pathogens, particularly bacteria.	17
TLR13	Endosome	Ribosomal RNA (rRNA)	Senses bacterial rRNA, contributing to host defense mechanisms.	18

TLRs in Autoimmune Diseases

TLRs are involved in autoimmune disorders like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) as well as type-I Diabetes mellitus (TIDM). In such disorders, abnormal TLRs activation results in inflammations and excessive immune responses, causing damage to tissues and progression of diseases. because of their roles in autoantigen recognition such as DNA and RNA, TLR-7 & TLR-9 are mainly involved in SLE (19). The autoimmune disorder TIDM is known by the destruction of pancreatic insulin producing β-cells. Several studies propose that TLRs, especially TLR-2 & TLR-4 contribute to autoimmunity initiation and propagation in TIDM. The pro-inflammatory cytokine release can be triggered by the activation of TLR-2 & TLR-4, resulting in the destruction of pancreatic β-cells. In TIDM, TLR-9 is also involved in autoreactive T-cell activation (20). TLRs participate in the dysfunction of β-cell via inflammation induction. For example, endoplasmic reticulum (ER) stress can be induced and apoptosis is triggered by the activation of TLR-4 in β-cells.

TLR-2 also contribute to β-cell deaths via promotion of production of inflammatory cytokines. Some studies indicated that protections against diabetes-induced β-cell loss showed TLR antagonisms or TLR knockout mice (21). In T2DM, there is a sign of chronic low-grade inflammation. TLRs, especially TLR-4 can be activated by increased circulating free fatty acid & lipopolysaccharide (LPS) levels from intestinal microbiota resulting in insulin resistances. TLR-2 & TLR-9 were also correlated with adiposities and insulin resistance development in T2DM. The activation of TLR-4 by LPS is implicated in cytokine induction like TNF-α, a cytokine that impairs the signaling of insulin and exacerbates metabolic dysfunctions (22). In T2DM, TLR-4 was widely investigated because it plays a significant role in the resistance of insulin. TLR-4 activation by LPS or free

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fatty acids results in NF-κB & other signaling molecule recruitments, leading to inflammatory cascade promotion which impair the sensitivity of insulin. To improve the sensitivity of insulin in diabetes, TLR-4 antagonists were explored as possible treatment agent (23).

Epidemiology

Toll-like receptor (TLR) plays an essential role in the immune responses and is broadly investigated in diabetes context. TLR is a central mediator of the immune responses and plays a key role in both type-I and type-2 diabetes as seen in Table (2).

Table (2): Summary of former studies on TLR types with their roles in immunity

TLR	Diabetes Type	Findings	Mechanism	Reference
TLR1	T2D	Involvement in Inflammatory Pathways	Plays a role in the activation of inflammatory pathways that contribute to insulin resistance and pancreatic beta-cell dysfunction in type 2 diabetes.	4
	T2D	Involvement in Inflammatory Pathways	TLRI activation contributes to insulin resistance via inflammatory cytokine production (TNF-α, IL-6).	24
	T2D	Genetic Polymorphisms and Diabetes Risk	Polymorphisms in TLRI gene associated with increased risk of T2D.	25
	T2D	Obesity and TLR1 in Adipose Tissue	TLRI activation in adipocytes by free fatty acids links to obesity-induced inflammation and insulin resistance.	26
	T1D	TLR1 in Autoimmune Processes	TLRI activation may contribute to autoimmune beta cell destruction in TID by modulating immune responses.	27
	T2D	Therapeutic Targeting of TLRI	Inhibiting TLRI signaling pathways could reduce inflammation and improve insulin sensitivity in T2D.	28
TLR2	T2D	Involvement in Inflammatory Pathways	Contributed to inflammation induction and resistance of insulin. Increased expression of TLR-2 in macrophages and fatty tissues participates in the inflammatory environments observed in type-2 Diabetes mellitus.	29
	T2D	TLR2 Expression in Adipose Tissue	Upregulation of TLR2 in adipose tissue contributes to inflammation and insulin resistance in type 2 diabetes.	30
	T2D	TLR2 in Diabetic Nephropathy	TLR2 activation exacerbates inflammation and fibrosis in the kidneys, leading to nephropathy.	31
	T2D	TLR2 and Insulin Resistance	TLR2 knockout mice showed improved insulin sensitivity and reduced inflammation.	23
	T1D	LR2 and Inflammation in Diabetic Monocytes	TLR2 activation leads to increased cytokine production in monocytes from diabetic patients.	32
	T1D	Immune activation, β-cell destruction	Activation of TLR-3 is associated with the apoptosis of beta cells in the pancreas and with inflammations. The role of TLR-3 in diabetes is not understood yet, but it is believed that it exacerbates dysfunctions of beta cells when accompanied with chronic hyperglycemias.	33

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TLR3	T1D	Immune activation, β-cell destruction	TLR3 contributes to β-cell apoptosis and autoimmunity in TID.	34
	T2D	Inflammatory signaling, insulin resistance	TLR3 activation induces chronic inflammation, contributing to insulin resistance and obesity.	35
	T1D & T2D	β-cell apoptosis	TLR3 activation induces cytokine release leading to β-cell apoptosis and dysfunction.	36
	T2D	Chronic inflammation, insulin resistance	TLR3 signaling exacerbates inflammation and impairs insulin signaling, contributing to insulin resistance in adipocytes and macrophages.	37
	T2D	Genetic variation, susceptibility	SNPs in TLR3 associated with susceptibility to T2D, modulating immune responses.	38
	T1D & T2D	Potential therapeutic target	Targeting TLR3 with antagonists could reduce inflammation and improve insulin sensitivity.	39
TLR4	T1D & T2D	inflammatory cytokine release	In both type-I & type-2 diabetes, TLR-4 is a serious inflammation mediator. The lipopolysaccharide (LPS) is recognized and release of inflammatory cytokines is triggered by TLR-4 resulting in resistance of insulin. The expression of TLR-4 can be increased by high fat diets in liver and fatty tissues, leading to dysregulation of metabolism.	40
	T1D	TLR4 and Inflammation	TLR4 plays a central role in inflammatory response in diabetes.	41
	T2D	TLR4 in Obesity and Insulin Resistance	Elevated TLR4 expression in adipose tissue links to insulin resistance.	42
	T2D	TLR4 in Beta Cell Dysfunction	TLR4 activation promotes beta-cell apoptosis in diabetes.	43
	T2D	TLR4 in Diabetic Complications	TLR4 contributes to complications such as nephropathy and retinopathy.	44
	T2D	TLR4 Inhibition as Therapy	TLR4 inhibitors may reduce insulin resistance and inflammation.	45
	T2D	TLR4 and Gut Microbiota	Gut microbiota influences TLR4 activation and inflammation.	46
	T2D	Hyperglycemia and TLR4 Activation	Chronic hyperglycemia induces TLR4 activation and worsens insulin resistance.	47
TLR5	T2D	modulate the immune response	The role of TLR-5 in diabetes is still not completely investigated, however, it appeared to modulate the immune responses and affect intestinal microbiota. Insulin resistance and inflammation can be affected by a change in the composition of microbiota.	48
	T2D	TLR5 Overview	TLR5 is involved in immune responses and inflammation.	4
	T2D	Inflammation in Diabetes	TLR5 activation contributes to insulin resistance.	49
	T1D & T2D	Diabetic Complications	TLR5 may contribute to diabetic nephropathy and retinopathy.	50
	T2D	Gut Microbiota	Dysbiosis can activate TLR5, leading to insulin resistance.	51

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	T2D	Polymorphisms	TLR5 gene variants may affect diabetes susceptibility.	52
	T2D	Therapeutic Targeting	Inhibition of TLR5 can alleviate insulin resistance.	53
TLR6	T2D	Investigate the role of TLR6 in inflammation associated with diabetes	TLR6 is upregulated in diabetic models, contributing to chronic inflammation, particularly in type 2 diabetes.	54
	T2D	Study the effect of TLR6 genetic variation in diabetic patients	Certain genetic variations in TLR6 are linked with an increased risk of diabetes and diabetic complications.	55
	T1D & T2D	Explore TLR6 expression in diabetic neuropathy	Elevated TLR6 expression contributes to the progression of diabetic neuropathy.	56
	T1D & T2D	Investigate TLR6 role in diabetic wound healing	TLR6 activation impairs wound healing in diabetic mice by exacerbating inflammation.	57
	T2D	Assess TLR6 in diabetic cardiovascular complications	Increased TLR6 activity exacerbates cardiovascular complications in diabetes by promoting inflammation.	58
TLR7	T1D	inflammatory cytokine release	TLR-7 is implicated in innate immune responses and can participate in the release of inflammatory cytokines. It is correlated with autoimmune Diabetes mellitus, particularly in type-I diabetes context.	59
	T1D	TLR7 activation leads to increased inflammatory cytokines, potentially contributing to autoimmune responses.	TLR7 activates NF-κB and other inflammatory pathways, which may exacerbate autoimmune beta-cell destruction.	60
	T1D	Deficiency of TLR7 protects against autoimmune diabetes in NOD mice, suggesting a pro-inflammatory role of TLR7 in TID.	TLR7 activation in NOD mice worsens the autoimmune response by enhancing ThI/ThI7 responses.	61
	T2D	TLR7 is implicated in the development of insulin resistance and inflammation in adipose tissue.	TLR7 activation leads to increased inflammatory cytokines like TNF-α and IL-6, contributing to insulin resistance in adipose tissue.	62
	T2D	TLR7 influences macrophage	TLR7 affects macrophage polarization, promoting a pro-inflammatory MI phenotype, which	63

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		polarization and increases the production of pro-inflammatory cytokines, linking it to insulin resistance.	contributes to chronic low-grade inflammation in T2D.	
	T1D & T2D	TLR7 signaling is linked to pancreatic islet inflammation and beta-cell dysfunction in both T1D and T2D.	TLR7 promotes inflammatory cytokine production in pancreatic islets, leading to beta-cell dysfunction and insulin resistance.	64
	T2D	TLR7-mediated immune responses are involved in the progression of diabetes complications such as nephropathy.	TLR7 activation exacerbates diabetic nephropathy by promoting renal inflammation and fibrosis.	65
TLR8	T2D	mediating inflammation	TLR8 also plays a role in mediating inflammation and insulin resistance. It is thought to contribute to the development of metabolic disorders through its activation of the NF-kB pathway.	66
	T2D	TLR8 expression is increased in the immune cells of diabetic patients, contributing to chronic inflammation.	TLR8 activation triggers the production of pro-inflammatory cytokines such as TNF-α, IL-Iβ, and IL-6, leading to insulin resistance.	67
	T2D	TLR8 signaling induces the activation of NF-κB and MAPK pathways in macrophages, promoting chronic inflammation in T2D.	TLR8 activation in macrophages promotes the release of inflammatory cytokines, contributing to insulin resistance and inflammation in adipose tissue.	68
	T2D	TLR8 activation accelerates inflammation in adipose tissue, leading to increased risk of insulin resistance and obesity.	TLR8 influences macrophage polarization, promoting MI pro-inflammatory macrophages, which are linked to insulin resistance.	69
	T2D	TLR8 deficiency reduces pancreatic islet inflammation and improves insulin	TLR8 deficiency reduces inflammation in pancreatic islets and improves insulin sensitivity by inhibiting the activation of pro-inflammatory cytokine pathways.	70

		sensitivity in a mouse model of T2D.		
	T1D & T2D	TLR8 activation exacerbates systemic inflammation and beta-cell dysfunction in diabetic mice.	TLR8 activation in immune cells enhances the release of pro-inflammatory cytokines, which contribute to beta-cell dysfunction and impaired insulin secretion.	7I
	T2D	TLR8-induced macrophage activation worsens insulin resistance and endothelial dysfunction in diabetes.	TLR8 activation induces MI macrophage polarization, increasing insulin resistance and endothelial dysfunction, both of which contribute to diabetic complications.	72
	T1D & T2D	TLR8 enhances the expression of inflammatory cytokines, aggravating systemic inflammation and beta-cell dysfunction.	TLR8 activation increases inflammatory cytokine expression in pancreatic islets, contributing to the dysfunction of beta-cells and the progression of both T1D and T2D.	73
TLR9	T1D	inflammatory cytokine response	TLR9 activation in response to DNA and methylated CpG motifs can contribute to beta-cell apoptosis and insulin resistance, especially under chronic inflammatory conditions.	74
	T1D	TLR9 activation in diabetic mice exacerbates pancreatic inflammation and accelerates beta-cell destruction.	TLR9 activation induces a pro-inflammatory cytokine response that contributes to beta-cell apoptosis and autoimmunity.	75
	T2D	TLR9 is involved in the induction of insulin resistance in obese mice through increased macrophage activation.	TLR9 activation promotes macrophage polarization to the MI phenotype, leading to chronic inflammation and insulin resistance in adipose tissue.	76
	T2D	TLR9-induced inflammatory response in the liver contributes to insulin resistance in T2D models.	TLR9 activation in liver cells induces inflammation, leading to insulin resistance and hepatic dysfunction.	77
	T2D	Inhibition of TLR9 signaling improves glucose metabolism and reduces	TLR9 inhibition reduced the production of inflammatory cytokines and improved insulin sensitivity in adipose and liver tissues.	71

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	inflammation in diabetic mice.		
T2D	TLR9 activation exacerbates insulin resistance by inducing adipose tissue inflammation in obesity.	TLR9 activation in adipose tissue increases the release of pro-inflammatory cytokines, contributing to insulin resistance.	78
T2D	TLR9-induced inflammation worsens both pancreatic islet function and systemic insulin resistance in T2D.	TLR9 activation in both immune cells and pancreatic islets increases the release of pro-inflammatory cytokines, worsening insulin resistance and beta-cell dysfunction.	79
T2D	TLR9 activation in immune cells increases systemic inflammation, contributing to vascular complications in diabetes.	TLR9-induced inflammation in immune cells leads to increased vascular inflammation, which contributes to endothelial dysfunction and complications in T2D.	80

DISCUSSION

This study reviewed 71 previous studies on the role of TLRs in the pathogenesis of diabetes mellitus for type 1 and type 2 as shown in Table 1. This table showed that the TLRs are impartment components of innate immune system and it have impartment role in the progression of diabetes mellitus by promoting chronic inflammation and insulin resistance. TLRI activation contributes to insulin resistance via inflammatory cytokine production (TNF-α, IL-6) (24). TLR2 activation leads to increased cytokine production in monocytes from diabetic patients (32). TLR3 contributes to β-cell apoptosis and autoimmunity in T1D (34). In both type-I & type-2 diabetes, TLR-4 is a serious inflammation mediator (40). TLR5 is involved in immune responses and inflammation (4). Increased TLR6 activity exacerbates cardiovascular complications in diabetes by promoting inflammation (58). TLR-7 is implicated in innate immune responses and can participate in the release of inflammatory cytokines (59). TLR8 activation triggers the production of pro-inflammatory cytokines such as TNF-α, IL-Iβ, and IL-6, leading to insulin resistance (67). TLR9 activation induces a pro-inflammatory cytokine response that contributes to beta-cell apoptosis and autoimmunity (75).

CONCLUSION

In conclusion, Toll-like receptors (TLRs) play a pivotal role in the pathogenesis of both type 1 and type 2 diabetes mellitus by mediating chronic inflammation and impairing insulin signaling. Previous research has highlighted the critical involvement of TLR2, TLR4, and TLR9 in modulating disease pathophysiology, establishing these receptors as promising therapeutic targets. Modulation of TLR signaling pathways may attenuate inflammatory responses, enhance insulin sensitivity, and preserve pancreatic β-cell function, thereby presenting novel therapeutic strategies for diabetes management and its associated complications. It is recommended to investigate how TLR activation in pancreatic islets triggers immune cell infiltration (e.g., macrophages, dendritic cells), and to study TLR4 inhibitors (e.g., TAK-242) in T2DM models.

Conflict of Interest

The authors declare that there is no conflict of interest.

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OPEN ACCESS Review Article

Pulp Capping Agents in Operative Dentistry: an Updated Review

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ABSTRACT

Background: Pulp capping is a restorative technique that helps preserve the tooth pulp after significant injury during cavity preparation, preventing it from degenerating when it was exposed or nearly exposed.
Objective: The aim of this article is to review the literature to assess the current trends and future directions of dental pulp capping materials and it mainly focus on the classification of materials along with their mechanisms.
Methods: A comprehensive search was made to identify relevant previous studies in this area in PubMed and Google Scholar databases.
Results: The procedure of vital pulp capping primarily depends on the ability of dental pulpal tissue to heal. A wide array of materials has been used for pulp capping. Calcium hydroxide and Mineral Trioxide Aggregates (MTA) are the most commonly used pulp capping materials in dentistry, and they have had significant clinical success.
Conclusion: In recent years various other materials like Bone morphogenic protein, Bio dentin, Lasers are also introduced clinically. Further *in vitro* and *in vivo* studies are necessary to examine and validate about calcium ion releasing ability, together with the cytotoxic effect and the clinical significance of these next-generation materials.

Keywords: Pulp capping agents, calcium hydroxide, MTA, Biodentine, lasers, and TheraCal.

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INTRODUCTION

Pulp capping depends mainly on the healing capability of pulpal tissue. [1-3] This process was affected by various factors, including age, periodontal condition, & the stage of root development. The primary goal of pulp therapy was to preserve the integrity and health of the teeth and their supporting structures, with the specific aim of maintaining pulp vitality. [4-6] Pulp exposure can occur due to mechanical causes, iatrogenic causes, dental caries, or trauma. A protective substance was applied, and this was called a pulp capping procedure, which was done in order to promote pulp healing and preserve its vitality and function [7]. Vital Pulp Therapy (VPT) was a procedure that involved applying a protective biomaterial known as a pulp capping agent to the remaining thin layer of dentin. This was done in cases of near-to-exposed pulp (indirect pulp capping), directly exposed coronal pulp (direct pulp capping), or when part of the coronal pulp tissue was removed (pulpotomy) [3, 7].

Calcium hydroxide and mineral trioxide aggregate are the commonly used pulp capping agents in dentistry, both showing substantial clinical success. In recent years, alternative materials such as bone morphogenetic proteins have also been explored for their potential in pulp therapy, and Biodentine and lasers have also been introduced into clinical practice [5-8].

Despite ongoing advancements, there is still no consensus on the most suitable pulp capping material for specific clinical situations. The aim of this article is to review the literature to assess the current trends and future directions of dental pulp capping materials and it mainly focus on the classification of materials along with their mechanisms.

METHODS

A comprehensive search was made to identify relevant previous studies in this area in PubMed and Google Scholar databases. The following keywords were used during the search: “pulp capping agents”, “Calcium hydroxide”, “MTA”, “Biodentine”, “Lasers”, and “TheraCal”. In this review, 50 previous studies were summarized.

RESULTS

Classification of Pulp Capping Materials

Pulp capping materials are classified as conventional, experimental, and hybrid.

These are further classified based on the composition, experimental analysis, site of action, and combination (Figure 1).

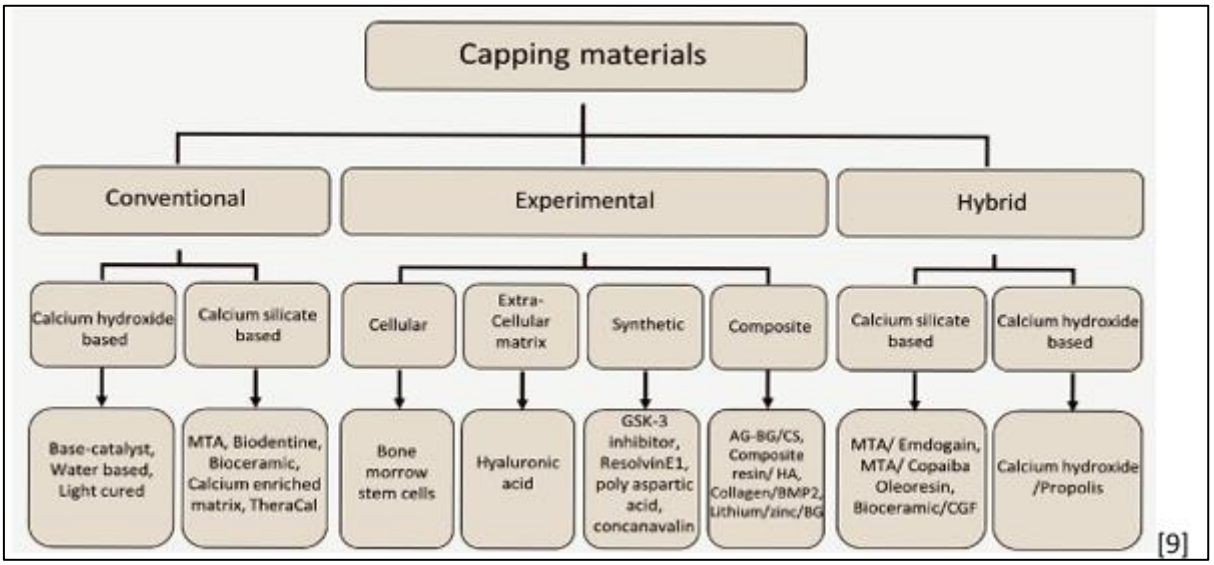

Figure 1: Classification of pulp capping materials [9].

Calcium hydroxide

Calcium hydroxide was used in dentistry since 1920s. It was a strong base with a pH of 12.9 and could be slightly soluble in aqueous solutions, releasing Ca^{2+} and OH^- ions.

Mechanism: The elevated pH resulting from OH^- ions offers strong antimicrobial properties, preventing bacterial infiltration into the pulp. Additionally, the high pH irritates the pulp tissue, leading to superficial formation of three-layered necrotic zone on the exposed pulp surface. This, in turn, stimulates mineralization in response to the necrotic area. Furthermore, the presence of Ca^{2+} provides a calcium source, supporting the mineralization process [10].

Aqueous Calcium Hydroxide

Historically, powdered Calcium hydroxide could be applied directly on the exposed pulp, where it forms a paste upon contact with pulpal tissue fluid. However, this technique was now infrequently used. The rate of success in direct pulp capping with calcium hydroxide tend to decrease over extended follow-up periods.

Calcium-hydroxide-based cement

A cement-type formulation with setting properties was developed in 1960s. Dycal, one of the most well-known commercial cement, consists of a catalyst paste and a base paste, which are mixed in 1:1 proportion [11].

Advantages: Considered as gold standard for pulp capping materials, exhibits excellent antibacterial properties, promotes mineralization, and demonstrates low cytotoxicity.

Disadvantages: Highly soluble in oral tissue fluids, promotes extensive dentin formation, potentially leading to obliteration of the pulp chamber, lacks adhesive properties, and susceptible to degradation after acid etching [12].

Hybrid Calcium Hydroxide-Based Propolis

Propolis, also known as "Russian penicillin," was a natural substance gathered by honeybees from trees and shrubs, its color ranges from yellow-brown to dark brown. Propolis acts as an anti-inflammatory agent by inhibiting prostaglandin synthesis. Moreover, it contains essential elements such as iron and zinc, which are important for collagen synthesis. Propolis made from resins gathered by bees from tree cracks and leaf buds, acts as both an antimicrobial and anti-inflammatory agent [13].

Mechanism: Propolis encourages the formation of a dentin tissue barrier by activating enzyme systems, boosting cell metabolism, improving blood circulation, and aiding collagen synthesis. It also interferes with microbial cell walls and cytoplasm, preventing microbial cell division and acts as anti-microbial agent [14].

Advantages: Antioxidant, anti-inflammatory, antifungal, antiviral and anti-bacterial properties, forms dental pulp tissue collagen by reducing both pulp inflammation and degeneration, and stimulate formation of reparative dentin

Disadvantages: Showed mild to moderate inflammation in 2-4 weeks with partial dentin bridge formation [15].

Calcium Silicate-Based Cements

Calcium silicate-based cements, also known as hydraulic cements, were developed as pulp capping materials to overcome the limitations of calcium hydroxide. In 1993, Mineral Trioxide Aggregate (MTA) was the first commercially available calcium silicate-based cement. To enhance its mechanical properties, calcium carbonate was added to the powder. Another pulp capping material like tricalcium silicate (commonly referred to as bioceramic cement), was available as BioAggregate. Additionally, IRootSP, another type of bioceramic cement originally designed as a root canal sealer, could be provided in a pre-mixed flowable tube also successfully used as a pulp capping material [16].

Hydraulic cements

Mineral trioxide aggregate (MTA)

The original ProRoot MTA Gray (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA), was launched in 1998. In this formulation, bismuth oxide serves as a radiopacifier, calcium sulfate acts as setting modifier.

Mechanism: MTA shares similarities with calcium hydroxide and can therefore be classified as a calcium hydroxide-releasing material, expected to exhibit comparable properties [17].

Advantages: It possesses sealing ability, bioactivity, biocompatibility, and the capacity to promote hard tissue formation. Additionally, MTA could be considered superior to calcium hydroxide because it causes less inflammatory response, induces a thicker and uniform dentin bridge, and reduced necrosis of pulpal tissue.

Disadvantages: highly soluble, poor handling, and coronal tooth discoloration [18].

Modified MTAs and MTA-like materials

Several formulations of MTAs have been developed to address the drawbacks of the original formulation, with most aiming to reduce setting time by altering the composition / particle size [19].

MTA was now categorized into two types: traditional gray MTA and white MTA. The primary differences lie in the amounts of Al_2O_3, Feo & MgO and white MTA (WMTA) does not contain iron [20].

Mechanism: Gray MTA functions by releasing calcium ions when mixed with moisture, generating an alkaline environment that supports the attachment and proliferation of cells involved in hard tissue formation. This process stimulates the body to create a "biologic seal" of hydroxyapatite-like material around the MTA, aiding in sealing and promoting tissue healing due to its bioactive properties [5].

Endo Sequence Root Repair Material

ERRM, a new aluminum-free bioceramic material, was introduced in recent decades as modified MTAwith improved handling and clinical outcomes. It serves as an excellent alternative to MTA. Unlike MTA, ERRM does not cause tooth discoloration because it uses zirconium oxide for radiopacity than bismuth oxide. It could be ready-to-use and was available under various names, including iRoot SP, and iRoot BP4 Plus (iRBP), iRoot FS (iRFS) & iRootBP [21].

Mechanism: The osteogenic property of iRoot BP Plus was comparable to that of MTA. iRBP promotesosteogenic differentiation in bone marrow mesenchymal stem cells by enhancing the gene expression for protein levels of dentin sialophosphoprotein, osteopontin ,osterix, and runt-related transcription factor-2. It stimulatesbone marrow mesenchymal stem cells differentiation through the mitogen-activated protein kinase and autophagy pathways. Additionally, iRoot BP4 Plus induces mRNA expression for odontoblastic markers such as osteocalcin, dentin sialoprotein, and dentin matrix protein-1 [22].

Advantages: Lesser cytotoxicity compared with MTA and Dycal, and antibacterial property.

Disadvantages: The biological activity of cells and alkaline phosphatase (ALP) activity gradually decreased when exposed to ERRM [21].

TheraCal

TheraCal was a newly developed resin modified, light-cured, calcium silicate-filled base/liner. TheraCal LC could be a single-paste, calcium silicate-based material designed for pulp capping procedure and as a liner beneath restorative materials, cement. TheraCal was classified as a fourth-generation calcium silicate material [23].

Mechanism: TheraCal LC releases calcium ions, whose bioavailability was essential for promoting the proliferation and differentiation of dental pulp cells and the formation of mineralized hard dental tissues. The concentration of calcium ions released by TheraCal LC was sufficient to stimulate dental pulp cells and activate odontoblasts [24].

Advantages: Protects the tooth pulpal complex, bonds to moist deep dentin, strong, no solubility, and possess high radiopacity [21].

Disadvantages: TheraCal LC was opaque and has a whitish color [5].

Bioceramics

Bioceramics encompass materials such as alumina, bioactive glass, zirconia, glass ceramics, hydroxyapatite, and resorbable calcium phosphates. In dentistry, these are used for filling bony defects, apical filling, root repairs, perforation sealing, as endodontic sealers, and for tissue regeneration. Based on their interaction with tissues, bioceramics are classified as either “bioinert” or “bioactive” [19, 25].

Mechanism: They function either as human tissues or can resorb and regenerate natural tissues [26].

Advantages: Biocompatible [9].

Disadvantages: brittleness, and poorly elastic in nature [9].

Biodentine

Biodentine, often refers to "dentine in a capsule "or "biocompatible and bioactive dentine substitute," developed to overcome limitations of calcium hydroxide and mineral trioxide aggregate [14].

Mechanism: Biodentine possesses mechanical properties similar to natural dentin, making it preferred material for procedures aimed at regenerating the dentin-pulp complex. It has a positive effect on vital dental pulp cells, stimulates the formation of tertiary dentin, and promotes reparative dentin production when in direct contact with the vital dental pulp [19].

Advantages: Best antimicrobial activity, biocompatible, stimulates tertiary dentin formation, strong, less solubility and tighter seals compared to Ca(OH)2, and lesser setting time.
Disadvantages: Lacks adhesion [2].

Enamel Matrix Protein (Emdogain-EMD)

Enamel Matrix Derivative was a bioactive molecule primarily contains amelogenin. EMD was derived from developing pig teeth, as their enamel proteins closely resemble those of humans.
Mechanism: EMD has been reported to increase alkaline phosphatase activity and stimulates the release of bone matrix proteins in osteoblasts. Amelogenin and amelin proteins play a vital role in odontoblast differentiation and dentin formation during dentinogenesis. EMD also promotes endothelial cells of pulp capillaries and odontoblasts to produce a hard tissue barrier upon the exposed pulp [27].
Advantages: EMD plays important role in odontogenesis, promotes pulp healing and regeneration by assisting odontoblast differentiation and hard tissue formation.
Disadvantages: The regeneration potential of EMD in endodontics was not fully understood, and further research was required.

Laser Applications

Lasers emits coherent, collimated, monochromatic light, making them valuable in dentistry. Recently, laser therapy has claimed attention as a non-pharmaceutical approach for vital pulp therapy (VPT). Various laser types, including the CO_2 laser, have been used on irradiate exposed pulp tissue, aiming to produce dentinal bridge formation [28].
Mechanism: Lower-intensity laser applications have been reported to decrease the inflammatory response in injured tissues without causing adverse effects. Their application enhances tertiary dentin formation on the dentin surface and also by providing sterilization [19].
Advantages: Deposition of secondary dentin, sterilization of targeted tissue, and bactericidal effects.
Disadvantages: Causes thermal injury to pulp in high doses, and technique sensitive [29].

Novel Endodontic Cement (NEC)

A newly developed endodontic cement (NEC), formulated by Asgary, consists of various calcium compounds [30].
Mechanism: It could be similar to MTA.
Advantages: Shorter setting time, biocompatible, do not cause tooth staining, better handling characteristics compared to MTA, and induces a thicker dentin bridge with less pulpal inflammation than MTA.
Disadvantages: Poor physical properties [31].

Bone Sialoprotein (BSP)

Bone sialo protein (BSP) was a non-collagenous protein found in mineralized dentinal connective tissues, including dentin, cementum, and calcified cartilage.
Mechanism: Bone sialoprotein (BSP) enhances the cell differentiation that effectively produce an organized extracellular matrix.
Advantages: NEC induces the formation of secondary dentin which was well mineralized and homogeneous [5].
Disadvantages: The cost of their clinical application can be an impediment.

Zinc Oxide Eugenol Cements

Zinc oxide eugenol was a sedative and analgesic material which was applied on dentin, it reduces microbial metabolism and restricts the diffusion of toxic substances to the pulp tissues [19].
Mechanism: When ZOE was applied to a tooth cavity, small amounts of eugenol diffuse through the dentinal tubules to the pulp. At low concentrations, eugenol demonstrates anti-inflammatory and obtundent properties, aiding in pulpal healing when used as a temporary filling material. However, at higher concentrations, it can be cytotoxic to pulp tissues.
Advantages: Zinc oxide eugenol cement destroys or inactivates microorganisms, kills microorganisms present in carious lesions, and good initial seal.
Disadvantages: The calcific bridges formation was not possible with zinc oxide-eugenol (ZOE) due to its lack of calcium [5].

Glass Ionomer Cement

Glass-ionomer cement (GIC) composed of a basic silica glass and an acidic polyacrylic polymer, which hardens through acid-base reaction between the components, as described by McLean, Nicholson, Wilson and Kent. GIC combines the advantages of both silicate and polycarboxylate cements and was commonly available in a powder-liquid forms.
Mechanism: Glass-ionomer cement (GIC) strongly bonds with enamel and dentin, provides sustained

fluoride release, demonstrates good biocompatibility, and has a coefficient of thermal expansion comparable to that of natural tooth structure [32].
Advantages: Amazing bacterial seal, anticariogenic due to release of fluoride, coefficient of thermal expansion and elastic modulus are similar to dentin, and excellent biocompatibility with pulp tissue [33].
Disadvantages: May Cause long-term inflammation with no formation of dentinal bridges, exhibits cytotoxic nature upon direct contact with cells, and also possesses poor physical characteristics [5].

Growth Factors and Proteins

Growth factors are natural polypeptide or protein hormones that provide essential cellular processes for tissue repair. They play a crucial role migration, mitogenesis, matrix synthesis, and remodeling during dental tissue repair [5].
Mechanism: Growth factors are polypeptides that bind with specific receptors on the surface of target cells, initiating intracellular signaling cascades. They can act in a paracrine or autocrine manner, transmitting signals to the nucleus and stimulating genetic mechanisms that influence cellular behavior and activity [19].
Advantages: Stimulates the formation of both osteodentin and tubular dentin, results in more uniform reparative dentin development, demonstrates greater effectiveness than calcium hydroxide ($Ca(OH)_2$) in promoting mineralization, among growth factors, and only TGF-β1 has been shown to effectively induce reparative dentin formation
Disadvantages: Potential for side effects, high production costs restricts clinical use, Ineffective in stimulating reparative dentin in inflamed pulp, short biological half-life, and requires high concentrations to achieve therapeutic effectiveness [34].

Castor Oil Bean (COB)

Castor oil bean (COB), derived from *Ricinus communis* polyurethane, was a polyester with amino radical that imparts bactericidal properties and ensures biocompatibility with living tissues. Originally developed as a biomaterial for bone repair and regeneration following localized bone damage, COB has shown favorable properties that make it a promising candidate for use in pulp capping procedures [15].
Advantages: Exhibits good antibacterial properties, demonstrates lower cytotoxicity, induces less inflammatory response compared to calcium hydroxide cement, promotes tissue healing, and offers superior sealing ability compared to MTA and GIC.
Disadvantages: Bioinert, and further clinical trials are needed [21].

Adhesive Systems

Adhesive resins are nearly as effective as calcium hydroxide in promoting dentin bridge formation and wound healing.
Mechanism: Primers in self-adhesive resin system are designed to simultaneously demineralize the inorganic structure and infiltrate the exposed collagen matrix, thereby preventing the collapse of demineralized dentin. When microleakage was minimized and bacterial contamination was controlled, the pulp has the potential to undergo natural healing [19].
Advantage: Prevents microleakage.
Disadvantage: Delayed response.

Stem Cells

Stem cells from human exfoliated deciduous teeth (SHED) and Dental pulp stem cells (DPSCs) are distinct populations of stem cells capable of self-renewal and multilineage differentiation. Notably, SHED demonstrates a significant high proliferation rate compared to DPSCs and bone marrow-derived mesenchymal stem cells (BMMMSCs) [21].
Advantages: Dentin pulp complex regeneration, and SHED was superior to DPSCs.
Disadvantages: Technique sensitive, and less economic [20].

Enzymes

Heme-oxygenase-1

Heme oxygenase-1 (HO-1) was a key enzyme that regulates heme catabolism. Its expression in oxidatively stressed dental pulp cells and odontoblasts indicates that pulp can respond to oxidative stress at the molecular level. The induction of HO-1 plays a protective role by mitigating hypoxic stress and nitric oxide-induced cytotoxicity [21].
Advantages: HO-1 provides cytoprotection to human pulp cells by shielding them from pro-inflammatory cytokines and nitric oxide. It also helps prevent cytotoxicity and oxidative stress caused by H_2O_2 in these cells.
Disadvantages: slow response.

Simvastatin

Statin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, was a first-line drug for hyperlipidemia. It enhances of function osteoblast through the BMP-2 pathway while suppressing osteoclast activity, promoting bone formation. Consequently, statins may enhance odontoblast function, potentially leading to improved dentin formation.

Advantages: Statins possess anti-inflammatory properties, promote angiogenesis, and enhance odontoblast function, and all of which may contribute to reparative dentin formation.

Disadvantages: Further evaluation studies are needed, and higher concentrations can cause damage to pulp tissue [21].

Corticosteroids and Antibiotics

Corticosteroids such as Cleocin, hydrocortisone, and Ledermix paste (a combination of calcium hydroxide and prednisolone), along with antibiotics like penicillin, neomycin, and Keflin (cephalothin sodium), have been used for pulp capping to minimize or prevent pulp inflammation [21].

Advantages: decreased pulp inflammation. And vancomycin + Ca(OH)2 combination stimulates reparative dentin bridge formation.

Disadvantages: contraindicated in patients at risk from bacteremia [21].

Hydroxyapatite

Nano-hydroxyapatite (Nano-HA) was synthetic calcium phosphate ceramic, exhibiting thermodynamic stability, excellent biocompatibility with pH of 7.0. It serves as an effective scaffold for newly formed mineralized tissue. In the early stages, vascularity increases in the Nano-HA group but gradually declines over time. Due to its ability to promote complete dentinal bridge formation and its favorable cellular and vascular responses, Nano-HA was considered a promising alternative for direct pulp capping procedure.

Advantages: Act as scaffold for the newly formed mineralized tissue (Biocompatible).

Disadvantages: Mild inflammation along with superficial pulpal necrosis [35].

Other Materials

Calcicur (80%) and Sealapex (60%) demonstrate significantly less success rate compared to ZOE or Vitapex [13]. Vitapex was composed of 30% calcium hydroxide, 40.4% iodoform, and 22.4% silicone oil. This pre-mixed, radiopaque material. It was also easily removable and absorbable if accidentally extruded beyond the apex, making it a popular and widely used choice in dental procedures [13]. Calcium hydroxide pastes with iodoform (Vitapex, Endoflas).There are a number of calcium hydroxide (CH) pastes that contain iodoform. CH provides the high pH >10 environment that, along with iodoform, has a high bacteriostatic effect [5].

Properties

A list of materials are included along with their proportion taken, mixing and setting time in Table 1.

Table 1: list of materials along with proportion taken, mixing and setting time

Material	Proportion taken	Mixing time	Setting time	References
Calcium hydroxide	1:1 base and catalyst paste	10-15 seconds	2.5-5.5 minutes	[3,28,36]
MTA	4:1 /2:1powder to liquid	1 minute	Initial: 165 minutes Final: 4 to 6 hours	
Endo sequence root repair material	Pre mixed	Direct application	2.5 to 10 minutes	
Theracal		45 seconds	Around 5 minutes	
Biodentine	5:1 powder to liquid	30secs in amalgamator	9-12 minutes	

Zinc oxide eugenol	4:1/6:1 powder and liquid	1-1.5 minutes	4-10 minutes
Glass ionomer cement	According to manufacture instructions	45 seconds	4-5 minutes

Composition

A list of pulp capping materials with composition are represented in Table 2.

Table 2: List of pulp capping materials with composition

PULP CAPPING MATERIALS	COMPOSITION	References
Calcium hydroxide cement	Base-paste: I-methyl-trimethylene disalicylate, calcium sulphate, titanium dioxide, calcium tungstate or barium sulfate Catalyst-paste: Zinc stearate, Zinc oxide calcium hydroxide, Ethylene toluene, sulfonamide	[3,15,19,21,28,37]
Propolis	Flavonoids, phenolics, other various aromatic compounds	
Calcium silicate based cements	Powder: Di calcium silicate, tricalcium silicate, calcium carbonate, zirconium dioxide. Liquid: calcium dioxide, water, superplasticizing agent like modified polycarboxylate	
MTA	75% Type I Portland cement, 20% bismuth oxide, 5% calcium sulfate dihydrate. Portlandcement-contains-55 wt% 3CaO, SiO_2(tricalciumsilicate), 19 wt% 2CaO.SiO_2 (dicalciumsilicate), 10 wt%3CaO.Al_2O_3(tricalcium aluminate), 7 wt%-tetracalciumaluminoferrite ($4CaO•Al_2O_3•Fe_2O_3$), 2.8 wt% magnesium oxide, 2.9 wt% sulfate, and 1.0 wt% free calcium oxide	
Endo Sequence Root Repair Material	Calcium silicate, zirconium oxide, monobasic calcium phosphate, tantalum oxide, fillers and thickening agents	
Theracal	45% wt mineral material (type III Portland cement),approximately 45% resin , 10% wt radiopaque component, 5% wt hydrophilic thickening agent (fumed silica)	
Biodentine	Powder: tricalcium-silicate, dicalciumsilicate, calcium-oxide, calciumcarbonate, ironoxide, zirconium oxide Liquid: water-soluble-polymer, water & calcium chloride	
Novel Endodontic cement	Calcium oxide, Calcium phosphate, Calcium sulphate, Calcium silicate, Calcium chloride, Calcium carbonate	

Zinc oxide eugenol cement	Powder: 69% Zinc oxide• 29% Rosin (to reduce brittleness), 0.7% Zinc acetate (accelerator), 1% Zinc stearate (plasticizer) Liquid: Mainly eugenol with some olive oil as a plasticizer
Glass ionomer cement	Powder: silica, alumina, fluorides like calcium fluoride, aluminum fluoride, and sodium aluminum fluoride. Liquid: poly acrylic acid with copolymers, and also contains tartaric acid and water
Castor oil bean	Natural polyol containing three hydroxyl radicals with 81-96% triglyceride of ricinoleic acid
Adhesive system	Primer: 10-methacryloyloxydecyl dihydrogen phosphate (MDP), silica, N,N-diethanol p-toluidine, canforoquinone, Hydroxy ethylmethacrylate(HEMA), dimetacrylate monomer,bisphenol A glycidyl methacrylate (BisGMA), canforoquinone

DISCUSSION

Pulp capping is defined as the treatment of an exposed vital dental pulp in which the wound is sealed with a dental capping material to facilitate the formation of a secondary dentin along with the maintenance of vitality of pulp [1,21]. These material plays key role in the success of treatment. Therefore, these materials should possess the ideal properties like stimulate reparative dentin formation, maintain pulpal vitality, release fluoride to prevent secondary caries, bactericidal or bacteriostatic, adhere to dentin, adhere to restorative material, resist forces during restoration placement and during the life of restoration, sterile, radiopaque, and provide bacterial seal [21,38]. Several materials had been introduced as pulp-capping agent, each having its properties and drawbacks.

Dental pulp is a highly vascular and contains loose connective tissue that contains various cells such as undifferentiated mesenchymal cell, fibroblast, odontoblasts, and other cells. These are self-renewal cells that can be transformed into odontoblasts, osteoblasts neural cells, and adipocytes. Therefore cytotoxicity of the material and dentin bridge formation are important factors considered to select the capping materials due to the fact that it can affect the healing and general health of the pulp. André Luiz Fraga Briso.et.al in 2006 conducted a study and concluded that, the response of the dental pulp capping with MTA was superior to calcium hydroxide, at a 1% significance level with MTA showing lower rates of infection and necrosis [34]. In another study conducted by Pathak et al., to compare the sealing ability and microleakage of RMGIC, MTA, and Biodentine, all three materials exhibiting some degree of microleakage. However, Biodentine demonstrated the least microleakage and was superior to the others [35]. Even in study conducted by Jalan et al., to evaluate response of pulp to direct pulp capping agents like calcium hydroxide and Biodentine, the Biodentine formed thicker dentinal bridges with less inflammation [39-42].

The non-collagenous protein in BSP can mineralize connective tissues such as dentin, cementum and calcified cartilage tissues by regulating hydroxyapatite crystal formation in bones and teeth. Jose et al., conducted a study to evaluate capacity to maintain tissue viability and concluded that Bone sialoprotein (BSP) promotes cell differentiation, enabling cells to secrete extracellular matrix more effectively and organized than any other pulp capping material used to date. [20]. Mahgoub stated that, ERRM possesses antimicrobial effects comparable to MTA. Additionally, the use of ERRM extends beyond root repair, as it has been proven to promote tissue regeneration in both apexification and apexogenesis [32]. The role of CGF in the proliferation and

mineralization of dental pulp cells (DPCs) was evident. Tian et al., conducted a study and concluded that, CGF was a promising pulp capping material for treating pulp injuries, as it enhances both the proliferation and mineralization of DPCs [43,44]. Afkhami et al., stated that, the evaluation of histological results of different dental lasers in combination with pulp capping materials shows enhanced success rate of VPT [39].

Selvendran.et.al, conducted a study for evaluating the success and efficacy of calcium silicate materials compared to calcium hydroxide (CH), leading to the conclusion that calcium silicate-based materials are superior to CH due to their enhanced ability to promote dentin bridge formation [45-47]. Ulusoy et al., stated in their article that, the hard dental tissue formation with Emdogain was twice as pronounced as in those treated with calcium hydroxide at both 2 and 4 weeks [19]. Qureshi et al., compared propolis, MTA and Dycal to evaluate histological properties in dental pulp of humans and concluded that bridge formation was similar with Propolis and MTA, when compared to Dycal [21]. Bostanci et al., conducted a study for evaluation of properties like physicochemical properties and radiopace nature of various light-curing pulp capping material, leading to the conclusion that bioactive pulp capping materials offer significant advantages over others by promoting dentin-like tissue formation, largely due to their high content of calcium and fluoride ions [48-50].

Gaps in Pulp Capping Agents Research

Despite advances in pulp capping agents, there remain important scientific knowledge gaps, including the lack of long-term clinical data (>10 years) for a direct comparison between materials like MTA, Biodentine, and bioceramics and limited understanding of their bioactive processes in dentin bridge development. Optimal material properties—combination of antibacterial efficacy, sealing, and biocompatibility—are not defined, and infection control measures to prevent bacterial microleakage have insufficient evidence. Regenerative therapies such as growth factor-based and stem cell therapy are hampered by clinical translation, and diagnostic equipment to foretell success (e.g., biomarkers or high-tech imaging) is primitive. Patient-specific factors (age, systemic disease) and the effect of pulp inflammation status on the outcome must be studied further, as well as unclear guidelines on direct vs. indirect pulp capping techniques and caries removal regimens. Also, cost barriers for new materials and the unavailability of standard clinical protocols hinder broader application. Upcoming research needs to focus on biomimetic and smart bioactive materials, scaffolds printed through 3D printing, and AI-assisted diagnostics to enhance predictability and availability in pulp capping therapy.

CONCLUSION

This review article classifies the pulp-capping agents into various groups as conventional, experimental, and hybrid materials. These materials exhibit varying degrees of the inflammation, which may decrease over time. Additionally, several experimentally developed materials—both alone and combined with conventional products have been studied to evaluate their effectiveness. Within the limitations of this review, most materials did not show superiority compared with the standard of care, CH. While MTA and Biodentin might be a valuable alternative to CH for direct pulp caps, no firm evidence was reached to strongly support any recommendations. These experimental materials show promising potential benefits along conventional agents, they still do not fully meet the criteria for ideal pulp-capping agents. Materials with new compositions should be evaluated comprehensively before their clinical application. Therefore, further *in vitro* and *in vivo* studies are necessary to examine and validate about calcium ion releasing ability, together with the cytotoxic effect and the clinical significance of these next-generation materials.

Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS Review Article

Pharmacologic and Clinical Applications of Commonly Administered Medications to Neonates: Focus on Vitamin K, Vitamin D, and Hepatitis B Vaccine Un Updated Review

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ABSTRACT

Background: The neonatal period is highly susceptible, and early pharmacological treatments can significantly reduce morbidity and mortality. Three commonly used neonatal interventions—vitamin K, vitamin D, and the hepatitis B vaccine—are highlighted by this review as being pharmacological in nature, clinically used, having recommended doses, and being safe.
Methods: A systematic search was conducted in PubMed, ScienceDirect, Google Scholar, and the Cochrane Library using MeSH terms and keywords (e.g., "vitamin K in neonates"). The literature was restricted to that published in the English language during 2015–2025 and involving human neonates.
Results: Vitamin K: The world standard is intramuscular (IM) injection of 0.5–1 mg at birth to prevent vitamin K deficiency bleeding, although alternative oral administration is less uniform. Vitamin D: A daily dose of 400 IU is recommended for all neonates, with up to 1,000 IU in preterm or deficient infants, to prevent rickets and hypocalcemia. Hepatitis B vaccine: Early vaccination within 12–24 hours of birth is essential, especially for babies born to HBsAg-positive mothers, who should also receive hepatitis B immunoglobulin (HBIG). The vaccine is highly effective and safe.
Conclusion: Vitamin K, vitamin D, and the hepatitis B vaccine are essential components of neonatal preventive care. Strengthening health systems, improving parent and healthcare worker education, and increasing access are vital for optimal outcomes.

Keywords: Vitamin K, Vitamin D, hepatitis B vaccine, newborn, neonatal pharmacology.

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INTRODUCTION

The neonatal period, the first 28 days of life, represents a window of extreme need for pharmacologic interventions for morbidity and mortality associated with perinatal and congenital illnesses [1]. Because neonatestheirimmature body functions present special pharmacokinetic and pharmacodynamic challenges that require special attention in drug ordering [2]. Despite these obstacles, certain medications are routinely administered to nearly all infants of the world soon after birth in order to effect a smooth transition from fetal to postnatal life [3].

Among the most popular agents utilized within the neonatal setting are vitamin K, vitamin D, and the hepatitis B vaccine [4-6]. Such drugs are recommended by key health agencies like the World Health Organization (WHO), American Academy of Pediatrics (AAP), and Centers for Disease Control and Prevention (CDC) due to their established use in preventing serious disorders like vitamin K deficiency bleeding (VKDB), nutritional rickets, and perinatal hepatitis B virus (HBV) infection [7-9].

Vitamin K is required for activation of coagulation factors and is given intramuscularly to prevent hemorrhagic disease of the newborn, a condition that can be fatal if left untreated [10, 11]. Vitamin D is crucial in mineralization of bone and for calcium balance, and supplementation is especially warranted in exclusively breastfed infants since human milk is poorly supplied with the vitamin [12]. The hepatitis B vaccine, administered in most instances within the first 24 hours of life, provides early immunological protection against vertical spread of HBV, a possible etiology of chronic liver disease [13].

This review attempts to address the pharmacologic properties, modes of action, clinical applications, and safety profiles of these three key neonatal drugs. Understanding their roles within the context of neonatal physiology is important in a bid to optimize therapeutic gain and deliver safe and effective therapy at this vulnerable phase of life.

METHODS

This review was conducted to examine and summarize the pharmacological considerations, clinical utilization, and current recommendations for three medications most commonly administered to neonates: vitamin K, vitamin D, and the hepatitis B vaccine. The approach taken followed a systematic process of identification, selection, and synthesis of the literature.

Literature Search Strategy

A systematic literature search was performed using four electronic databases, including PubMed, ScienceDirect, Google Scholar, and the Cochrane Library. The Medical Subject Headings (MeSH) and keywords used in the search included "vitamin K in neonates," "neonatal vitamin D supplementation," "hepatitis B vaccine newborn," "pharmacokinetics in neonates," "neonatal pharmacology," and "guidelines for neonatal drug administration." Boolean operators such as "AND," "OR," and "NOT" were used to limit the search.

Inclusion and Exclusion Criteria

The following criteria were met by the articles included: Published between the years 2015 and 2025 in the English language, they were on human neonates. had pharmacological properties, clinical guidelines, safety, or efficacy of the drugs under consideration and were randomized controlled trials, observational studies, review articles, or official guideline documents.

Exclusion criteria: non-neonate, non-human or animal studies, articles, and those with no full texts available.

Data Synthesis and Extraction

Qualitatively, the relevant information was extracted and synthesized. Significant themes such as drug mechanism of action, pharmacokinetics, dosing recommendations, clinical use, and safety profiles were extracted and compiled into coordinated sections. Global and regional guideline suggestions by WHO, CDC, and national pediatric societies were reviewed and compared.

RESULTS

Vitamin K

Pharmacologic Profile

Vitamin K_1 (phylloquinone) is the primary preparation employed for prophylaxis in neonates [14]. Pharmacokinetically, IM injection leads to rapid and sustained plasma levels, with a half-life of roughly 1.5 to 3 hours, and extensive hepatic storage that gives protection for weeks [17]. Oral administration is less reliable and requires dosing repetition [18]. As a fat-soluble vitamin, its

absorption in adults is [D2] dependent on bile salt and pancreatic enzyme [15]. Neonates have immature biliary and pancreatic function and a sterile gut, and therefore there is impaired absorption of oral vitamin K, particularly in infants who are exclusively breastfed [16].

Mechanism of Action

Vitamin K acts as a cofactor for the enzyme γ-glutamyl carboxylase, which carboxylates glutamic acid residues of the clotting factors to form γ-carboxyglutamate that can bind calcium and bind to phospholipid membranes. This enables activation of clotting factors II, VII, IX, and X, and proteins C and S [19].

Clinical Indications and Global Recommendations

The first-line use of vitamin K in neonates is the prevention of vitamin K deficiency bleeding (VKDB), formerly hemorrhagic disease of the newborn [20]. VKDB has three patterns of presentation: Early VKDB: ≤24 hours; associated with maternal drug intake (e.g., anticonvulsants, anti-tubercular drugs). Classic VKDB: Days 1–7; usually due to inadequate ingestion of vitamin K by the mother. Late VKDB: >2 weeks to 6 months; typically, severe with intracranial hemorrhage [20]. The WHO and AAP recommendation is an IM injection of 1 mg of vitamin K_1 in term neonates at birth. 0.5 mg IM in preterm or low-birth-weight neonates. Alternative oral administration (e.g., 2 mg at birth with repeated doses) is less potent but more prone to parental failure to comply [21]. See Table 1.

Variable intramuscular (IM) vitamin K availability and injection resistance across culture s in low-middle-income countries lead to the utilization of inferior oral regimens that put patients at risk of late-onset VKDB. Lack of good health system organization and suboptimal parental education also discourage universal coverage (22).

Table 1. Recommendation for Vitamin K Prophylaxis of the Newborn Infant

Population	Route	Dose & Schedule	Key Recommendation	Source
All term newborns	IM	0.5–1.0 mg once at birth	All newborn infants receive a single IM dose of 0.5 to 1.0 mg of vitamin K.	AAP (2022) NSH V4.0
All term newborns	Oral	1 mg once at birth	Effective against classic VKDB but fails to prevent late-onset unless multi-dose regimens are used	AAP (2022)
Term Infants (≥34 weeks)	IM	1mg	All healthy infants of 34 weeks and above should receive 1mg (0.1ml) Vitamin K (Konakion MM Paediatric) as soon as is practical after birth.	NSH V4.0
Preterm Infants (<34 weeks)	IM	0.5mg	All infants under 34 weeks gestation should receive 0.5mg (0.05ml via the included special syringe) Vitamin K (Konakion MM Paediatric) intramuscular (or intravenously on medical advice).	NSH V4.0
Preterm infants <1000 g	IM	0.3–0.5 mg/kg once at birth	The AAP has recommended a single IM dose of vitamin K of 0.3 to 0.5 mg/kg for preterm infants weighing less than 1000 g.	AAP (2022)
At Risk Infants	Oral	20mg	Mothers at high risk (taking potentially hepatic enzyme-inducing drugs during pregnancy, i.e., phenytoin, phenobarbitone,	NSH V4.0

			carbamazepine, primidone, topiramate, rifampicin, isoniazid, and warfarin) should be identified so that oral vitamin K 20 mg is given daily for 4 weeks prior to delivery.	
Breastfed infants	Oral	2 mg	In most countries with oral vitamin K supplementation policies, a late dose of oral vitamin K is recommended at 4 to 12 weeks to prevent late-onset VKDB in these infants.	AAP (2022) NSH V4.0

Adapted from AAP (2022) & NSH Neonatal Clinical Guideline V4.0 [23, 24].

Safety and Adverse Effects

Vitamin K is usually well tolerated. Side effects occur infrequently and consist of reaction at the injection site, pain, and bruising. Anaphylactic and hypersensitivity reactions are extremely rare. Prior suggestions linking IM vitamin K with childhood leukemia have been refuted by large population-based studies [25].

Vitamin D

Pharmacologic Profile

Vitamin D_3 (cholecalciferol) is used due to greater potency and longer half-life [26]. It is converted in the liver after intestinal absorption by 25-hydroxylation to 25(OH)D, which is the major circulating form and index of vitamin D status. It is further metabolized in the kidneys to the active hormone $1,25(OH)_2D$ (calcitriol) [27]. Maternal status provides significant dependence of neonatal vitamin D status [28]. Premature birth, dark skin, lower sunlight exposure, and extended exclusive breastfeeding are risk factors for deficiency [29]. Pharmacokinetics: 25(OH)D half-life of 2–3 weeks. Fat-soluble and fat-stored in adipose tissue. Oral supplement preferable in neonates [26].

Mechanism of Action

Vitamin D is bound by vitamin D receptors (VDRs) in the intestine, bone, kidney, and immune cells [30]. It promotes the absorption of calcium and phosphate, enhances mineralization of the bone, and regulates immune function [31]. In neonates, adequate vitamin D is required to avoid nutritional rickets, skeletal deformities, hypocalcemia, and seizures [8].

Dosage and Clinical Use Recommendations

AAP and the Endocrine Society recommend 400 IU/day of vitamin D_3 from the initial days of life for all babies, including those breastfed solely [32]. Preterm infants, especially when they have limited sun exposure or low birth weight, may require 800–1,000 IU/day [32]. WHO advises vitamin D supplementation specifically in areas of high prevalence of deficiency [33]. Deficiency in Sudan and sub-Saharan Africa in general is underreported, presumably because of maternal deficiency and cultural restrictions of sun exposure [34]. See Table 2 about recommendations for the treatment of low vitamin D in newborn infants.

Vitamin D: Motherly deficiency and limited supplementation programs in LMICs are accountable for rates of neonatal vitamin D deficiency that are overwhelmingly high. Cultural restrictions in sun exposure and poor healthcare infrastructure in rural areas aggravate the problem. (35)

Safety and Adverse Effects

Vitamin D has a wide therapeutic range. Its toxicity is rare but may be seen with prolonged high doses and causes hypercalcemia, irritability, vomiting, polyuria, and nephrocalcinosis. Preterm and formula-fed babies on multiple preparations of vitamin D must be monitored [36].

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Table 2. Recommendation for Treatment of low vitamin D In Newborn Infant

Category	Age / Population	Replacement Dosing	Maintenance / Prevention Dosing
Preterm Infants	≥ 1,500 g	Mild–Mod (20–29 ng/mL)400–800 IU/day	400 IU/kg/day (max 400 IU)
	< 1,500 g	All levels200–400 IU/day via enteral feeds	
	< 37 weeks gestation	Deficient (< 20 ng/mL)800 IU/day for 1 month then reassess	
Term Infants (< 3 months)	All feeding types	Deficient / Severe1,000 IU/day × 3 months	400 IU/day
		Mild–Mod (20–29 ng/mL) 400 IU/day × 3 months	
Infants (3–12 months)	All feeding types	Deficient / Severe1,000 IU/day × 3 months OR 50,000 IU "stat" once then reassess in 1 month	400 IU/day
		Mild–Mod400 IU/day × 3 months	400 IU/day

Adapted from (RCH). Vitamin D deficiency CPG [36, 37].

Hepatitis B Vaccine

Composition of the vaccine and pharmacologic profile

The hepatitis B vaccine is a non-live, recombinant vaccine prepared from yeast cells genetically modified to secrete hepatitis B surface antigen (HBsAg) [39]. It is preserved in aluminum-based adjuvants to enhance immunogenicity [40]. Pharmacokinetically, it is administered intramuscularly and produces an adaptive immune response in weeks with the development of neutralizing anti-HBs antibodies [41].

Mechanism of Action

Upon administration, the HBsAg is digested by antigen-presenting cells (APCs) and presented to helper T cells, leading to B cell proliferation and the generation of antibodies [42]. The development of anti-HBs antibodies (>10 mIU/mL) is considered to be protective against HBV infection [43].

Clinical Indications and WHO Recommendations

Hepatitis B is the leading cause of chronic liver disease globally. Neonatal infection, especially from vertical (mother-to-child) transmission, has a 90% probability of evolving into chronic infection [44]. WHO recommends a birth dose (0.5 mL IM) within the first 24 hours of life, ideally within 12 hours. And three additional doses at 6, 10, and 14 weeks with the pentavalent vaccine schedule. In neonates of HBsAg-positive mothers: Administer hepatitis B vaccine and HBIG (0.5 mL each) at separate sites within 12 hours of birth. And finish with a complete vaccine schedule [45]. See Table 3 about hepatitis B vaccine schedules for infants.

Hepatitis B Vaccine: Delayed birth doses and poor screening rates of pregnant women for HBsAg in LMICs limit the effectiveness of the vaccine. Challenges of cold chain storage and far-flung delivery sites contribute to the barriers in receiving timely administration. (46)

Safety and Adverse Effects

The hepatitis B vaccine is highly safe. Local pain, erythema, or low-grade fever are frequent side effects. Severe reactions such as anaphylaxis occur very rarely (estimated <1 per million doses), and there is no causal relation between vaccines and autism or neurodevelopmental disorders, as concluded by multiple studies [47].

Table 4. Hepatitis B vaccine schedules for infants, by infant birthweight and maternal HBsAg status

Birthweight	Maternal HBsAg status	Single-antigen vaccine		Single-antigen + combination vaccine	
		Dose	Age	Dose	Age
≥2,000 g	Positive	1	Birth (≤12 hrs)	1	Birth (≤12 hrs)
		HBIG	Birth (≤12 hrs)	HBIG	Birth (≤12 hrs)
		2	1–2 months	2	2 months
		3	6 months	3	4 months
				4	6 months

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	Unknown*	1	Birth (≤12 hrs)	1	Birth (≤12 hrs)
		2	1–2 months	2	2 months
		3	6 months	3	4 months
				4	6 months
	Negative	1	Birth (≤24 hrs)	1	Birth (≤24 hrs)
		2	1–2 months	2	2 months
		3	6–18 months	3	4 months
				4	6 months
<2,000 g	Positive	1	Birth (≤12 hrs)	1	Birth (≤12 hrs)
		HBIG	Birth (≤12 hrs)	HBIG	Birth (≤12 hrs)
		2	1 month	2	2 months
		3	2–3 months	3	4 months
		4	6 months	4	6 months
	Unknown	1	Birth (≤12 hrs)	1	Birth (≤12 hrs)
		HBIG	Birth (≤12 hrs)	HBIG	Birth (≤12 hrs)
		2	1 month	2	2 months
		3	2–3 months	3	4 months
		4	6 months	4	6 months
	Negative	1	Hospital discharge or age 1 month	1	Hospital discharge or age 1 month
		2	2 months	2	2 months
		3	6–18 months	3	4 months
				4	6 months

adapted from CDC [47].

HBIG = hepatitis B immune globulin; HBsAg = hepatitis B surface antigen.

The final dose in the vaccine series should not be administered before age 24 weeks (164 days).

DISCUSSION

This review critically examined pharmacokinetics, mode of action, and clinical practice guidelines for neonatal administration of vitamin K, vitamin D, and the hepatitis B vaccine. While these interventions are still the pillars of neonatal practice, examination also revealed disparities in access, variability in practice, and systemic barriers to timely and proper use. Through a blend of pharmacological evidence and real-life challenges, this review highlights not just the science underpinning these interventions but also the need for improved implementation plans and policy advocacy so that there can be universal and equitable neonatal coverage (48-50).

Vitamin K prophylaxis is generally advocated across the globe for the evident prevention of vitamin K deficiency bleeding (VKDB) in the form of potentially fatal late-onset VKDB [20, 51]. Intramuscular (IM) injection proved more bioavailable and longer in duration of protection compared to oral regimens, as confirmed through several comparative studies [21]. Nonetheless, some countries continue to administer oral vitamin K due to cultural opposition towards neonatal injections, which carries the risk of enhanced noncompliance and decreased efficacy [52].

Similarly, vitamin D supplementation in neonates, particularly those who are being exclusively breastfed, plays a crucial role in the prevention of rickets and complications of hypocalcemia [8]. Pharmacological reasoning has been supplied by the low level of vitamin D in human milk and high prevalence of maternal deficiency in the majority of low- and middle-income nations [53]. But the dose variation of recommendations from 400 IU/day to as much as 1,000 IU/day among preterm infants implies the lack of consensus concerning the optimal regimens for specific neonatal subgroups [32].

The hepatitis B vaccine is still one of the most successful interventions in neonatal care, and early vaccination has been shown to greatly reduce vertical transmission [54]. The use of both vaccine and hepatitis B immunoglobulin (HBIG) in infants whose

mothers are HBsAg-positive is an extremely effective combination strategy [55]. In practice, however, it has been shown that early use, particularly in the first 12 hours, remains low in the majority of resource-limited settings [55].

Despite shared clinical consensus regarding their efficacy, several controversies persist; vitamin K: the safety of IM injection, once linked (although disproven) to leukemia, remains an influence upon parental preference [56]. Secondly, the lack of a single-dose regimen that is as effective as IM dosing, taken orally, persists as a significant gap in our understanding [57].

Vitamin D: Large, randomized trials are extremely limited in neonates from geographically widespread areas [58]. Such trials are lacking from Africa and Southeast Asia, which are likely to have a high prevalence of deficiency but where reporting is poor [58]. The ideal dose in very-low-birth-weight infants and in comorbid patients is under-researched [59].

Hepatitis B Vaccine: The challenge of ensuring maternal screening for HBsAg status and integration of on-time HBIG administration into routine delivery care poses logistic hurdles. Additionally, the emergence of HBV variants might have implications for future vaccine efficacy, an issue not yet sufficiently examined in neonates [5].

The early pharmacologic interventions depicted early are not only preventive; they are crucial in the prevention of infant morbidity and mortality [61-63]. Their universal use reflects an investment by a system in public health, maternal-child integration of care, and practice based on evidence. Universal coverage, however, is thwarted in rural or underserved settings by logistical, educational, and cultural barriers. Neonatologists, midwives, and public health workers should be well-trained and well-equipped to employ these agents early and appropriately [61-63].

CONCLUSION

Pharmacologic and clinical use of vitamin K, vitamin D, and hepatitis B vaccine among newborns is a reflection of urgent, evidence-based interventions that significantly reduce the risk of potentially life-threatening conditions such as VKDB, nutritional rickets, and vertical hepatitis B transmission. Despite their proven efficacy, there remains a challenge to guarantee equal application, particularly where low resources and ethnic diversity prevail.

Intramuscular prophylaxis with vitamin K remains the gold standard for VKDB prevention, but diverse oral dosing practices and parent resistance highlight the need for improved communication and alternative regimens. Vitamin D supplementation is critical in exclusively breastfed infants and preterm infants, but divergent dosing recommendations and underreporting of deficiency necessitate stronger data in different populations. The hepatitis B vaccine, especially in combination with HBIG in infants of HBsAg-positive mothers, is the cornerstone of infection prevention, but delays in making the birth dose available restrict its best utilization in most settings.

In order to optimize neonatal outcomes, there is a need for concerted systemic effort towards optimizing the education of healthcare professionals, parents' education, and policy advocacy. Additional research is also required in order to standardize dosing in special populations as well as improve access to adequate delivery systems. These early interventions are not only life-saving pharmacologic interventions but also integral parts of long-term public health agendas.

Recommendations

Promote universal administration of vitamin K, vitamin D, and the hepatitis B vaccine as a routine procedure of neonatal care. Promote education and awareness among parents and health workers regarding the importance and safety of the medicines. Enhance national policy and guidelines to enable timely administration, especially in low-resource settings. Encourage further studies on individualized dosing and pharmacokinetics in special neonatal populations (e.g., preterm or low birth weight infants).

Conflict of Interest

The authors declare that no conflict of interest

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