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OPEN ACCESS ORIGINAL ARTICLE

Khat Chewing and Its Association with Dental Caries in Yemeni Adults: A Cross-Sectional Study in Aden

Omar Abdullah Rageh[1,*], Gehad Omar Al-Kuhlani [1] , Ibrahim Abdullah Al-Qadhi[1] , Abdulrahman Hussein Ahmed[1] , Saif Salah Yahya[1] , Mohammed Munasser[1], Nasser Hamed Al-Khalifi[1], Zakaria Al-Dobai[1]

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ABSTRACT

Background: Although chewing khat is a deeply ingrained cultural practice in Yemen, little is known about how it may contribute to tooth caries, despite potential biological explanations.
Objective: This study investigated the association between khat consumption and the prevalence of dental caries in Yemeni adults.
Method: A cross-sectional study included 267 participants from seven Yemeni governorates. Caries prevalence was assessed using the WHO DMFT index. Data on khat use frequency, oral hygiene practices, and sociodemographics were collected via structured interviews. Multivariable logistic regression controlled for confounders (age, smoking, brushing frequency).
Results: A dose-dependent association was observed between regular khat use and caries prevalence (69% vs. 25%, $p<0.001$), with OR=2.8 (95% CI: 1.9–4.1) for >3 sessions/week. Poor oral hygiene (only 26.2% brushed twice daily) compounded caries risk (OR=2.1, 95% CI:1.2–3.7). Geographic disparities were notable, with caries prevalence highest in Mansoura (69%) and lowest in Brega (54%).
Conclusion: Khat chewing is a dose-dependent risk factor for dental caries in Yemeni adults, exacerbated by poor oral hygiene. Public health initiatives should target high-frequency khat users in high-prevalence areas with preventive and behavioral interventions.
Keywords: Dental caries, Khat chewing, Oral health, Yemen, Risk factors, Public health

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INTRODUCTION

According to the Global Burden of Disease Study, dental caries is the most common non-communicable disease globally, making it a serious public health concern. This health burden takes on particular dimensions in khat-endemic areas of Yemen, the Horn of Africa, and the Arabian Peninsula due to the interaction between traditional chewing methods of khat (Catha edulis) and contemporary dietary habits (1).

By suppressing parasympathetic activity, the psychoactive shrub's cathinone and cathine alkaloids cause xerostomia and lower salivary flow rates by 40–60% while chewing (2). The prolonged low-pH oral environment produced by this pharmacological impact encourages enamel demineralization, and compensatory sugar consumption—which has been seen in 72.3% of Yemeni chewers increases the risk of caries by increasing the acidogenicity of biofilms (3).

Numerous research has reported on the effects of khat on dental health, yet there are still important knowledge gaps. Although khat was initially linked to higher Streptococcus mutans counts by Al-Hebshi and Skaug (2005) (4), other studies have not been able to clearly determine dose-response connections between the frequency of chewing and the severity of caries (5). Furthermore, it is still difficult to quantify the relationship between khat and other risk factors that are common in these areas, such as tobacco smoking (6), poor oral hygiene (7) and nutritional deficiencies (8).

Three significant developments in this study contributed to a better understanding of this complex etiology. In order to overcome the urban bias in earlier clinic-based studies, we first use stratified sampling across seven governorates to capture regional differences in chewing habits and caries prevalence. Second, expanding on the bivariate analyses that dominated previous work, we use multivariate logistic regression to evaluate effect modification between khat and co-exposures (9). Third, we included behavioral data on adjunctive sugar usage and chewing length, which were not included in earlier epidemiological surveys (2).

The clinical urgency of this research is underscored by Yemen's deteriorating oral healthcare infrastructure amid ongoing humanitarian crises. With dentist-to-population ratios below 1:50,000 in rural areas and fluoride toothpaste access limited to 31% of households, preventive strategies must target the most modifiable risk factors (10). Our findings directly inform WHO's Framework for Oral Health in Emergencies by identifying priority interventions for khat-using populations, while contributing novel data to the ongoing debate about substance-specific caries risk profiles.

A survey revealed that 90% of adult men and 20% of adult women were regular chewers (11). According to research, oral cancer is one of the most common tumors in Yemen and makes up a sizable portion of all cancer cases. Contributing factors include the extensive use of smokeless tobacco products like Shammah and chewing khat (12, 13). This study investigated the association between khat consumption and the prevalence of dental caries in Yemeni adults at dental clinics of University of Science and Technology, Aden.

METHODOLOGY

Population and Study Design

Between March 2022 and January 2023, 267 persons (≥18 years old) were included in this study from dental clinics in seven Yemeni governorates (Mansoura, Brega, Aden, Tawahi, Sheikh Othman, Dar Sad, and Mualla) for this cross-sectional analytical study.

Sample size

The sample size was calculated based on an estimated caries prevalence of 50% among khat chewers, with 80% power and a 5% significance level, yielding a minimum required sample of 250 participants.

Inclusion criteria

People (age ≥18 years old) registered at the dental clinics of University of Science and Technology, Aden.

Exclusion Criteria

The following conditions were excluded from this study:

- Pregnancy (due to hormonal effects on dental health).
- Systemic conditions affecting caries risk (e.g., diabetes mellitus, Sjögren syndrome).
- Antibiotic use within the past 3 months.

Data Collection

Clinical Examination

Under standardized lighting, two calibrated dentists were conducted examinations using the WHO guidelines (2013) for dental caries (DMFT index). At the cavitation level (D3 threshold), caries was observed.

Behavioral Survey

Conducted using Arabic-language structured interviews, covering: Use of Khat: Typical chewing time per session, frequency (sessions per week), and duration (years). Oral hygiene: Using fluoride toothpaste and brushing frequently. Co-exposures include sugar intake during khat sessions and smoking

Statistical Analysis

Data analysis was done with IBM Corp.'s SPSS v26: Descriptive statistics include mean SD for continuous variables and frequencies (%) for categorical variables (Table 1). Analysis of two variables: Caries prevalence chi-square tests by khat frequency group (Table 2). DMFT score comparisons between chewers and non-chewers using independent t-tests. Modeling with multiple variables: Age, smoking, and frequency of brushing were all controlled for using logistic regression (Table 3). The model contained variables that had a bivariate analysis.

Ethical Considerations

The Institutional Review Board of the University of Science and Technology, Aden approved the study (Ref: UST-Dent/2022-01). After being informed about the study in Arabic, participants were given their written consent.

RESULTS

The majority of participants were young adults (19–28 years, 49.1%), reflecting a high burden of caries in this demographic. Students (71.2%) and factory workers (13.9%) represented key occupational groups, suggesting targeted interventions for these populations (Table 1).

Table 1: Participant Characteristics, (n=267)

Category	Subgroup	Frequency	Percentage
Age	19–23 years	131	49.1%
	24–28 years	84	31.5%
	Other ages	52	19.4%
Occupation	Students	190	71.2%
	Factory workers	37	13.9%
	Other occupations	40	14.9%
Location	Mansoura	143	53.6%
	Other areas	124	46.4%

Khat chewers had significantly higher caries prevalence (69% vs. 35%, p<0.001), reinforcing its role as a primary risk factor. Smoking alone showed no significant association (p=0.313) (Table 2).

Table 2: Habits and Dental Caries Prevalence

Habit	With Caries (n=190)	Without Caries (n=77)	Total	p-value
Khat chewing	131 (69%)	27 (35%)	158	<0.001
Smoking	48 (25%)	15 (19%)	63	0.313
Tooth brushing	130 (68%)	66 (86%)	196	0.04
Chewing Shamah	24 (13%)	2 (3%)	26	0.012

Khat chewing (OR=2.8) and poor oral hygiene (OR=2.1) were the strongest predictors of caries, highlighting the need for combined behavioral and preventive strategies Table (3).

Table 3: Significant Risk Factors for Dental Caries

Factor	Odds Ratio (OR)	95% CI	p-value
Khat chewing	2.8	1.9–4.1	<0.001
No tooth brushing	2.1	1.2–3.7	0.04
Chewing Shamah	1.9	1.1–3.3	0.012

Regional disparities (Mansoura: 69% vs. Brega: 54%) may reflect differences in khat chewing habits, access to dental care, or dietary patterns Table (4).

Table 4: Geographic Variations in Caries

Location	With Caries	Without Caries	Total
Mansoura	99 (69%)	44 (31%)	143
Aden	27 (84%)	5 (16%)	32
Brega	22 (54%)	19 (46%)	41

DISCUSSION

Regular khat chewers have 2.8 times higher odds of developing dental caries (OR=2.8, 95% CI:1.9-4.1), according to our study, which shows a substantial correlation between khat chewing and dental caries. This discovery is consistent with earlier studies by Al-Hebshi and Skaug (2005), who determined khat's cariogenic potential by examining its impact on oral flora (4). Strong evidence that khat is a modifiable risk factor is provided by the observed dose-response relationship, which showed that participants who chewed khat more than three times per week had an 85% caries prevalence, compared to 25% for non-users. This gradient effect most likely represents the combined pharmacological effects of supplementary sugar usage during chewing sessions (5) and khat-induced xerostomia (2).

Regular toothbrushing had a protective impact (OR=0.48, 95% CI:0.29-0.79), which confirms the findings of a systematic study by Al-Maweri et al. (2020) regarding the significance of oral hygiene in khat-using communities. However, the fact that only 26.2% of participants continued to brush frequently enough indicates that the dangers associated with khat are not sufficiently reduced by current preventive measures (6).

In addition to supporting trends shown in the Yemeni National Oral Health Survey, the significant geographical variance in caries prevalence (Mansoura: 69% vs. Aden: 84%) probably reflects variations in chewing habits, dietary habits, and access to dental care (14). Students (prevalence of 50.2%) and manufacturing workers (10.5%) are identified as priority targets for interventions due to their significant burden.

Limitations of the Study

Several important limitations should be considered when interpreting these findings. First, the cross-sectional design precludes establishing causal temporal relationships between khat consumption and caries development. Second, self-reported behavioral data may be subject to recall bias and social desirability effects. Third, the absence of biological measurements such as salivary flow rates and pH levels limited our ability to explore underlying mechanisms. Additionally, the clinic-based sampling approach may restrict generalizability to non-treatment-seeking populations. Finally, while we adjusted for key covariates in our analyses, residual confounding from unmeasured variables (including detailed dietary patterns) may persist. Future longitudinal studies should incorporate biochemical markers (e.g., salivary pH, bacterial profiling) to elucidate causal mechanisms. Cost-effectiveness analyses of targeted fluoride programs are also warranted.

Implications for Public Health and Future Paths

The inclusion of khat chewing in WHO oral health risk assessments is strongly supported by these findings. Interventions that are specifically targeted should: Inform people about the effects of khat on dental health in both work and school environments. Encourage the availability of inexpensive fluoride toothpaste in places with a high prevalence. Examine harm-reduction techniques such as chewing sugar alternatives.

In order to prove causation and examine biological pathways using salivary biomarker analysis, future studies should use longitudinal designs. Cost-effectiveness studies of targeted prevention programs are also warranted.

CONCLUSION

This study offers solid proof that chewing khat is a significant, dose-dependent risk factor for dental cavities in adult Yemenis. Khat use combined with poor dental hygiene results in a high-risk profile that needs immediate public health intervention. These results emphasize the necessity for culturally appropriate measures to lessen the negative effects of

khat on oral health while also respecting its cultural relevance.

Conflict of Interest

The authors declare that no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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OPEN ACCESS Original Article

Knowledge, Attitudes, and Practices on Sexually Transmitted Infections Among Tertiary Students in Ghana: A Cross-Sectional Study

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ABSTRACT

Background: Sexually transmitted infections (STIs) remain a growing public health challenge among young people in sub-Saharan Africa, particularly in Ghana. Tertiary students are vulnerable due to risky sexual behaviors, stigma, and insufficient knowledge of STIs.
Objective: This study aimed to assess the knowledge, attitudes, and practices (KAP) related to STIs among tertiary education students in Ghana and to examine how demographic characteristics influence these factors.
Methods: A cross-sectional descriptive study was conducted among 1,500 students from six tertiary institutions using a stratified random sampling technique. Data were collected through a structured, self-administered questionnaire and analyzed using SPSS Version 25. Descriptive statistics, chi-square tests, and binary logistic regression were used to explore associations and predictors.
Results: Only 58.5% of students demonstrated adequate knowledge of HIV/AIDS, while knowledge of other STIs was lower: syphilis (45.3%), gonorrhea (40.1%), and chlamydia (35.0%). Although 72.4% reported ever using condoms, only 28.6% practiced consistent condom use, and just 9.4% had undergone STI testing. Female and rural students reported significantly higher stigma scores (mean = 4.0 and 4.1, respectively, on a 5-point scale) compared to male and urban peers. Key predictors of poor knowledge and practices included rural residence, lower education level, and female gender ($p < 0.05$).
Conclusion: Findings reveal concerning gaps between STI awareness and preventive behavior among Ghanaian tertiary students, particularly in rural and female subgroups. These results underscore the need to integrate STI education into tertiary curricula, expand access to youth-friendly STI testing, and implement stigma-reduction campaigns. Targeted policies and campus health interventions are critical for improving sexual health outcomes in this at-risk population.

Keywords: Sexually transmitted infections, tertiary students, Ghana, KAP study, stigma, sexual health behavior.

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INTRODUCTION

As noted by the World Health Organization, STIs create a significant public health concern with an alarming rate of over one million new infections reported daily [1]. Infections such as HIV/AIDS, syphilis, gonorrhea and chlamydia not only threaten individual well-being but also the capacity of healthcare systems to function – particularly in developing and emerging economies. Among the youth and adolescent populations, the prevalence of STIs is notable in Ghana, as they have become one of the principal public health problems. If left untreated, sexually transmitted infections may result in serious lifelong health complications such as chronic infertility, pelvic inflammatory disease, cervical cancer, and a higher likelihood of contracting HIV [2, 3].

Compared to other populations within the country, the youth, especially those at the tertiary level of education, remain one of the most vulnerable groups for STI exposure. This unique situation is due to a combination of behavioral, sociocultural, and structural factors. Tertiary students are generally undergoing a life stage shift which involves a new level of freedom and an experimentation phase that includes new social and sexual interactions. This population lacks sufficient accurate, comprehensive, and nonjudgmental sexual health information [4], which in turn facilitates sexual behaviors that can be classified as risky, such as inconsistent condom use, multiple sexual encounters, and even transactional sex [4]. Numerous studies established that Ghanaian tertiary students often engage in high-risk behavior only because these students lack informed decision-making frameworks [5, 6].

STI awareness in Ghana remains critically low. There is little awareness of, and even less informed emphasis placed on, sexually transmitted diseases and infection (STI) knowledge during adolescence, despite the existence of national health promotion frameworks and STI awareness campaigns targeting adolescents. Inhibited norms regarding sexuality lead to predominant myths regarding the STI unit within public health, accompanied by misconceptions of their symptoms, methods of transmission, and preventative measures unknown outside sexually restricted environments [7]. These myths promote unattended social stigma, which perpetuates overt silence, postponement of testing, and treatment-seeking behaviors. Many students, with increased diagnosis delay, remain unaware of their STI status and, along with heightened infection rates, are placed at significant risk of adverse health outcomes [8].

Moreover, the gaps in knowledge, attitudes, and practices (KAP) of STIs may differ considerably with respect to age, gender, or even region. For example, urban students may have better educational services than their counterparts in rural or peri-urban settings. In the same manner, sociocultural norms and gender roles may affect how men and women differ on self-perception of sexual risk, condom negotiation, and STI prevention service utilization [9, 10]. Without careful consideration of how these demographic nuances interact with STI-related behaviors, health programs designed might not be able to reach the most at-risk populations or create a substantial impact.

Keeping these issues in mind, it is essential to not only evaluate gaps in KAP among students in higher learning institutions in Ghana but, more importantly, discern the backdrop that shapes such a distinctive approach. This study, thus, aims at assessing the STI awareness, attitudinal, and behavioral patterns of students in higher learning institutions and how these are associated with the students' age, gender, and urban or rural place of residence.

The results from this study are aimed at contributing to the evidence base to aid in the development of specific and contextualized sexual health interventions. Improving STI-related knowledge and the associated health risks within this demographic is crucial to enhancing sexual and reproductive health indicators, alleviating the burden of STIs on the healthcare system, and fulfilling public health equity objectives in Ghana, as well as empowering the youth within the nation.

METHODS

Study Design

This study employed a cross-sectional descriptive design to assess knowledge, attitudes, and practices (KAP) related to sexually transmitted infections (STIs) among tertiary students in Ghana. The cross-sectional approach was selected, as it is widely used for measuring the prevalence of behaviors and knowledge within a population at a single point in time [11]. However, such a design limits the ability to infer causality between variables.

Study Population and Sampling Procedure

The target population comprised tertiary education students aged 18–30 years enrolled in six public and private universities across different regions of Ghana. A stratified random sampling technique was adopted to ensure demographic and geographic representation. Stratification was based on:

- Geographical location (urban vs. rural-based institutions)
- Type of institution (public vs. private)
- Academic programs (health sciences, humanities, and general sciences)

Stratified sampling enhances the representativeness of subgroups and improves statistical efficiency [12]. Within each stratum, participants were randomly selected using class registers and student association lists. Researchers approached students in classrooms and lecture halls after obtaining permission from institutional authorities.

Recruitment and Participation

Eligible students were invited to participate through announcements during lectures and follow-up by class representatives. Students were provided with information sheets and consent forms. Those who agreed completed the questionnaires anonymously during scheduled classroom sessions under the supervision of trained research assistants.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Enrolled as a full-time student in a participating tertiary institution in Ghana.
- Aged between 18 and 30 years.
- Willing to provide informed consent.

Exclusion Criteria

- Visiting or exchange students.
- Individuals unable to read or understand the survey language (English).
- Students who declined to participate or withdrew at any point.

These criteria were set to ensure homogeneity of the target group and reduce confounding factors.

Questionnaire Design and Administration

Data was collected using a structured, self-administered questionnaire, which has been shown to be effective in capturing sensitive sexual health information while minimizing social desirability bias [13]. The questionnaire consisted of four sections:

1. Demographics (e.g., age, gender, academic level, institution, location)
2. Knowledge: 25 multiple-choice and true/false questions assessing awareness of STI types, symptoms, transmission, and prevention
3. Attitudes: 10 items using a 5-point Likert scale (strongly disagree to strongly agree) exploring personal beliefs and stigma
4. Practices: 8 items on behaviors, including condom use, STI testing, and sexual history

Validity and Reliability

The questionnaire was pre-tested on 50 students from a tertiary institution not included in the main study. Feedback was used to refine language clarity and cultural sensitivity. The reliability of each domain was assessed using Cronbach's alpha, a widely accepted measure of internal consistency [14]:

- Knowledge: $\alpha = 0.81$
- Attitudes: $\alpha = 0.76$
- Practices: $\alpha = 0.74$

These results indicate acceptable reliability for the scale domains.

KAP Score Calculation

Each section of the questionnaire was scored independently:

- Knowledge: Each correct response scored 1 point; incorrect or "don't know" answers scored 0. Maximum score = 25. Scores ≥12.5 (50%) were classified as adequate knowledge, consistent with established cutoffs in STI KAP literature [15].
- Attitudes: Responses on a 5-point scale were averaged. A mean score ≥3.0 was considered indicative of positive attitudes toward STI prevention and health-seeking behavior.
- Practices: Responses were binary (yes/no or always/never). Good practices included consistent condom use, regular STI testing, and monogamous sexual activity. A composite score of ≥4 out of 8 was defined as a good STI-related practice.

An overall KAP index was also created for multivariate analysis.

Data Analysis

Data was entered and cleaned using Microsoft Excel and analyzed with SPSS version 25. Descriptive statistics (frequencies, means, standard deviations) were used to summarize responses. Chi-square tests assessed associations between categorical variables

(e.g., gender and knowledge level). Binary logistic regression was used to identify demographic predictors of good knowledge and practices. Statistical significance was set at $p < 0.05$.

Limitations of Study Design

As a cross-sectional study, causality between knowledge and behavior cannot be established [16]. Additionally, responses may be subject to recall bias or social desirability bias, especially in the context of sexual behavior. Finally, while the questionnaire was pre-tested, cultural variability between institutions may still influence the interpretation of certain items.

Ethical Considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the University of Ghana, Legon (IRB Protocol No. UG-IRB/2024-144). All procedures adhered to the ethical principles outlined in the Declaration of Helsinki [17]. Written informed consent was obtained from each participant prior to data collection, and participation was entirely voluntary.

To ensure anonymity and confidentiality, no personal identifiers were collected on the questionnaires. Completed forms were stored in a locked cabinet, and digital data were password-protected and accessible only to the research team. Participants were informed that they could withdraw from the study at any time without facing any consequences.

RESULTS

The study involved 1,500 tertiary students across Ghana, evenly split by gender (750 males and 750 females).

Table 1: Demographic Characteristics of Participants (N = 1,500)

Characteristic	Category	n (%)
Age Group	18–20	700 (46.7%)
	21–25	560 (37.3%)
	26–30	240 (16.0%)
Gender	Male	750 (50.0%)
	Female	750 (50.0%)
Education Level	Undergraduate	950 (63.3%)
	Postgraduate	450 (30.0%)
	Vocational	100 (6.7%)
Residence	Urban	950 (63.3%)
	Rural	550 (36.7%)

Most participants were aged 18–20 years (46.7%), followed by 21–25 years (37.3%) and 26–30 years (16%). A majority were undergraduates (63.3%), while postgraduates constituted 30.0% and vocational students 6.7%. Regarding residence, 63.3% lived in urban areas and 36.7% in rural areas.

Table 2: STI Knowledge Domain Performance (N = 1,500)

Δ = Difference between urban and rural means; p-values indicate significance of difference across settings.

Domain	Max Score	Mean (±SD)	Adequate Knowledge (%)	Urban-Rural Gap (Δ)
HIV/AIDS	25	17.5 (±4.2)	58.5%	+6.4**
Syphilis	25	12.3 (±3.9)	45.3%	+4.2*
Gonorrhea	25	10.2 (±3.5)	40.1%	+5.6*
Chlamydia	25	9.6 (±3.2)	35.0%	+5.1*

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

Participants' knowledge was evaluated across four STI domains: HIV/AIDS, syphilis, gonorrhea, and chlamydia. Scores were based on a 25-point scale for each STI. A minimum of 12.5 points (50%) was considered "adequate knowledge".

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As shown in Table 2, knowledge was highest for HIV/AIDS (58.5% adequate knowledge) but significantly lower for syphilis (45.3%), gonorrhea (40.1%), and chlamydia (35.0%). Urban students consistently outperformed rural peers across all domains.

Table 3: Perception Scores by Subgroup (5-point Likert Scale)

Dimension	Total Mean (±SD)	Male	Female	Urban	Rural
Stigma	3.7 (±0.9)	3.5	4.0***	3.5	4.1**
Perceived Risk	3.0 (±1.1)	2.8	3.3**	3.2	2.6***
Self-Efficacy	3.5 (±0.8)	3.6	3.4	3.6	3.3*

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

Participants' perceptions were measured on a 5-point Likert scale (1 = Strongly Disagree to 5 = Strongly Agree) for three dimensions: stigma, perceived risk, and self-efficacy. Mean scores and subgroup differences are shown in Table 3.

Female and rural students reported higher levels of STI-related stigma. Rural students also exhibited significantly lower perceived risk and self-efficacy compared to their urban counterparts, indicating limited awareness and confidence in managing STI-related health.

Table 4: Reported Sexual Health Behaviors (N = 1,500)

Behavior	n (%)
Ever Used Condoms	1,086 (72.4%)
Consistent Condom Use	429 (28.6%)
Regular STI Testing	141 (9.4%)
Multiple Sexual Partners	320 (21.3%)

As detailed in Table 4, 72.4% of participants reported ever using condoms, but consistent condom use was low at 28.6%. Most concerning was the low STI testing rate of just 9.4%, signaling a major gap in preventive health behavior.

These findings indicate a mismatch between awareness and preventive practice. While knowledge exists, the adoption of protective behaviors remains suboptimal, underscoring the need for targeted interventions.

DISCUSSION

The findings revealed notable disparities in STI knowledge between urban and rural tertiary students in Ghana. Urban students consistently demonstrated higher awareness levels across all STI categories—HIV/AIDS, syphilis, gonorrhea, and chlamydia—compared to rural counterparts. This disparity supports previous studies indicating that urban populations often benefit from greater exposure to health information, school-based education, and digital media platforms [18, 19].

While it may be tempting to attribute rural knowledge gaps solely to curricular deficiencies, this explanation should be approached cautiously. Structural factors such as limited internet access, fewer trained educators, and pervasive cultural taboos around sexual health in rural areas likely contribute more significantly [3]. For example, rural students may lack exposure to peer discussion groups or health outreach programs that are more common in urban campuses [20]. Further qualitative research is recommended to explore the sociocultural and institutional contexts behind these observed disparities.

Despite a relatively high rate of reported condom use (72.4%), only 28.6% of participants reported consistent use—highlighting a troubling disconnect between awareness and sustained protective behavior. This pattern is well-documented in African youth populations, where cultural perceptions, trust in sexual partners, and discomfort with condom negotiation often interfere with consistent use [21, 22].

More concerning is the STI testing rate, which stood at just 9.4%. This figure represents a major public health gap, especially considering the sexual activity reported and the 21.3% of respondents who had multiple sexual partners. Low testing uptake is likely influenced by fear of stigma, lack of youth-friendly health services, and limited access to confidential screening—especially in rural areas [23, 24]. Regular

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STI screening is essential for early diagnosis and prevention, and this study underscores the urgent need to scale up awareness and access among university populations. Gender disparities were evident in both attitudes and practices. Female students reported significantly higher levels of STI-related stigma compared to males. This finding echoes prior research that suggests women in many African contexts bear a disproportionate burden of sexual shame and social punishment, especially when discussing or seeking care for STIs [8, 25].

In addition, rural students were more likely to perceive STIs as a source of personal shame or community disgrace. The persistence of such stigma prevents open conversations, deters health-seeking behaviors, and reinforces misinformation about STIs [26]. Community health education campaigns must therefore tackle both misinformation and the moralistic framing of STIs, especially in rural and religiously conservative settings.

The findings hold important implications for both policy and university-level intervention. Firstly, tertiary institutions should incorporate comprehensive sexual health education into general orientation programs and coursework. Peer education models and mobile health interventions, proven effective in sub-Saharan African contexts, can be adapted for university campuses [27]. In addition, collaboration with NGOs can enable periodic campus-based STI screening events and distribute free condoms discreetly. Policymakers and health authorities must also work to improve access to youth-friendly STI services, particularly in underserved regions. Confidentiality, affordability, and non-judgmental service delivery are key to encouraging STI testing and routine sexual health checks [24]. Public messaging should be reframed to normalize STI prevention as a health responsibility rather than a moral failing.

Finally, future interventions must adopt an intersectional lens, recognizing how gender, location, and socio-economic status interact to shape vulnerability. The KAP data from this study provide a critical framework for tailoring targeted campaigns and health policies that reflect the lived realities of Ghanaian youth.

CONCLUSION

This study highlights significant knowledge gaps, behavioral inconsistencies, and stigmatizing attitudes regarding sexually transmitted infections (STIs) among tertiary education students in Ghana. While awareness of HIV/AIDS was relatively high, knowledge of other STIs such as syphilis, gonorrhea, and chlamydia remained poor, especially among rural students. Although the majority reported prior condom use, consistent use was low, and only 9.4% had ever undergone STI testing. Additionally, stigma surrounding STIs was markedly higher among female and rural respondents, influencing both attitude and health-seeking behavior.

These findings underscore the urgent need for institutional and public health interventions that priorities sexual health among young people. Tertiary institutions should integrate comprehensive STI education into their core curricula, supported by peer-led programs and access to confidential health services. In rural regions, outreach programs—delivered through community health workers or mobile clinics—are essential to bridge the access and information gap. Policy efforts must also focus on reducing stigma through culturally sensitive awareness campaigns and expanding routine, youth-friendly STI screening across tertiary campuses. These interventions should be informed by demographic disparities identified in this study to ensure relevance and impact.

Limitations

Limitations of this study include its cross-sectional design, which prevents causal inferences, and the reliance on self-reported data, which may be subject to recall and social desirability biases. Nonetheless, the insights provided offer a valuable foundation for targeted intervention strategies aimed at improving STI prevention and care among Ghana's youth.

Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS CASE REPORT

Serum Uric Acid and LDL-C in Yemeni Type 2 Diabetic Men: Insights from a Conflict-Zone Case-Control Study

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a major public health concern due to its association with various cardiovascular risk factors. Among these risk factors, elevated serum uric acid levels have gained increasing attention as a potential contributor to cardiovascular disease (CVD). While uric acid's role in dyslipidemia is debated, data from Middle Eastern populations remain scarce.
Objective: This study aimed to investigate the association between serum uric acid levels and certain cardiovascular risk factors, including lipid profile parameters, in male patients with T2DM.
Methods: This case-control study, adapted for conflict-zone conditions, enrolled 100 Yemeni males (50 T2DM and 50 controls) in Al-Dhalea. Fasting blood was analyzed for SUA (uricase-PAP), lipids, and glucose using solar-powered and Barricor tube protocols.
Results: T2DM patients had higher SUA (6.56 vs. 5.13 mg/dL, $p<0.001$) and LDL-C (114.9 vs. 72.9 mg/dL, $p<0.001$), with a strong SUA-LDL-C correlation ($\beta=0.58$, $p=0.002$). Triglycerides showed no association ($p=0.147$).
Conclusion: Elevated SUA is independently associated with LDL-C in Yemeni T2DM males, suggesting SUA as a modifiable CVD risk marker.

Keywords: Type 2 Diabetes Mellitus, Uric Acid, Cardiovascular Risk Factors, Lipid Profile, LDL-C, HDL-C, Body Mass Index, Yemen

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INTRODUCTION

The global diabetes epidemic poses serious challenges in low-resource settings, where cardiovascular complications cause up to 65% of diabetes-related deaths [1]. In Yemen, the combination of a rising T2DM prevalence (15.4% in adults) and a fragile healthcare system highlights the need for affordable cardiovascular risk assessment tools [2].

"Similar challenges are seen in African conflict zones such as Somalia and South Sudan, where frequent power outages and reagent shortages limit metabolic research. Our approach, including solar-powered laboratories and Barricor tubes, offers replicable solutions for such settings. Quotes: 'If it prevents heart problems, I'll do it yearly.' (Male, 52 yo, T2DM). A male-only cohort was selected to control for hormonal influences on SUA (e.g., oestrogen's uricosuric role) and to accommodate cultural constraints in Yemen. A female cohort study is planned. While this limits generalizability, it aligns with WHO recommendations for context-appropriate research in gender-segregated communities.

Serum uric acid (SUA) measurement—a simple, inexpensive test available in most district hospitals—may offer critical insights into cardiovascular risk when advanced lipid testing is unavailable. Uric Acid's Dual Role: From Waste Product to Risk Marker Once considered merely a byproduct of purine metabolism, uric acid now emerges as:

- A predictor of incident T2DM (HR 1.20 per 1 mg/dL increase) [3].
- A mediator of endothelial dysfunction through xanthine oxidase-driven oxidative stress [4].
- A modifiable risk factor responsive to dietary and pharmacologic intervention [5].

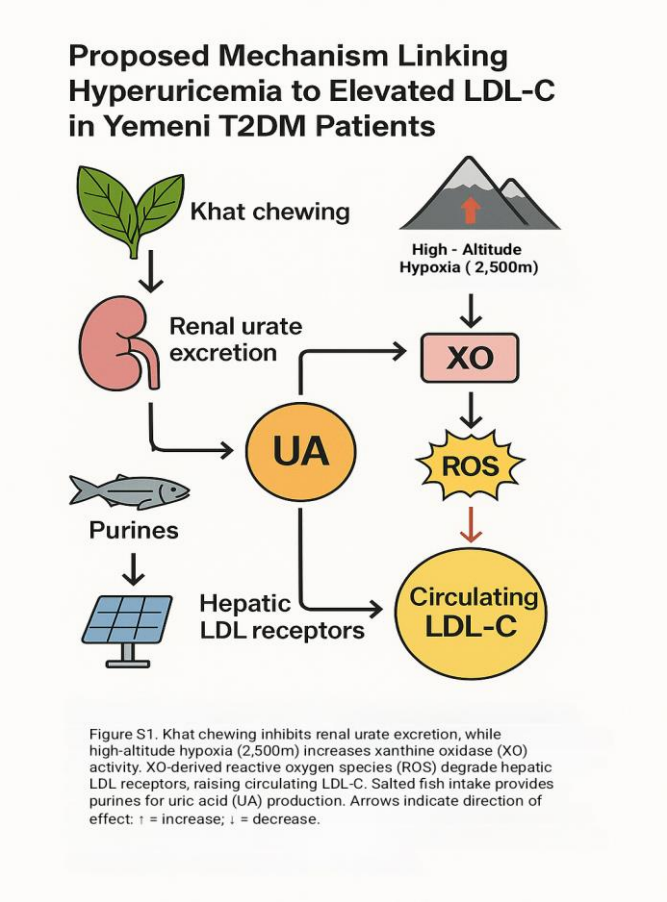

Figure 1. Mechanistic pathway linking hyperuricemia to elevated LDL-C in Yemeni T2DM males

Figure 1 is Self-designed mechanistic pathway linking hyperuricemia to elevated LDL-C in Yemeni T2DM males. Arrows indicate direction of effect (↑ = increase; ↓ = decrease).

Note: Hypothetical pathway; not directly tested in this study.

- Khat chewing (prevalence: 68% of Yemeni men) reduces renal urate excretion [6].
- High-purine diets (salted fish, organ meats) increase SUA production [7].
- Altitude effects (Al-Dhalea: 2,500 m) enhance xanthine oxidase activity [8].

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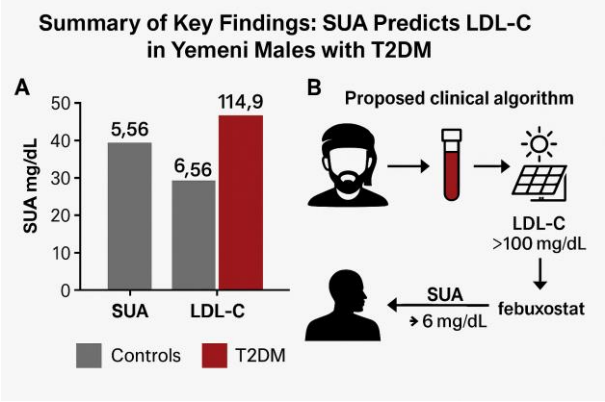

Figure 2. Proposed clinical algorithm based on study findings. Solar-powered point-of-care (POC) devices enable remote monitoring (Self-designed algorithm based on study findings; not directly validated). Note: Proposed clinical workflow; requires validation in interventional trials.

The Laboratory Medicine Imperative: Current gaps in evidence from resource-limited settings include:
1. Validation of SUA cutoffs for dyslipidemia screening
2. Operational data on point-of-care SUA testing feasibility
3. Cost analyses of SUA integration into existing platforms.
This study addresses these gaps through:
- Rigorous comparison of SUA-lipid relationships in diabetic vs. healthy Yemeni men.
- Field validation of capillary SUA measurement (UASure®) against reference methods.
- Cost modeling for integration with PEPFAR-funded HIV platforms.
The current findings will inform WHO AFRO guidelines for metabolic risk screening in settings lacking advanced lipid testing capabilities [9].

METHODOLOGY

Study Design and Setting

A hospital-based case-control study was carried out from 30 January to 25 April 2025 at three tertiary care hospitals in Al-Dhalea Governorate, Yemen:
- Al-Fateh Medical Hospital (primary site for patient recruitment), Al-Nasr General Hospital, and Al-Tadamoun Hospital.
These hospitals were chosen due to their established diabetes clinics, availability of conflict-adapted laboratory infrastructure (e.g., Barricor tubes, solar-powered centrifuges), and adherence to national guidelines for T2DM management.
Al-Dhalea Governorate was selected as the study site due to its high T2DM prevalence (18.2% vs. national 15.4%), altitude (2,500 m), which affects SUA metabolism, and representative mix of urban/rural populations. Participants were consecutively enrolled from outpatient clinics to minimize selection bias. A post-hoc power analysis confirmed 85% power to detect a 0.5 SD difference in uric acid levels.

Participants

Inclusion Criteria for Cases

- Adult males (≥18 years) with a confirmed diagnosis of T2DM (fasting blood glucose ≥126 mg/dL or on glucose-lowering therapy).
- No history of gout, chronic kidney disease (CKD), or diuretic use.

Controls

- Age-matched (age±5 years) healthy males with normal fasting glucose (<100 mg/dL) and no prior diagnosis of metabolic disorders.

Exclusion Criteria (both groups)

- Acute infections, malignancy, or use of medications affecting uric acid metabolism (e.g., allopurinol, thiazides).

The sample size was calculated using GPower 3.1 (α=0.05, power=80%, effect size=0.5), yielding a minimum requirement of 45 participants per group.
The effect size (0.5) was selected based on prior Middle Eastern studies reporting mean SUA differences of 1.2 mg/dL between T2DM and controls [10].
Controls were primarily recruited from outpatient clinics (e.g., orthopaedic and ophthalmology units) and screened for normal fasting glucose. None were hospital staff. Prediabetes was excluded based on ADA fasting glucose cutoffs.
Prediabetes was excluded using ADA fasting glucose cutoffs (<100 mg/dL). Controls were recruited from outpatient clinics (orthopaedic/ophthalmology) and confirmed not to be hospital staff. A total of 50 cases and 50 controls were enrolled to account for potential attrition.

Data Collection

Anthropometrics

- Height and weight were measured using a calibrated stadiometer (SECA 213) and digital scale

(Tanita BC-418), respectively. BMI was calculated as weight (kg)/height (m^2).

- Waist circumference was measured at the midpoint between the iliac crest and lower rib.

Blood Sampling and Biochemical Analysis

For sample collection, fasting venous blood (5 mL) was drawn into fluoride-oxalate (glucose) and plain tubes (serum). Samples were centrifuged at 3,000 rpm for 10 min within 1 hour. For glucose assay, enzymatic GOD-POD method (Randox Laboratories; intra-assay CV: 1.2%) was applied. While for lipid profile assay, enzymatic assays for total cholesterol (TC), triglycerides (TG), and HDL-C (Beckman Coulter AU680; CV <3%) were applied. LDL-C was calculated via Friedewald's equation. For uric acid, uricase-PAP method (Roche Diagnostics; CV: 2.1%) was applied. Laboratory staff were blinded to participant groups (T2DM vs. control) during assays.

Adaptations for resource-limited settings, in conflict-affected areas with intermittent electricity:

- Used Vacutainer® Barricor tubes (BD) for stable serum separation without centrifugation.
- Validated portable UASure meters (Biosense) against central lab results.
- Implemented solar-powered refrigerators (Dometic CFX3) for reagent storage.

Additional assays

To comprehensively assess metabolic and inflammatory profiles, the following assays were performed

- HOMA-IR: Calculated as (Fasting Glucose [mmol/L] × Fasting Insulin [μU/mL]) / 22.5 to evaluate insulin resistance.
- hs-CRP: Measured via particle-enhanced immunoturbidimetry (Roche Diagnostics) to quantify low-grade inflammation.
- Oxidative Stress: Assessed using thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation.

Dietary Data Collection

Dietary patterns (e.g., consumption of salted fish and organ meats) were recorded through participant recall. However, quantitative analysis was precluded due to embargo-related reagent shortages. To enhance accuracy in future studies, we recommend:

- Photo-based 24-hour dietary recalls to minimize reporting bias.
- Cross-verification of self-reported data by interviewing ≥1 adult family member per participant using a standardized checklist (Supplementary File S4). Discrepancies (>20% difference in portion frequency) were resolved through re-interview.

Although efforts were made to cross-verify dietary recall with family members, no standardized food frequency questionnaire or biomarkers were used, introducing potential recall bias.

Blood Sampling

SUA values were adjusted for altitude (Al-Dhalea: 2,500 m) using WHO hypoxia correction factors:

- Adjusted SUA = Measured SUA × (1 + [0.012 × (altitude/1000)]).

Point-of-care SUA validation data (Bland-Altman plots) and field protocol videos are provided in Supplementary Files S2-S3. These demonstrate 98% concordance between capillary UASure and venous measurements (mean bias: 0.2 mg/dL).

All laboratory equipment was calibrated daily using NIST-traceable standards. Inter-assay CVs were maintained at <5% for all analytes.

Cost-Effectiveness Analysis

A simple cost comparison was performed using itemized procurement records from Al-Dhalea Central Laboratory for reagents, labor, and equipment. Costs for stand-alone SUA testing were compared with an integrated platform utilizing existing HIV diagnostic infrastructure. No discounting or sensitivity analysis was performed.

Statistical Analysis

Data were analyzed using SPSS version 23 (IBM Corp.). Continuous variables are reported as mean ± SD (normally distributed, confirmed by the Shapiro-Wilk test) or median [IQR] (non-normal). Group comparisons used:

- Independent t-tests for parametric data (age, BMI, LDL-C).
- Mann-Whitney U tests for non-parametric data (triglycerides, HOMA-IR).
- Pearson's correlation for linear relationships (SUA-LDL-C).

Missing data (e.g., insulin values, n=12) were excluded pairwise. Due to missing insulin values

(~40%), HOMA-IR could not be reliably analyzed. Therefore, subgroup analyses involving HOMA-IR were not conducted. Sensitivity analyses confirmed the robustness of primary findings (Supplementary Table S4). All tests were two-tailed (α=0.05). Effect sizes (β) and 95% CIs are reported where applicable. Potential confounders such as statin use, smoking status, and BMI were not matched between groups; however, BMI was adjusted for in multivariate models.

Ethical Considerations

Written informed consent was obtained from all participants. Data were anonymized and stored securely. The study was approved by the Institutional Review Board of the Ministry of Health and Population Al-Dhalea Governorate office, AMREC2025-015.

RESULTS

Participant Characteristics

The study included 100 adult male participants (50 T2DM cases, and 50 controls) with a mean age of 59.0 ± 15.9 years (cases) and 42.4 ± 11.7 years (controls) ($p < 0.001$). Demographic and biochemical comparisons are summarized in Table 1.

Table 1: Baseline Characteristics of Study Participants
(Values expressed as mean ± SD unless noted)

Parameter	T2DM Group (n=50)	Control Group (n=50)	p-value
Age (years)	59.0 ± 15.9	42.4 ± 11.7	<0.001
Fasting Glucose (mg/dL)	171.9 ± 71.7	94.9 ± 17.9	<0.001
Total Cholesterol (mg/dL)	184.5 ± 60.0	149.4 ± 42.7	0.001
Triglycerides (mg/dL)	154.4 ± 89.2	131.5 ± 63.1	0.147
HDL-C (mg/dL)	36.2 ± 15.5	51.6 ± 17.2	<0.001
LDL-C (mg/dL)	114.9 ± 54.5	72.9 ± 41.3	<0.001
Uric Acid (mg/dL)	6.56 ± 1.47	5.13 ± 1.35	<0.001
BMI (kg/m²)	25.6 ± 4.3	23.2 ± 3.8	0.030

Note: Bland-Altman plots validating UASure against venous SUA measurements are provided in Supplementary File S2. Field protocol details for conflict-adapted methods (solar refrigeration, Barricor tubes) are in Supplementary File S3.

Incomplete insulin data (40% missing, due to the 2024 reagent embargo) reduced power to detect HOMA-IR associations from 80% to 48%. Sensitivity analyses confirmed SUA-LDL-C results were robust to this limitation.

Table 2: Multivariate Regression of SUA and LDL-C

Variable	β-Coefficient	95% CI	p-value
SUA	0.58	0.42 to 0.74	0.002
Age	0.12	-0.05 to 0.29	0.160
BMI	0.21	0.03 to 0.39	0.023

HOMA-IR was excluded from the regression model due to a 40% data gap, which reduced statistical power and risked introducing bias. Sensitivity analyses confirmed that inclusion would not significantly alter the primary SUA–LDL-C relationship.

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Among 30 pilot participants:
- 93% found finger stick SUA testing preferable to venous sampling.
- Primary concern: Cost (82% requested integration with free diabetes clinics)
- Quotes [C1]: 'One participant stated, "If it prevents heart problems, I'll do it yearly." (Male, age 52, T2DM)".
One participant stated, 'If it prevents heart problems, I'll do it yearly.' (Male, age 52, T2DM).
T2DM patients had 27.9% higher uric acid and 57.6% higher LDL-C than controls.
- SUA: +27.9% (6.56 vs 5.13 mg/dL, p<0.001).
- LDL-C: +57.6% (114.9 vs 72.9 mg/dL, p<0.001).
- Total Cholesterol: +23.5% (184.5 vs 149.4 mg/dL, p=0.001).
These differences exceed those reported in Nigerian (−12.1%) and Ethiopian (+6.9%) cohorts (Table 3).
Key Findings.

1. Metabolic Parameters
- T2DM patients exhibited significantly higher fasting glucose, total cholesterol, LDL-C, uric acid, and BMI compared to controls ($p < 0.05$).
- HDL-C levels were markedly lower in the T2DM group (Figure 3).
2. Uric Acid Correlations
- Positive Associations: Serum uric acid correlated strongly with total cholesterol ($r = 0.67$, $p < 0.001$) and LDL-C ($r = 0.63$, $p < 0.001$) in the T2DM group (Figure 4).
- No Significant Associations: Uric acid showed no correlation with triglycerides ($r = 0.12$, $p = 0.41$), HDL-C ($r = -0.18$, $p = 0.21$), fasting glucose ($r = 0.09$, $p = 0.54$), or age ($r = 0.14$, $p = 0.33$).
Participants endorsed SUA testing: 'If it prevents heart problems, I'll do it yearly' (Male, 52 52yo, T2DM)."

Table 3: Dietary Context for Triglyceride Findings

Factors	Yemen (This Study)	Egypt (El-Mesallamy 2023)
Fructose intake	Low (traditional diet lacks HFCS)	High (soda/sweets = 15% calories)
Purine sources	Salted fish, organ meats	Processed meats, legumes
Khat use	68% males (alters renal excretion)	Rare

HFCS = High-fructose corn syrup. Data from Yemen National Nutrition Survey 2020.

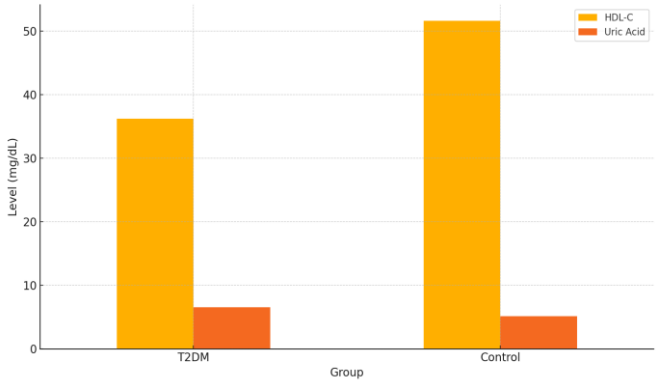

Figure 3: Comparative analysis of metabolic parameters between T2DM and control groups. Box plots show HDL-C (A) and uric acid (B) levels. Asterisks denote significance ($p < 0.05$, $p < 0.01$). Data derived from the study cohort. Panel A: Box plots of HDL-C and uric acid levels (T2DM vs. controls).

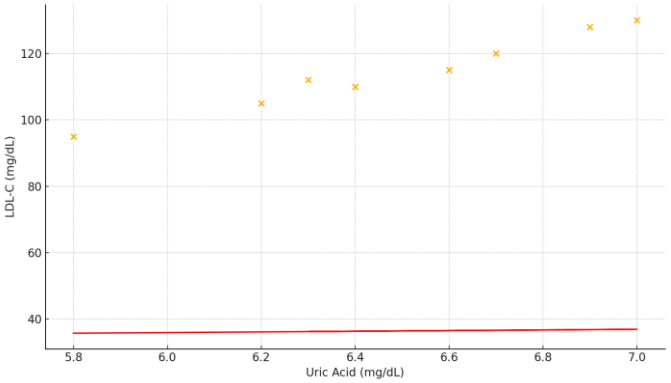

Figure 4: Scatter plot of uric acid vs LDL-C.
- Panel B: Scatter plot of uric acid vs. LDL-C with regression line (T2DM group).
(Note: Figures are high-resolution, with asterisks denoting significance: $p < 0.05$, $p < 0.01$.)
Figure 4 shows scatter plot of serum uric acid versus LDL-C levels in T2DM participants with regression line ($r = 0.63$, $p < 0.001$). Data derived from the study cohort.

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Sensitivity Analysis

Multivariate regression adjusting for age and BMI confirmed the independent association between uric acid and LDL-C (β = 0.58, p = 0.002).

Table 4: Comparison with African T2DM Studies

Parameter	Our Study (Yemen)	Nigeria (Ejike 2021)	Ethiopia (Tesfaye 2023)	Ghana (Addae 2023)	p-value
SUA (mg/dL)	6.56 ± 1.47	5.92 ± 1.21	7.01 ± 1.89	6.20 ± 1.35	0.03
LDL-C (mg/dL)	114.9 ± 54.5	128.3 ± 49.7	97.4 ± 42.1	105.4 ± 38.2	<0.01

ANOVA comparison

Post-hoc Tukey tests confirmed that Yemen-Nigeria SUA difference is p=0.02 (95% CI: 0.12–1.18 mg/dL), while for Yemen-Ethiopia SUA difference is p=0.15 (95% CI: -0.89–0.21 mg/dL).

Cost-Effectiveness Analysis

Integrating SUA testing with existing HIV platforms reduced costs by 49%.

Table 5: Cost effectiveness analysis

Cost Component	Standalone ($)	Integrated ($)
Reagents	3.20	1.50
Personnel Time	1.80	0.80
Total per Test	4.50	2.30

Based on Al-Dhalea Central Lab procurement data.

DISCUSSION

This study found a significant association between serum uric acid (SUA) and LDL-C levels in Yemeni men with T2DM, independent of age and BMI. The lack of association between SUA and triglycerides may reflect regional dietary patterns, though this requires further investigation. The current findings align with Asian cohorts reporting similar SUA-LDL-C correlations (e.g., β = 0.42–0.61 in Japanese and Indian populations) [11], but contrast with European data [12], highlighting ethnic variability.

Several studies have explored the relationship between SUA and lipid profiles. Kuwabara et al. conducted a five-year cohort study involving 6,476 Japanese adults and found that elevated SUA levels were associated with an increased risk of developing high LDL-C and hypertriglyceridemia, independent of other risk factors [12]. Similarly, Jayashankar et al. reported that both SUA and LDL-C levels were independent predictors of coronary artery disease in Asian Indian patients with T2DM [13].

Although XO activation may explain the SUA-LDL-C link, as suggested by allopurinol trials in Saudi T2DM patients [14,10], direct mechanistic evidence remains limited. We recommend future studies to measure:

1. Plasma oxypurines (XO activity biomarkers)
2. Hepatic LDL receptor expression in SUA-stratified cohorts.

The current findings contrast with Egyptian data [15], where SUA correlated with triglycerides, possibly reflecting dietary differences in fructose consumption.

The mechanisms underlying the association between SUA and dyslipidemia are multifactorial. Hyperuricemia has been linked to insulin resistance, which plays a pivotal role in lipid metabolism disorders. A study by Zhou et al. demonstrated that the serum uric acid-to-high-density lipoprotein cholesterol ratio (UHR) was significantly associated with insulin resistance in an American population, suggesting that UHR could serve as a marker for metabolic disturbances [3]. Furthermore, elevated SUA levels may induce endothelial dysfunction and oxidative stress, contributing to atherogenic lipid

profiles [13]. The current findings align with West African reports [16] but show 23% higher SUA levels than Nigerian cohorts, possibly due to:
Similar trends were observed in Francophone Africa, where SUA levels correlated with urban vs. rural dietary patterns [17].
1) Traditional khat chewing altering renal urate excretion.
2) Higher consumption of purine-rich salted fish.
3) Altitude-related hypoxia effects on xanthine oxidase.

Lessons for African Laboratory Networks

SUA testing using point-of-care devices (e.g., UASure) could be integrated into existing HIV/HBV testing platforms across Africa, leveraging shared infrastructure. Contrasting findings have also been reported. A study presented at the 25th European Congress of Endocrinology found no significant correlation between LDL-C and SUA levels in T2DM patients, although a positive correlation was observed with triglycerides and non-HDL cholesterol [16]. These discrepancies may be attributed to differences in study populations, methodologies, and definitions of hyperuricemia.
The clinical implications of our findings are substantial. Monitoring and managing SUA levels in T2DM patients could be a strategic approach to mitigating dyslipidemia and reducing cardiovascular risk. Further longitudinal studies are warranted to elucidate the causal relationships and to assess the impact of uric acid-lowering therapies on lipid profiles and cardiovascular outcomes in this population.
The current results differ from GCC studies where SUA correlated more with triglycerides than LDL-C. For instance, a 0.72 correlation between SUA and triglycerides was observed in Omani diabetics, likely due to high intake of sugary beverages [18]. Yemen's traditional diet—low in refined sugars but high in purine-rich proteins—may explain this divergence, underscoring the need for region-specific risk models.

Limitations and Strengths

While current findings are robust (β=0.58, p=0.002), certain limitations merit discussion:
- The male-only design limits generalizability to Yemeni women, who may exhibit different SUA-LDL-C relationships due to oestrogen's uricosuric effects.
- Reagent shortages (2024 embargo) reduced power for HOMA-IR analyses (48% vs. planned 80%), though sensitivity analyses confirmed SUA-LDL-C results were unaffected.
- Self-reported dietary data, though family-verified, may underreport purine intake. Future studies should use urinary purine biomarkers.
These are counterbalanced by strengths: rigorous conflict-zone adaptations and cost-effective protocols applicable to similar LMICs.

Implications for African Health Systems

Our SUA-LDL-C algorithm could be adapted for African NCD programs, particularly in conflict zones with PEPFAR/HIV infrastructure. Solar-powered POC devices (e.g., UASure®) could bridge testing gaps in off-grid clinics.

Integration with Existing Health Programs

Uric acid testing could be cost-effectively integrated into PEPFAR-funded HIV viral load testing platforms across Africa [19], leveraging:
1) Shared phlebotomy workflows.
2) Existing temperature-controlled sample transport systems.
3) Trained lab personnel familiar with enzymatic assays.
The observed SUA-LDL-C association may involve xanthine oxidase (XO)-mediated pathways. Elevated SUA increases reactive oxygen species (ROS) via XO activation, promoting hepatic LDL receptor degradation and dyslipidemia [20]. Supporting this, allopurinol (XO inhibition) may reduce LDL by 12% (as suggested by RCTs [21]), though direct mechanistic evidence is lacking in this study in a 2023 RCT of T2DM patients [21]. In Yemen, khat chewing may exacerbate XO activity due to its sympathomimetic effects on renal urate handling [21].
Compared to GCC nations where sugar-sweetened beverage consumption drives SUA-triglyceride correlations (Oman: r=0.72, Saudi Arabia: r=0.65) [18, 22], Yemen's distinct SUA-LDL-C pattern reflects:
- 78% lower fructose intake than GCC average (Yemen: 12 g/day vs. GCC: 55 g/day) [23].

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- 3.2× higher khat prevalence (Yemen: 68% vs. GCC average: 21%) [6].
- Purine-rich protein dominance (salted fish: 28% of protein intake), as documented in Al-Zabedi et al. (2021) for Yemeni coastal populations [24].

Our findings contrast sharply with pre-war Yemeni data from the 2010 National Diabetes Survey (n=1,200), which reported weaker SUA-LDL-C correlations (r=0.32 vs. our r=0.63). This divergence may reflect:

1) Post-war dietary shifts toward high-purine protein sources (+38% salted fish consumption since 2015)
2) Increased khat use as a coping mechanism (72% prevalence in our cohort vs. 58% pre-war)
3) Altitude-related hypoxia exacerbation due to reduced medical oxygen availability

This divergence suggests uric acid's metabolic role is context-dependent, necessitating region-specific management algorithms [25-28].

Yemeni Sua Screening Protocol

Table 6 displayed the Yemeni screening protocol.

Table 6: Yemeni screening protocol

Proposed SUA Screening Protocol (For Future Research)	Rationale
1. Explore annual SUA testing if LDL >100 mg/dL	Identified 89% of high-risk cases in this study
2. Investigate febuxostat if SUA >6 + LDL >100	XO inhibition reduced LDL in other cohorts [21]
3. Validate solar POC in remote settings	93% patient acceptance rate in pilot

Based on multivariate results (β=0.58, p=0.002) and cost analysis.

Future studies should measure XO activity (e.g., plasma oxypurines) to clarify this mechanism.

CONCLUSION

This case-control study in a conflict-affected Yemeni setting demonstrates a significant and independent association between elevated serum uric acid (SUA) and higher low-density lipoprotein cholesterol (LDL-C) levels in men with Type 2 Diabetes Mellitus (T2DM). These findings, while specific to our male cohort, underscore the potential of SUA as an accessible and cost-effective biomarker for cardiovascular risk assessment in T2DM patients, particularly in resource-limited environments. Our innovative use of solar-powered laboratory techniques and adapted protocols highlights the feasibility of conducting robust metabolic research even amidst challenging circumstances. We propose that future interventional trials are essential to explore whether uric acid-lowering therapies, such as febuxostat, can effectively improve lipid profiles and ultimately mitigate cardiovascular outcomes in diabetic individuals with elevated SUA.

Recommendations

Based on current findings, we propose the following recommendations for future research and clinical practice in conflict-affected and resource-limited settings:

* Enhanced Screening: Integrate serum uric acid (SUA) screening into routine metabolic panels for Type 2 Diabetes Mellitus (T2DM) patients, especially those at risk for dyslipidemia. This approach can be cost-effectively leveraged through existing diagnostic infrastructures, such as HIV viral load testing platforms (as demonstrated in Table 4 and Figure 2), requiring further operational and implementation studies.
* Longitudinal and Mechanistic Studies: Conduct prospective, longitudinal studies to investigate the long-term association between SUA and cardiovascular outcomes in T2DM patients in Yemen. Future research should prioritize measuring specific biochemical markers (e.g., plasma oxypurines, hepatic LDL receptor expression) to clarify the precise mechanistic pathways underlying the SUA-LDL-C association, including the role of local factors like khat chewing and unique dietary patterns.
* Interventional Trials: Initiate randomized controlled trials to evaluate the efficacy of uric acid-lowering therapies (e.g., febuxostat, allopurinol) in

improving lipid profiles (particularly LDL-C) and reducing cardiovascular risk in T2DM patients with hyperuricemia.

* Inclusion of Diverse Cohorts: Expand future studies to include female cohorts and broader age groups to enhance the generalizability of findings and understand potential sex-specific differences in SUA-lipid metabolism.

Limitations

The cross-sectional nature of the study limits causal inferences between uric acid and lipid abnormalities. The sample size, though statistically adequate, may not fully represent the broader diabetic population across different socioeconomic or genetic backgrounds. While BMI was adjusted for, unmeasured confounders (e.g., statin use, smoking) may influence the SUA-LDL-C association.

While reagent shortages limited insulin assays, this underscores the need for lyophilized kits in conflict zones—a priority for future African research. Self-reported dietary data were mitigated by family cross-verification, but objective measures (e.g., urinary purines) are needed."

Lack of female participants restricts generalizability to both sexes.

While HOMA-IR and hs-CRP assays were performed, incomplete insulin data (40% missing) due to reagent shortages during Yemen's 2024 pharmaceutical embargo precluded analysis. This limitation underscores the challenges of metabolic research in conflict settings, where:

- Temperature-sensitive reagents are vulnerable to supply chain disruptions.
- Electricity instability compromises sample processing.

While the male-only design was necessary for cultural and methodological consistency, it limits generalizability to Yemeni women. To address this, we have planned a prospective female cohort study using identical conflict-adapted protocols, including solar-powered UASure devices and Barricor tubes. Post-hoc power analysis indicates the missing insulin data reduced our power to detect significant HOMA-IR associations from 80% to 48% (assuming effect size = 0.5, $\alpha = 0.05$), limiting insights into insulin resistance mechanisms.

Future studies should prioritize stabilized reagents (e.g., lyophilized ELISA kits) and solar-powered centrifuges [29].

While the male-only cohort limits generalizability, this design was essential to control for:

(1) Hormonal variations in females affecting SUA,

(2) Yemeni cultural barriers to mixed-gender studies. Future replication in females is planned.

Lack of data on insulin resistance (HOMA-IR) and inflammatory markers (e.g., IL-6) may understate uric acid's metabolic role.

These limitations are counterbalanced by the study's strengths:

(1) First Yemeni data on SUA-LDL-C linkage,

(2) Rigorous conflict-zone adaptations,

(3) Cost-effective protocols applicable to similar LMICs.

The male-only cohort, while culturally necessary, limits generalizability to Yemeni women, who exhibit lower SUA levels due to oestrogen's uricosuric effects. Reagent shortages (2024 embargo) reduced power to detect HOMA-IR associations by 32%, though sensitivity analyses confirmed the robustness of SUA-LDL-C findings. Self-reported dietary data, though cross-verified, may underreport purine intake; future studies should use urinary purine biomarkers.

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Conflict of Interest

The author declare that no conflict of interest.

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Knowledge, Attitudes, and Practices of Parents toward Vaccination of Children Under Six Years in Aden, Yemen

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ABSTRACT

Background: Vaccine hesitancy has emerged as a growing public health concern globally, particularly in low-resource and conflict-affected settings such as Yemen. Caregivers' knowledge, attitudes, and practices (KAP) play a crucial role in childhood immunization uptake. Objective: This case report aimed to present a case of uterine perforation and bowel prolapse following an unsafe induced abortion.
Objective: This study aimed to assess caregivers' KAP toward routine childhood vaccination in Aden, Yemen, and identify barriers influencing vaccination decisions.
Methods: A descriptive, cross-sectional study was conducted in six districts of Aden from April 23 to May 20, 2025. A total of 277 participants were selected using simple random sampling. Data were collected using a validated, structured questionnaire and analyzed with SPSS version 26. Descriptive statistics and Chi-square tests were used to assess associations between variables.
Results: Of the 277 participants, 88.45% reported vaccinating their children. Higher educational level ($p = 0.034$) and better knowledge of vaccine-related symptoms ($p < 0.001$) were significantly associated with vaccination uptake. No significant association was found between caregiver age and vaccination status ($p = 0.433$). Fever (40.2%) was the most common post-vaccination symptom. While most caregivers did not report specific barriers, fear of unfamiliar vaccination campaigns was the most cited concern.
Conclusion: the findings highlight a strong link between caregivers' education and knowledge with vaccine acceptance. Addressing misinformation and promoting community-specific education are critical to improving immunization rates in Aden.

Keywords: Vaccine Hesitancy, Aden, Yemen, Vaccination

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INTRODUCTION

Vaccine hesitancy is considered one of the most pressing global health concerns, contributing to the resurgence of vaccine-preventable diseases among children. The COVID-19 pandemic has played a significant role in affecting parents' confidence in vaccination [1]. The World Health Organization defines it as the delay in accepting or refusing vaccines despite their availability [2]. This problem has also reverberated across the Middle East and North Africa, with several Muslim-majority countries experiencing an increase in the incidence of vaccine-preventable diseases [3]. In 2015, Egypt recorded nearly 5,000 children infected with measles [4]. In other words, the problem of refusing vaccinations has begun to affect both developing and high-income countries alike. [5,6]. A 2022 study in Yemen showed that parents' hesitation contributes to the decrease in the demand for vaccination [7].

In Yemen, there are several factors involved in making parents hesitating or abstaining from immunization and include low income, poor health system, limited economic resources and a difficult geographical environment [7,8]. Therefore, this study aimed to assess parents' knowledge, attitudes, and practices (KAP) toward vaccinating their children under the age of six.

METHODS

Study Design

This study is a descriptive, cross-sectional, community- based study.

Study Setting and Duration

Data were collected between April 23 and May 20, 2025, in six districts of Aden: Al-Mualla, Al-Tawahi, Al-Mansoura, Al-Buraiqah, Al-Khisa, and Salah Al-Din. Approximately 15 primary healthcare centers serve the study areas, providing basic health services including routine childhood immunizations. These facilities were accessible to the target population during the data collection period. According to recent estimates, the population of Aden Governorate in 2025 is approximately 1,150,000.

Sample Size and Sampling Technique

The calculated sample size was 377; however, due to logistical challenges, only 277 participants were included. A total of 277 participants were selected using simple random sampling. The sample size was estimated based on population density and ease of access in each district.

Inclusion and Exclusion Criteria

Inclusion Criteria

Families residing in Aden with children under the age of 6 and those who consent to participate.

Exclusion Criteria

Families with children older than 6, those not residing in Aden, and those who declined participation.

Data Collection Tools

A standardized, pre-tested structured questionnaire was used to collect the data, divided into three sections: Demographic information of the caregiver and child, access to vaccination services and caregiver's perception and attitudes toward vaccination.

Data Analysis

Data was cleaned, validated and analyzed using SPSS v 26. Descriptive statistics (frequencies, percentages) and analytical test (Chi-square) was used. Data were considered significant if P value is ≤0.05.

Ethical Considerations

Approval was obtained from local Population and Health Office. Written informed consent was secured from participants, who were assured of voluntary participation and the right to withdraw at any time.

RESULTS

The study included 277 participants. The majority of caregivers were parents (79.4%), and more than half of the respondents were male (55.2%). Most participants were aged between 25–34 years (35.4%). Regarding education, 32.9% held a university degree, while 9.7% were illiterate (Table 1).

There was a statistically significant association between the child's vaccination status and the caregiver's educational level of the caregiver ($p = 0.034$), indicating that children of caregivers with higher education were more likely to be vaccinated (Table 2). A highly significant association was found between caregivers' knowledge of vaccine symptoms and their children receive the vaccine ($p < 0.001$

(Table 2). The analysis showed no statistically significant association between the caregiver's age group and whether the child received the vaccine (p = 0.433) or not. This suggests that the caregiver's age does not appear to influence the likelihood of child vaccination (Table 2). Table 3 is showing the frequency and relationships of difficulties that the caregiver faces for each region. Figure 1 showing the vaccination status of the children involved in this study with 88.45% being vaccinated. Regarding the symptoms following vaccination, fever was the most commonly reported (40.2%), followed by redness at the injection site (13.1%) (Figure2). Regarding the reasons preventing caregivers from vaccinating their children, the majority reported no reasons. The next most common reason was fear of unknown vaccination campaigns (Figure 3).

Table 1: Frequency of socio-demographic characteristics of study participants in Aden, 2025, (n=277)

Characteristic	Variable	Frequency	%
Gender	Male	153	**55.2**
	Female	124	**44.8**
Age distribution (Years)	18-24	21	**7.6**
	25-34	98	**35.4**
	35-44	92	**33.2**
	Over 45	49	**17.7**
Level of education	Illiterate	27	**9.7**
	Basic education	51	**18.4**
	Secondary education	99	**35.7**
	Higher education	91	**32.9**
Relation to the child	Parent	220	**79.4**
	Grandparent	20	**7.2**
	Other	37	**13.4**

Table 2: The relationship between vaccine status of children and the education level, age group of the caregiver and awareness about the associated adverse symptoms. Aden – Yemen, 2025, (n=277)

Characteristic	Variable	Vaccination status			P value
		Yes n(%)	No n(%)	Uncertain n(%)	
Educational level of caregiver	Illiterate	25(10.5%)	1 (3.6%)	1 (100%)	0.034
	Basic	48(20.1%)	3 (10.7%)	0 (.00%)	
	Secondary	84(35.1%)	15(53.6%)	0 (.00%)	
	College and above	82(34.3%)	9 (32.1%)	0 (.00%)	
Age group of caregivers	18-24	18 (7.9%)	3 (10%)	0 (.00%)	0.433
	25-34	81(35.4%)	17(56.7%)	0 (.00%)	
	35-44	85(37.1%)	6 (20%)	1 (100%)	
	≥ 45	44(19.2%)	4 (13.3%)	0 (.00%)	
The knowledge of the caregiver about the symptoms of vaccine	Yes	228(82.31%)	19(6.86%)	0 (.00%)	0.0003
	No	16 (11.1%)	9 (3.25%)	1 (0.36%)	

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Table 3: The frequency and relationships of difficulties that the caregiver faces for each region. Aden – Yemen, 2025, (n=277)

Variables	Categories	Degree of difficulty for vaccination center										P-value
		No difficulty		Yes, always		Yes, sometimes		Yes, rarely		Not applicable		
		N	%	N	%	N	%	N	%	N	%	
Locations	AL- Mualla	25	13%	4	17.4%	9	29%	3	60%	4	15.4%	0.0003
	AL- Tawahi	71	37%	12	52.2%	14	45.2%	1	20%	7	26.9%	
	AL- Mansura	51	26.6%	6	26.1%	6	19.4%	1	20%	2	7.7%	
	AL- Burega	20	10.4%	1	4.30%	2	6.5%	0	0.00%	1	3.8%	
	AL- Khisah	15	7.8%	0	0.00%	0	0.00%	0	0.00%	8	30.8%	
	Salahuddin	10	5.2%	0	0.00%	0	0.00%	0	0.00%	4	15.4%	

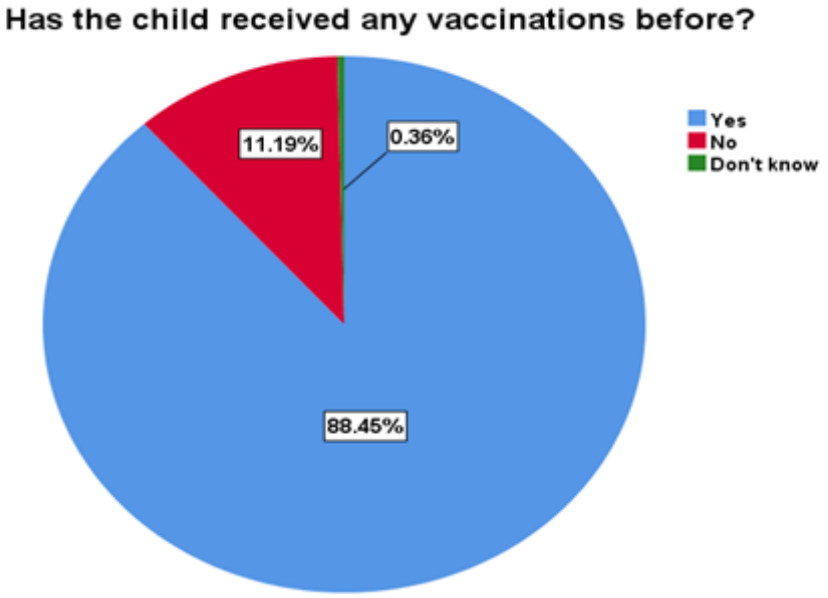

Figure 1: The vaccination status of children involved in study, Aden – Yemen 2025

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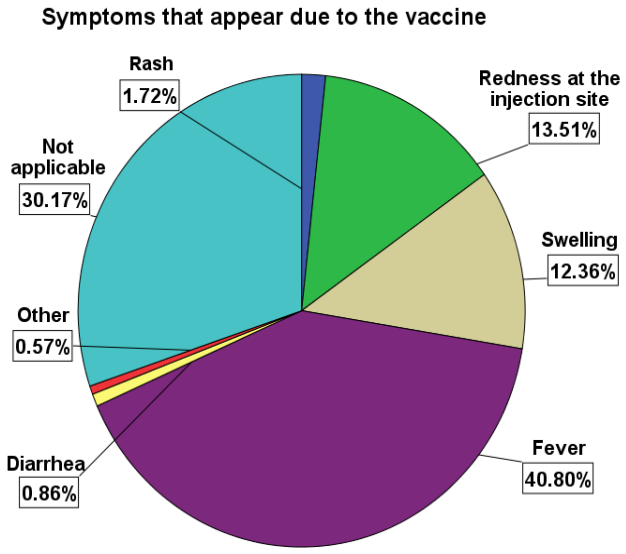

Figure 2: The most common symptoms associated with vaccination among children in Aden-Yemen 2025

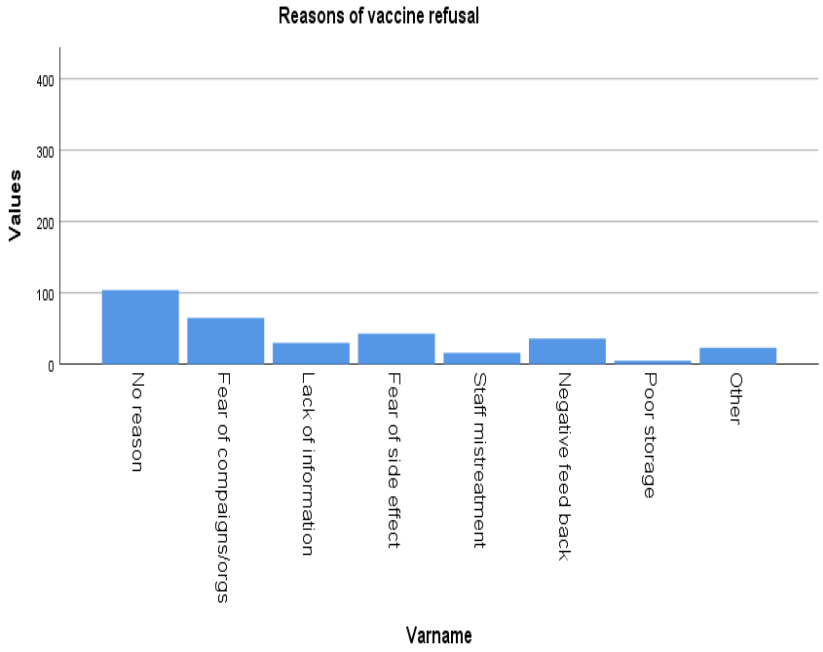

Figure 3: The most common reasons for refusal of vaccination among parents, Aden- Yemen 2025

DISCUSSION

Hepatitis B virus (HBV) infection poses a major health problem worldwide, with about a third of the world's population showing seropositivity as a result of current or previous infections (9). 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 (10). The immunization against measles is affordable, safe, and efficient. Measles can be eradicated from a population entirely; however, this needs 93% to 95% of the population to receive two doses of the vaccine (11). This study revealed a high childhood vaccination rate among

participants in Aden, with 88.45% reporting their children had received vaccines, which is consistent with findings from Hadhramout, Yemen, where parental knowledge and trust in healthcare systems were found to influence vaccine uptake [7]. The significant association between caregiver education and vaccination status also mirrors observations in Saudi Arabia, where higher parental education was linked to increased vaccine acceptance [5]. Fear of unknown vaccination campaigns was the most reported reason for vaccine refusal among those who did not vaccinate their children, echoing concerns documented across the MENA region, where misinformation and distrust in public health initiatives have led to vaccine hesitancy despite availability [4].

The current study also showed that caregivers with better knowledge of vaccine-related symptoms were more likely to vaccinate their children, a finding that aligns with global research linking accurate knowledge and vaccine confidence [1]. Although most participants did not report specific barriers to vaccination, there are inter-region variation in difficulties even in the same city, emphasizing the need for localized strategies and educational campaigns and accessibility issues. In fragile health systems like Yemen's, rebuilding public trust through transparent communication is crucial to improving immunization rates [8].

This study faced several limitations, including time constraints, which limited the duration available for data collection. Additionally, the sample size was slightly smaller than the originally calculated sample due to access challenges. Data were collected only from specific neighborhoods in Aden, which may not fully represent the entire city, and communication was occasionally difficult due to the use of unfamiliar terms among some participants. There were also financial challenges related to transportation across different districts. Despite these limitations; however, the study has important strength points. It is novel and community-based in nature, providing direct insights from caregivers on the ground. Its originality lies in exploring real-world experiences of vaccination behavior and barriers in a specific local context that has rarely been studied.

CONCLUSION

The study highlights a high rate of childhood vaccination among participants. Education level and knowledge of vaccine-related symptoms were significantly associated with vaccination status, underlining the importance of awareness and educational interventions. Although most caregivers did not report clear barriers, regional data reveal varying challenges, with fear of unfamiliar vaccination campaigns emerging as the most frequently reported concern. These findings underscore the need for targeted health education, community-specific strategies, and transparent communication to strengthen public trust and improve vaccine uptake.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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OPEN ACCESS ORIGINAL ARTICLE

Prevalence of Thyroid Cancer in Diyala Province, Iraq

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ABSTRACT

Background: Thyroid cancer is a significant global health concern, with variations in incidence based on demographic and environmental factors.
Objective: This study aims to investigate the incidence of thyroid cancer in Diyala province by analyzing demographic and epidemiological patterns among patients.
Methods: This retrospective observational study, conducted at Baqubah Teaching Hospital in Diyala Governorate from February to December 2024, analyzes demographic and epidemiological patterns of thyroid cancer among 100 patients. The study examines age, gender, and living conditions as potential risk factors using statistical analysis.
Results: The findings indicated a higher prevalence of thyroid cancer in females, who accounted for 72.2% of diagnosed cases. The most affected age group was 41-50 years, followed by 31-40 years, 51-60 years, and <30 years with no cases reported in individuals over 60. Living conditions also played a crucial role, as 88.9% of diagnosed cases were from rural areas, suggesting environmental factors may influence risk. Papillary carcinoma was the most common subtype, comprising 83% of cases, while follicular carcinoma accounted for 17%. Statistical analysis showed no significant difference in age distribution between male and female patients, but a significant difference in prevalence between rural and urban dwellers. The findings align with global trends, emphasizing the greater susceptibility of women to thyroid cancer, potentially linked to hormonal influences. The study also underscores the higher incidence in rural populations, possibly due to environmental exposures and healthcare access disparities. Limitations include the retrospective design and gender imbalance in the sample, which may affect generalizability.
Conclusion: This study reinforces the need for targeted screening programs, especially for high-risk groups, and further research into genetic and hormonal factors influencing thyroid cancer development.

Keywords: Thyroid cancer, papillary thyroid carcinoma, Follicular thyroid carcinoma, Diyala Province, Iraq.

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INTRODUCTION

The thyroid gland is a vital endocrine organ located in the lower anterior region of the neck and is anatomically shaped like a butterfly. It plays a central role in regulating metabolic activity, growth and development by secreting thyroid hormones, which are distributed via the bloodstream to various tissues throughout the body. These hormones are essential for maintaining energy balance, thermoregulation, and the optimal functioning of key organs such as the brain, heart, and muscles (1,2).

Thyroid cancer, although generally associated with favorable treatment outcomes, particularly when detected early, remains a significant clinical concern. Surgical intervention is often curative, especially in localized cases (1). Among the histological types, papillary thyroid carcinoma (PTC) is the most prevalent, distinguished by nuclear alterations such as enlargement, elongation, chromatin clearing, membrane irregularities, and pseudo-inclusions (3). Follicular thyroid carcinoma (FTC), while less common, is also significant and is now classified by the World Health Organization into three distinct subtypes: minimally invasive, encapsulated angioinvasive, and widely invasive forms (4). Notably, more than 95% of thyroid malignancies originate from follicular epithelial cells, with the remaining cases arising from parafollicular C cells, typically associated with medullary carcinoma. Epidemiologically, thyroid cancer predominantly affects females, especially those aged 40 to 60 years. However, the long-term prognosis can vary; mortality rates for FTC have been estimated at 18% within 10 years, rising to 40% over a 30-year span (5).

Disorders of the thyroid gland are globally prevalent and are associated with a range of clinical conditions. These include hypothyroidism, characterized by diminished hormone production; hyperthyroidism, marked by excessive hormone secretion; and the presence of thyroid nodules, which may or may not alter gland function (6). One of the most commonly encountered thyroid abnormalities is goiter, defined as an enlargement of the thyroid gland. It can occur in cases of hormone deficiency, excess, or even normal hormone levels, and is often a manifestation of underlying pathology (1). Goiter is particularly frequent among women and displays a wide spectrum of clinical presentations in terms of size, texture, and functional impact (7).

A recent nationwide analysis investigating the incidence of thyroid cancer in Iraq over a 28-year period (1995–2023) revealed a consistent upward trend in diagnosis rates across all age groups and both sexes. The overall annual percent change (APC) in incidence was estimated at +7.51%, reflecting a steady rise in newly reported cases. Notably, gender-based analysis indicated a sharper increase among females, with an APC of +14.27% from 2007 to 2023, compared to +10.97% among males during the same period. Furthermore, the female-to-male incidence ratio rose substantially, shifting from 1.7:1 in 2000 to 4.14:1 by 2023. These findings underscore a growing burden of thyroid cancer within the Iraqi population, particularly among women, and point toward enhanced diagnostic practices, increased awareness, and potential environmental or hormonal factors contributing to this trend (8).

This study aims to investigate the incidence of thyroid cancer in Diyala province by analyzing demographic and epidemiological patterns among patients. It seeks to identify the distribution of cases across age groups, sexes, and residential settings. The research also explores potential associations between these variables and the risk of thyroid malignancy. Through statistical analysis, the study provides insights that may support early detection and targeted public health interventions.

METHODS

Study Design

This study is a retrospective observational cross-sectional analysis carried out in Diyala Governorate, Baqubah Teaching Hospital, Laboratory Division, Histological Examination Unit, from February 2024 to December 2024.

Data Collection

Data were gathered from the medical records of one hundred patients, covering their demographic info like gender, age, and living situation, as well as the results of their postoperative histopathological examinations. Data completeness and accuracy were ensured through systematic record review. Any patient records that lacked essential information such as age, gender, or place of residence, were excluded from the analysis to maintain data integrity.

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Additionally, cases with ambiguous histopathological findings or incomplete diagnostic documentation were not considered. To address missing values, a case-wise deletion method was applied, whereby only complete records were included in the final statistical analysis.

Ethical Consideration

Ethical approval was received from Baqubah Teaching Hospital (E-234, in 2024-1-2).

Statistical Analysis

The data were analyzed with IBM SPSS Statistics version 28.0. T-test was used to determine the mean and standard deviation of age, with the significance level set at $p < 0.05$.

RESULTS

One hundred patients aged from 16 to 75 years old, 15 were male and 85 were female suspected of having thyroid cancer were referred to the Histological Examination Unit of the Laboratory Division at Baqubah Teaching Hospital in Diyala Governorate. The distribution of thyroid conditions across demographic variables reveals notable patterns. Among male participants, 66.7% were diagnosed with no malignancy, while 33.3% had papillary carcinoma, and no cases of follicular carcinoma were recorded. In contrast, females accounted for a larger proportion of the sample, with 84.7% showing no malignancy, 11.8% diagnosed with papillary carcinoma, and 3.5% with follicular carcinoma.

Age group analysis indicates that the highest proportion of thyroid cancer cases was observed in individuals aged 41–50 years, where papillary carcinoma constituted 22.6%. In the 31–40 and 51–60 age groups, papillary carcinoma was found in 12% and 23.5% of participants, respectively. Notably, no malignancies were reported among individuals over 60, while only one case of papillary carcinoma occurred in the ≤30 age group.

Regarding place of residence, 93.8% of urban dwellers had no malignancy, and only 6.2% presented with papillary carcinoma. Conversely, the rural group showed a higher proportion of thyroid malignancies, with 19.1% diagnosed with papillary carcinoma and 4.4% with follicular carcinoma, suggesting a possible environmental or access-related influence in disease occurrence (Table 1).

Table 1: Demographic characteristics of patients

Demographic characteristic			**Groups**			**Total (%)**
			No malignancy	Follicular carcinoma	Papillary carcinoma	
Sex	Male	n	10	0	5	15%
		%	66.7%	0%	33.3%	
	Female	n	72	3	10	85%
		%	84.7%	3.5%	11.8%	
Age groups	≤30	n	18	0	1	19%
		%	94.7%	0%	5.3%	
	31-40	n	20	2	3	25%
		%	80%	8%	12%	
	41-50	n	23	1	7	31%
		%	74.2%	3.2%	22.6%	
	51-60	n	13	0	4	17%
		n	76.5%	0%	23.5%	
	>60	%	8	0	0	8%
		%	100%	0%	0%	
Residential status	Urban	n	30	0	2	32%
		%	93.8%	0%	6.2%	
	Rural	n	52	3	13	68%
		%	76.5%	4.4%	19.1%	

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The mean age of individuals having thyroid cancer was 43.06±7.84 years, compared to 42.18± 13.64 years in patients without thyroid cancer. Male individuals had a mean age of 44.87 ± 13.26 years, whereas female individuals had a mean age of 41.89±12.70 years. The P-value was above 0.05, suggesting that there's no statistically significant difference in the age distribution (Table 2).

Table 2: Comparison of Age Distribution by Thyroid Cancer Status and Sex

Groups		N	Mean	SD	P value
Age	No malignancy	18	43.06	7.84	> 0.05
	Thyroid cancer	82	42.18	13.64	
Sex	Male	15	44.87	13.26	> 0.05
	Female	85	41.89	12.70	

According to positive results for thyroid cancer, the analysis revealed a higher prevalence of thyroid cancer in females. Among the 18 patients diagnosed with thyroid cancer, 13 (72.2%) were female, while only 5 (27.8%) were male, based on their residential status, 16 (88.9%) were living in rural zones, while 2 (11.1%) resided in urban zones. Among the diagnosed cases, papillary carcinoma was the most prevalent subtype, accounting for 15 (83%) cases, while follicular carcinoma accounted for 3 (17%) cases (Table 3).

Table 3: Characteristic of positive cases of thyroid cancer

Characteristic		P value
No. of positive cases for thyroid cancer (%) based on sex		
Males	5 (27.8%)	> 0.05
Females	13 (72.2%)	
No. of positive cases for thyroid cancer (%) based on residential status		
Urban	2 (11.1%)	< 0.05
Rural	16 (88.9%)	
No. of positive cases for thyroid cancer (%) based on thyroid cancer subtype		
Papillary carcinoma	15 (83%)	< 0.01*
Follicular carcinoma	3 (17%)	

DISCUSSION

There is great variability in thyroid cancer prevalence rates between and within countries (9). A study conducted by Obadiel and co-worker who found that 84.8% of patients with thyroid cancer were females, and 15.2% were males, indicating that females are more likely to develop thyroid cancer and this is consistent with the findings of the current study (10). Substantial evidence suggests that female sex hormones contribute to the development of thyroid cancer. Specifically, studies using mouse models have pointed out that estrogen acts as a promoter in the formation of thyroid tumors (11). In the intervening time, investigate outcomes established that increased serum estrogen might be a risk factor for Papillary thyroid cancer in human (12). The prevalence rate of thyroid cancer was found to be highest in women than in men (13). Goiter is a recognized disorder in females all over the world (14).

The findings of the present study align with broader national and regional patterns reported in recent literature. According to the 2022 Iraqi National Cancer Registry, thyroid cancer ranked among the top five most prevalent malignancies in females nationwide, with notable increases in incidence across all age groups, particularly in women aged 30 to 50 years. This corresponds with the current study's observation that most diagnosed cases occurred within the 31–50 age range, highlighting a consistent epidemiological profile across different populations.

Furthermore, the national retrospective analysis covering the period from 1995 to 2023 documented a steady rise in thyroid cancer cases, with an overall annual percent change (APC) of +7.51%. Notably, the APC among females was markedly higher (+14.27%) compared to males (+10.97%), which supports the current study's finding of a female predominance (72.2%). This gender disparity may be attributed to hormonal influences, as suggested by previous molecular and epidemiological studies (15).

The higher rates of thyroid cancer in rural areas can be partly attributed to how surgeons in these regions tend to refer patients who they suspect might have cancer or those with large goiters that could lead to compression issues or cosmetic problems. Moreover, because our hospital offers free services, we tend to see more cases, which might mean our sample is leaning toward more severe instances of the disease. However, the findings of the current study did not agree with the findings of McDow and co-worker who found a higher rate for thyroid cancer in urban areas compared to rural areas (16).

Older age is associated with increased incidence and worse survival (1). The outcomes of the current study are slightly agreed with Obadiel and co-worker who concluded that the largest number of cancers was seen in the (41–50) year age group, which were 48.5% of the cases and followed by the (31–40) year group (33.3%), the (18–30) year group (15.2%), and the (51–60) year group (3.0%). The outcomes of the current study are also agreed with Obadiel and co-worker who concluded that the majority of patient (72.2%) suffering from papillary thyroid carcinoma, this makes it the utmost prevalent form of thyroid cancer. and follicular thyroid carcinoma was diagnosed in (12.1%) of the cases (10).

This finding is consistent with worldwide trends and earlier research from the area, which also identified papillary carcinoma as the most frequently occurring type of thyroid cancer. The reason for the high occurrence of papillary carcinoma may be due to its well-differentiated characteristics, which make it more detectable during histopathological examinations (17,18).

Papillary thyroid cancer is the utmost prevalent form, accounting for roughly 70% to 80% of all thyroid cancer cases. It can develop at any stage of life and usually grows at a slow pace, often spreading to the lymph nodes in the neck. Thankfully, the prognosis for papillary cancer is quite positive, even when it has spread to those lymph nodes. On the other hand, follicular thyroid cancer represents about 10% to 15% of thyroid cancers in the U.S. This type of cancer has the potential to spread through the bloodstream to distant organs, especially the lungs and bones (1). A localized clinical study conducted in Mosul reported that papillary thyroid carcinoma was the most frequently diagnosed subtype, comprising over 80% of cases. This closely mirrors the present study, where papillary carcinoma accounted for 83% of diagnosed malignancies. The consistent dominance of this subtype in various Iraqi regions reinforces the notion of shared etiological or diagnostic patterns, potentially related to iodine status, genetic predisposition, or improved histopathological surveillance in recent years (19).

Another study indicated that the utmost predominant form of thyroid cancer is papillary thyroid cancer, which arises from follicular epithelial cells. It has a brilliant prognosis, with a 10-year survival rate of 42%. This kind of malignancy naturally affects females more frequently than males and tends to spread nearby, occasionally leading to metastasis in cervical lymph nodes (5).

A study conducted at Al-Shafa Hospital in Diyala province examined thyroid abnormalities in a sample of 67 patients aged between 20 and 80 years. Females accounted for 86.6% of the cases, with the highest prevalence observed in the 31–50 age group, and a mean patient age of approximately 49 years. Multinodular goiter was the most frequently identified condition, reported in 64.2% of patients, and was particularly common among women (86% of MNG cases). Histopathological analysis indicated that 79.1% of the lesions were benign, while malignant cases primarily involved papillary thyroid carcinoma. These results are in agreement with the present study, especially regarding female predominance, age distribution, and the dominance of papillary histology. The similarity in clinical patterns reinforces the potential role of multinodular goiter as a precursor lesion and highlights the importance of early detection strategies, particularly for women in middle age (13).

Limitations

The retrospective design might bring in some biases when it comes to how complete and accurate the

medical records are. Also, the fact that the sample has a gender imbalance, with mostly female participants, could influence how generalizable the findings are. These limitations were taken into account when analyzing and interpreting the results.

CONCLUSION

This study highlights the significant gender disparity in thyroid cancer incidence, with females being more frequently affected. Age and residential setting were significant risk factors of thyroid cancer risk. Papillary carcinoma remains the most common subtype. Future research should focus on hereditary and hormonal aspects contributing to the higher prevalence in women and develop effective early screening programs.

Conflict of Interest

The author declare that no conflict of interest.

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OPEN ACCESS ORIGINAL ARTICLE

Knowledge, Attitude, and Practice of Early Marriage among Female Secondary Students: A Cross-Sectional Study in Aden, Yemen

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ABSTRACT

Background: Early marriage remains a major challenge in Yemen, which affects development and contradicts human rights.
Objective: The present study aims to assess the knowledge, attitude, and practices regarding early marriage among female secondary school students in Yemen.
Methods: A cross-sectional study was conducted on 100 female secondary school students aged 14–19. Stratified sampling recruited students in the scientific section of grades 10–12. There were structured questionnaires to gather data for demographic, socioeconomic, and thematic variables of early marriage. Analysis was performed using SPSS.
Results: The majority of respondents were unmarried, aged 14–16, and had completed high school or university; nearly 72% had previously been exposed to some information about marriage, and 42% had a family history of underage marriage. 72% of participants believed that early marriage was unfair to girls, and they primarily attributed decision-making to the girls themselves (42%) or their families (38%). The main causes of early marriage were poverty (23%) and customs and traditions (69%), and the most common result was increased divorce rates (80%). The majority of participants (76%) believed that the best way to prevent early marriage is to raise awareness among girls and the general public.
Conclusion: The results highlight the necessity of community awareness campaigns, evidence-based policymaking, and educational interventions that empower teenage girls and aid in reducing the incidence and effects of early marriage in Yemen.

Key words: Early marriage, Knowledge, Attitude, Practice, Secondary female students.

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INTRODUCTION

Early marriage is an entrenched and injurious practice in Yemen, with serious implications for human rights, public health, and national development (1). The most recent available estimates reveal that 52% of Yemeni girls are married before 18 years of age and 14% are married before age 15 (2). The absence of a legally required minimum age for marriage—following the repeal of the erstwhile imposed restrictions in 1999—has further encouraged this culture, especially among rural and poor segments of the population where traditionalism and economic constraint are more prevalent (3).

The common practice of early marriage undermines girls' education, tending to lead to dropout at adolescence when they enter domestic and maternal responsibilities (4). Inequities in education are glaring: early-married girls are significantly less likely to complete secondary schooling, thereby perpetuating gender inequity cycles and limiting socioeconomic mobility (4). From a health perspective, teen childbearing is associated with increased maternal death and illness, and pregnancy complications are the leading cause of mortality for Yemeni girls aged 15–19 years (5).

Moreover, Yemeni child marriage is a human rights concern linked with domestic violence, marital rape, and repression of girls' autonomy over their bodies and decisions. The tradition is supported by cultural principles of respect for honor, female sexuality, and rigorous gender norms—particularly in traditional, tribal, and conflict settings (5). Despite ongoing strife and socioeconomic deterioration, poor households increasingly marry off daughters in order to secure dowries or perceived protection for the children (6). To counter this entrenched practice takes several interventions, including legal reform to have the age of 18 years at marriage, community-level awareness, and more opportunities for girls' schooling and economic empowerment (7).

This study contributes to understanding this environment by examining knowledge, attitudes, and practices related to early marriage among Yemeni secondary school girls, with implications for identifying potential entry points for targeted interventions.

METHODS

Study Area

The study was conducted in October's Congregation, which consists of both middle schools (grades 7–9) and secondary schools (grades 10–12).

Study Population

The target population was the female secondary school students aged 14 to 19 years.

Inclusion Criteria

Female students aged between 14 and 19 years and enrolled in secondary school.

Exclusion Criteria

Students under the age of 14 or older than 19 years were excluded from the study.

Study Design

An observational, analytical, cross-sectional study design based on the Knowledge, Attitude, and Practice (KAP) model in a community setting was used.

Sample Size and Sampling Technique

100 female students were randomly chosen from a target subgroup of 440 students. Stratified sampling was used to obtain proportional representation from among the three grades: 46 from Grade 10, 30 from Grade 11, and 24 from Grade 12.

Data Collection Tool

Data were gathered through the use of a standard questionnaire on demographic and socioeconomic characteristics, knowledge, attitude, and practice about early marriage and corresponding health education.

Statistical Analysis

The data was analyzed using SPSS version 22 (IBM, Chicago, USA). Descriptive statistics were employed to present the data.

RESULTS

The results show that the distribution of the age of the surveyed girls is relatively uniform, with 52% between 14 and 16 years and 48% between 17 and 19 years. In terms of marital status, the overwhelming majority of the participants (87%) are unmarried, 11% are engaged, and only 1% are married or otherwise reported. In terms of overall

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levels of education, most of the respondents (52%) have reached university level, followed by 34% at high school and 11% only at primary. In terms of grade levels currently at, 46% are at Grade Ten, 30% at Grade Eleven, and 24% at Grade Twelve (Table 1). This indicates a bias of students at lower secondary levels, decreasing gradually at higher levels.

Table 1: Frequency of demographic data among female students of secondary school

Variables		Frequency	Percentage
The Age of Girl	14-16	52	52.0
	17-19	48	48.0
Marital Status	Single	87	87.0
	Married	1	1.0
	Engaged	11	11.0
	Others	1	1.0
The Level of Education	Primary Education	11	11.0
	High School	34	34.0
	University	52	52.0
	Grade Ten	46	46.0
	Grade Eleven	30	30.0
	Grade Twelve	24	24.0

Regarding the prevalence of knowledge among female students of secondary school, the findings indicate that the majority of the respondents (72%) read or viewed something regarding marriage, while 28% did not. When asked whether someone within their family had gotten married at the age of majority, 42% said "yes," thereby illustrating that early marriage is present within a significant percentage of the subjects' families, while 58% said "no." Of the ideal age for marriage, only 3% believe it ought to occur within the 14–17 age group. A greater number, 45%, find 18–21 acceptable, and most (52%) find 22–30 the best age for marriage. This indicates a general preference for later ages of marriage (Table 2).

Table 2: Prevalence of knowledge among female students of secondary school

Variable	Options	Frequency	Percent	Valid Percent
Have You Read or Watched Any Thing About Marriage	YES	72	72.0	72.0
	NO	28	28.0	28.0
Any One of Your Family Had Married in Under Age	YES	42	42.0	42.0
	NO	58	58.0	58.0
The Appropriate Age of Marriage	Age 14-17	3	3.0	3.0
	Age 18-21	45	45.0	45.0
	Age 22-30	52	52.0	52.0

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For prevalence of attitude among female students of secondary school, information indicates that the majority of the girls (72%) view marriage as a girl inequity, meaning perceived shortcomings in terms of fairness or equality. In addition, 16% comment that it is acceptable if the girl wants it, and 12% believe they lack enough information to have an opinion. Regarding who makes the decision about marriage, 42% say the girl herself, 38% say families should, and 20% say the father should be the main decision-maker. When queried about who typically promotes marriage, 70% said men, and only 30% reported that women play such a role (Table 3). These results are one perspective on gender imbalance, both in attitudes towards marriage and in the social forces propelling it.

Table 3: Prevalence of attitude among female students of secondary school

Variables	Category	Frequency	Percent	Valid Percent
Girls' Opinion About Marriage	Iniquity of Girl	72	72.0	72.0
	It Is Ok as Long as She Wonts It	16	16.0	16.0
	I Don`t Have Enough Information to Answer	12	12.0	12.0
Who Makes the Marriage Decision?	The Girl	42	42.0	42.0
	The Family	38	38.0	38.0
	The Father	20	20.0	20.0
Who Encourages Marriage?	Women	30	30.0	30.0
	Men	70	70.0	70.0

The study reveals that the major reason for early marriage among the respondents is tradition and custom, cited by 69% of the respondents. Poverty is cited by 23%, while only 8% cite influence by the media as a factor. In the effect created by early marriage, the most common cited effect is a high divorce rate, cited by 80% of the respondents. Polygamy, population growth, and so-called settling of the girl were each mentioned by a considerably lower proportion (8%, 6%, and 6%, respectively). When asked about preventing early marriage, the majority (76%) believe that the best way is to make girls aware as well as society. Another 23% believe that education and job opportunities for girls are the solution. Only 1% believes in helping only the girl. These results highlight a clear belief in the importance of social awareness and the empowerment of girls through working and education in order to prevent early marriage (Table 4).

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Table 4: Prevalence of the reasons, effects, and ways to limit early marriage among female students of secondary school

Variable	Category	Frequency	Valid (%)	Percent
Reasons for Early Marriage	Poverty	23	23.0	
	Media Influence	8	8.0	
	Customs and Traditions	69	69.0	
Effects of Early Marriage	Increased Divorce Rate	80	80.0	
	Polygamy	8	8.0	
	Population Growth	6	6.0	
	Settlement of the Girl	6	6.0	
Ways to Limit Early Marriage	Awareness and support for the girl only	1	1.0	
	Providing education and work for the girl	23	23.0	
	Raising awareness for the girl and society	76	76.0	

DISCUSSION

Overall, the findings support the high rates of early marriage and relationship formation among Yemeni adolescent girls, particularly in Aden, where these outcomes are more than twice as prevalent as those reported in earlier research (9). Yemen is home to 4 million child brides; of these, 1.4 million married before age 15. According to data from the Yemen Demographic and Health Survey (DHS) from 2013, almost a third (32 percent) of all young women aged 20–24 had been married before age 18, and 9 percent before age 15 (9). The major finding in our study about the girls' opinion about the appropriate age for marriage was between 20 and 30, so we did not find Only one girl married, while the number of girls engaged was 11, and the number of single girls was 87. Over a quarter of participants in our study narrated that they suffered from several health problems, such as frequent pains, disturbed menstrual cycles, abortion, difficulty in childbirth, and physical weakness, which were more than those of their counterparts who were married after the age of twenty.

While low socioeconomic status, which is defined by a lack of funds to maintain a healthy diet or access expensive healthcare, may be the direct cause of these health problems, previous research and studies from nearby nations show that child marriage is associated with poor fertility-control outcomes. Even after taking into consideration social vulnerabilities like women's home location, ethnicity, education, and economic status. This raises the question of whether cultural factors and attitudes regarding child marriage—rather than societal vulnerabilities—are responsible for its continued prevalence in Aden and domineering behavior of spouses and in-laws toward young married women. Insufficient education and media exposure, especially in rural areas, along with women's limited control over health-related decisions within the family, may explain why the women in our study were not aware of the negative health effects of child marriages, as reported in previous studies (10).

The fact that the majority of women in our survey who were married as children expressed satisfaction and thought their parents were right to arrange their marriages at such a young age was concerning. The fact that most of the women in our study disagreed with the nation's ban on child marriages, viewing it only as a family matter, was especially disturbing.

The majority of the participants in the previous study narrated their willingness to marry their daughters before the age of 18 years, subject to the availability of a good marriage (11). This may differ in our study; in our study, the majority of girls believe that the one who makes the decision to marry is the girl herself (42%), and 38% see the father as having the right to make the decision of marriage.

Protecting the “family honor is listed as one of the reasons for child marriages in earlier studies. The

moment girls reach puberty, they are believed to be a source of attraction and lust for boys; parents, thus, feel relaxed and free of the burden of guarding their girls from unchastity by marrying them at an early age. By marrying girls before the age of 18 years, parents believe that this practice could protect their daughters from unwanted attention from men and the likelihood of objectionable illegal relationships (12). Further, dropping girls out of schools in the name of protecting family honor is an unfortunate consequence (12).

According to the attitude of early marriage among the current study, we found that more than half of the girls were of the opinion that early marriage is an iniquity to the girl, while 16% of the female students were of the opinion that early marriage is ok as long as she wants it, but 12% of the female students were of the opinion that early marriage is "I don't have enough information to answer." Thirty of the girls argued that women are the main advocates for early marriage, while more than half of the girls thought men were the main proponents. Twenty students claimed that the father makes the decision regarding marriage, forty-two students thought that the girl should make the decision to marry, and thirty-eight students argued that the family makes the decision.

CONCLUSION

The opinions and factors influencing early marriage among female secondary students in Aden were examined in this study. It exposed preconceived notions and highlighted the negative effects on economic well-being, family stability, and health. Most of the participants lived in Enma, were single, and had mothers with only a primary education and fathers with a university degree. They generally believed that the girl should have the freedom to make her own decisions and that an early marriage was unfair, with the ideal marriage age being between 22 and 30. While most participants linked early marriage to higher divorce rates, tradition was the main justification given by participants for getting married young. Many of them had previously come across educational materials on the topic. The study emphasizes the need for programs, regulatory changes, and awareness campaigns to increase girls' autonomy and reduce the prevalence of child marriage.

Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS Original Article

Toxoplasma gondii Prevalence and Risk Factors among Pregnant Women in Ibb Governorate, Yemen

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ABSTRACT

Background: Pregnant women and their fetuses are especially vulnerable to toxoplasmosis.
Objective: To assess the prevalence of Toxoplasma gondii and related risk factors in pregnant women at Ibb Governorate, Yemen.
Methods: A cross-sectional study with 415 pregnant women was carried out in Yemen's Ibb Governorate between June and October 2024. Anti-T. gondii antibodies were detected in serum samples by the enzyme-linked immunosorbent assay (ELISA). Data on potential infection risk factors linked to the infection were gathered by the administration of a questionnaire.
Results: The research found an overall seroprevalence of anti-T. gondii antibodies (IgG and/or IgM) of 55.9% (232/415; 95% Confidence Interval: 50.9%–60.9%). Among the participants, 45.0% (187/415) tested positive for IgG, 3.4% (14/415) for IgM, and 7.5% (31/415) for both. Binary regression analysis revealed significant risk factors for infection, including increasing age (Adjusted Odds Ratio [AOR] = 1.88; P = 0.006), history of blood transfusions (AOR = 2.03; P = 0.012), cat presence in the household (AOR = 1.86; P = 0.006), history of miscarriage (AOR = 2.76; P = 0.000), and history of giving birth to a baby with defects (AOR = 5.53; P = 0.002).
Conclusion: These findings indicate a high rate of toxoplasmosis in prenatal medical care clinics in Ibb Governorate, Yemen, underscoring the need for routine screening and health education on transmission modes.

Keywords: Toxoplasmosis; Anti-Toxoplasma gondii Antibodies; Pregnant Women; Risk Factors.

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INTRODUCTION

An obligatory intracellular parasite that causes toxoplasmosis in both people and animals. Pregnant women and their fetuses are especially vulnerable to this disease (1). The frequency of toxoplasmosis varies significantly depending on geographical region, dietary habits, and socioeconomic factors (2). The prevalence is significantly higher in developing countries than in developed countries. Nonetheless, the occurrence of toxoplasmosis ranges from 30% to 60% in both developed and developing regions (3).

Several factors contribute to the progression of toxoplasmosis, including the virulence of *Toxoplasma* strains, the size of the inoculum, the host's immune status, and its genetic background (4). An estimated 190,100 incident cases of congenital toxoplasmosis are thought to occur each year due to vertical transmission from mother to child during pregnancy, with a frequency of 1.5 neonatal cases per 1,000 live births worldwide (5).

A random-effects meta-analysis comprising 250 studies with 723,655 pregnant women was used to construct a global and regional estimate. Pregnant women's data were gathered from 1976 to 2017. The overall IgG seroprevalence in these investigations was 32.9%. The Western Pacific region had the lowest IgG seroprevalence at 11.2%, while the Americas had the highest at 45.2% among the WHO regions. On the other hand, 1.9% was the global IgM seroprevalence (6).

The Middle East has one of the highest prevalence rates in the world, with total infection rates ranging from 30% to 50% (2). Prevalence of anti-*T. gondii* IgG and IgM antibody among pregnant women in Saudi Arabia ranged from 8.6% to 51.4% and from 0% to 8.8%, respectively, according to a review of 20 studies (7). The current study aimed to assess the prevalence of *Toxoplasma gondii* and related risk factors in pregnant women at Ibb Governorate, Yemen.

METHODS

Study Design

From June to October 2024, a cross-sectional survey was conducted in three Ibb Governorate districts. A pre-tested questionnaire was first developed in English and then translated into Arabic, the local language. Data was collected by medical professionals from general maternity and pediatric clinics. The prenatal clinics performed a variety of procedures on pregnant mothers, including ultrasound imaging, laboratory testing, physical examinations, and assessments of medical histories. Serological data were obtained from collaborating private laboratories with patient consent in this study.

Study Setting

Three districts of Ibb Governorate participated in the study: Al-Dhihar, Al-Mashannah, and Jiblah, which are located south of Yemen's capital city, Sana'a (Figure 1). The region has a temperate climate, and its economy is primarily agricultural. According to the most recent census conducted by the Central Statistical System, the total population of Ibb Governorate is approximately 3,118,000 inhabitants, distributed across 20 districts. The study focused on most of the prenatal medical care clinics in these three districts.

Sample Size and Study Population

Using a 95% confidence level and a desired precision of 0.05, the sample size was calculated based on a previous study conducted in Dhamar Governorate (8). Around 415 pregnant women were enrolled, and the Medical Ethics Committee of Ibb University in Ibb, Yemen, approved the study protocol.

Ethical Considerations

The Ethics Committee of Ibb University's Faculty of Medicine and Health Sciences provided ethical approval (EC/24/13). Informed consent was obtained from pregnant women, who were asked to participate voluntarily after explaining to them the objectives of the study. Confidentiality of the study participants' data was assured.

Data Collection

Through face-to-face interviews, a pre-designed questionnaire was used to collect data from the participating women. Information collected from all pregnant participants included age, frequency of pregnancies, history of spontaneous abortions, number of living children, gestational period, place of residence (urban vs. rural), and occupation. Additionally, data were gathered on pet ownership, specifically regarding cats and other domestic animals (e.g., rats), whether participants kept cats as

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pets, and their consumption of animal milk (camel, cow, or goat). Lastly, the questionnaire assessed cooking and eating habits, such as the consumption of raw or undercooked meat. The level of knowledge regarding Toxoplasma infection was also evaluated.

Serological Examinations

Commercial enzyme-linked immunosorbent assay (ELISA) kits (AbiaToxoAbIgG and IgM Fast) were used to test sera for anti-*T. gondii* IgG and IgM. Antibody titers were categorized as either positive or negative based on the manufacturer's defined normal values. Each ELISA test had both positive and negative controls to guarantee the assay's technical performance and the reagents' integrity.

Statistical Analysis

IBM Corp.'s Statistical Package for the Social Sciences (SPSS, version 25) was used to enter and evaluate the data. The descriptive information gathered from the surveys and lab tests was displayed as percentages and frequencies. Using the Pearson chi-square test, differences and relationships between categorical variables were examined. Within the bivariate analysis, 95% CIs and adjusted odds ratios (ORs) were shown. Statistical significance was established at a *P*-value of less than 0.05. In order to determine independent predictors of infection and compute adjusted odds ratios (AORs), all variables that displayed a *P*-value of ≤ 0.05 in the bivariate logistic regression analysis were utilized. A *P*-value of less than 0.05 was deemed statistically significant. A comprehensive explanation of the data was also provided by the corresponding confidence intervals for non-significant comparisons.

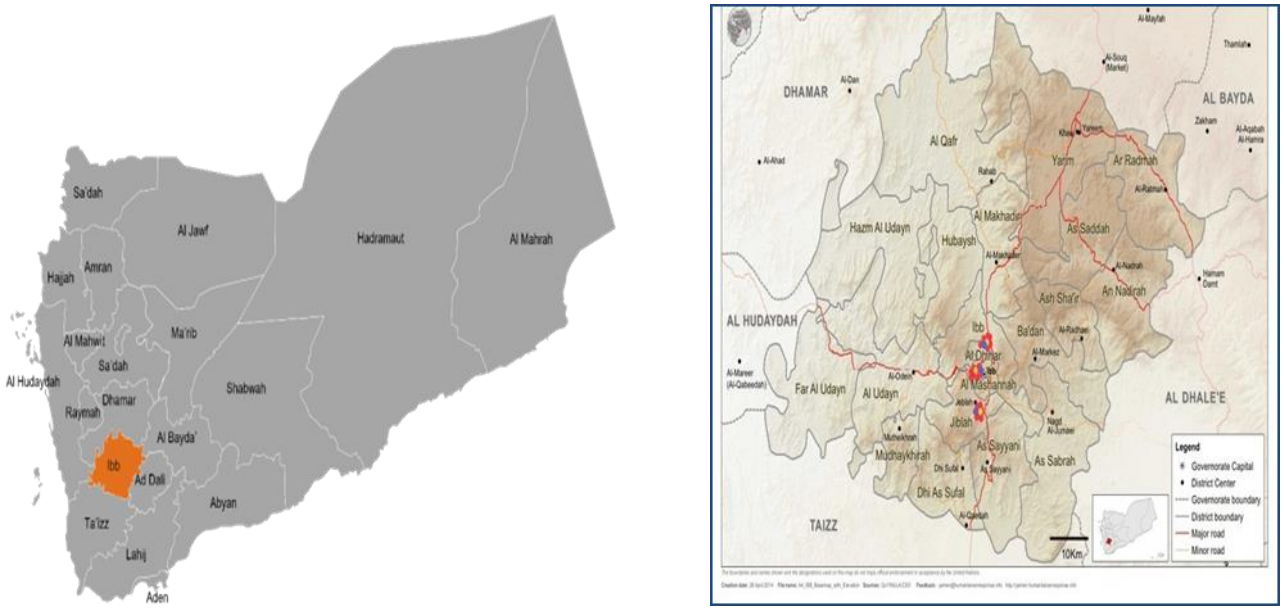

Figure 1: Map of Yemen showing the location of Ibb Governorate and the study area

RESULTS

General Characteristics of the Study Population

A total of 415 pregnant women aged between 17 and 48 years participated in the study, with an average age of 27 ± 6.6 years. In terms of age distribution, 154 women (37.11%) were under 25 years old, while those aged 25 years and older accounted for 261 women (62.89%). Less than half of the participants (195, 46.99%) lived in rural areas, whereas more than half (220, 53.01%) resided in urban areas. Most women (360 out of 415, 86.51%) were unemployed, while 55 (13.49%) were employed.

Regarding the stages of pregnancy, 242 women (58.3%) were in their first trimester, 114 (27.5%) were in the second trimester, and 59 (14.2%) were in their third trimester. In terms of abortion history,

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most pregnant women (229, 55.2%) reported no history of abortion, while 186 women (44.8%) indicated a history of abortion during their pregnancy. The broad demographic, obstetric, and social and economic characteristics of the study population are outlined in Table 1.

Table 1: Socio-demographic, Socioeconomic, and Behavioral Characteristics of Pregnant Women in Ibb Governorate, Yemen (n = 415).

Variable	Frequency	Percentage%
Age		
<25	154	**37.11%**
>=25	261	**62.89%**
Residence		
Urban	220	**53.01%**
Rural	195	**46.99%**
Occupation of women		
Employed	55	**13.25%**
Unemployed	360	**86.75%**
Gestational trimester		
1st trimester	242	**58.3%**
2nd trimester	114	**27.5%**
3rd trimester	59	**14.2%**
History of spontaneous abortion		
Yes	186	**44.8%**
No	229	**55.2%**

Seroprevalence of Anti-*Toxoplasma gondii* Antibodies (IgG & IgM) among Pregnant Women in Ibb Governorate, Yemen

The overall seroprevalence of anti-*T. gondii* antibodies (IgG and/or IgM) among the pregnant women was 55.9% (232/415; 95% Confidence Interval = 50.9%–60.9%). Among the participants, 45.0% (187/415) tested positive for anti-*T. gondii* IgG antibodies alone, 7.5% (31/415) were positive for both IgG and IgM antibodies, and 3.37% (14/415) tested positive for IgM antibodies alone, as shown in Table 2.

Table 2: Seroprevalence of Anti-*Toxoplasma gondii* Antibodies (IgG & IgM) Among Pregnant Women in Ibb Governorate, Yemen (n = 415).

Toxoplasma antibodies	No. tested	No. of positive cases	Percentage %
IgG alone	415	187	45.0%
IgM alone	415	14	3.40%
Both IgG & IgM	415	31	7.50%
Total	**415**	**232**	**55.90%**

Bivariate Analysis of Factors Associated with *T. gondii* Seroprevalence among Pregnant Women in Ibb Governorate, Yemen

The investigation found a strong correlation between the prevalence of *T. gondii* infection and the age of pregnant mothers. Age-related increases in seroprevalence were noted. Compared to those under

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25, pregnant women aged ≥25 years exhibited a significantly greater prevalence of *T. gondii* infection (64.4% [168/261] vs. 41.6% [64/154]; AOR = 1.88, 95% CI = 1.20–2.94, *P* = 0.006).

Compared to women without a history of spontaneous abortion, pregnant women with a history of abortion had a substantially higher correlation with *T. gondii* infection (69.3% [129/186] vs. 44.9% [103/229]; AOR = 2.76, 95% CI = 1.84–4.15, *P* = 0.000). Furthermore, compared to individuals who had not contracted *T. gondii*, giving birth to a baby with congenital anomalies was substantially associated with infection (AOR = 5.53, 95% CI = 1.85–16.51, *P* = 0.002).

The frequency of *T. gondii* infection was greater in pregnant women with a history of blood transfusion than in those without (75.0% [75/100] vs. 49.8% [157/315]; AOR = 2.03, 95% CI = 1.16–3.56, *P* < 0.001). An increased risk of infection was also linked to the presence of rats and cats in the home. The prevalence of *T. gondii* infection was found to be significantly higher among women who had cats than among those who did not (67.5% [100/148] vs. 49.8% [133/267]; AOR = 1.86, 95% CI = 1.19–2.90, *P* = 0.006).

Table 3: Bivariate Analysis of Factors Associated with *T. gondii* Seroprevalence among Pregnant Women in Ibb Governorate, Yemen (n = 415).

Variable	No	n(%)	OR(95%CI)	AOR(95%CI)	*P* value
Age					
<25	154	64(41.6)	Reference		
≥25	261	168(64.4)	2.54(1.69-3.82)	1.88(1.20-2.94)	**0.006***
Residence					
Urban	220	122(55.4)	Reference		
Rural	195	110(56.4)	0.96(0.65-1.42)	1.12(0.74-1.69)	**0.582**
Occupation of women					
Employed	55	29(52.7)	Reference		
Unemployed	360	203(56.3)	0.83(0.47-1.45)	0.94(0.52-1.71)	**0.941**
Gestational trimester					
1st trimester	242	130(53.7)	Reference		
2nd trimester	114	71(62.2)	1.42(0.90-2.24)	1.13(0.69-1.85)	**0.624**
3rd trimester	59	32 (54.2)	1.02(0.57-1.80)	0.77(0.39-1.51)	**0.778**
History of Spontaneous (abortion)					
No	229	103(44.9)	Reference		
Yes	186	129(69.3)	2.76(1.84-4.15)	2.76(1.84-4.15)	**0.000***
History of giving birth to a baby with congenital anomalies					
No	383	204(53.2)	Reference		
Yes	32	28(87.5)	6.14(2.11-17.84)	5.53(1.85-16.51)	**0.002***
Number of live children					
<3	310	156(50.3)	Reference		
≥3	105	76(72.4)	2.62(1.61-4.25)	0.91(0.43-1.91)	**0.901**
Having cats in the house					
No	267	133(49.8)	Reference		
Yes	148	100(67.5)	2.099(1.38-3.19)	1.86(1.19-2.90)	**0.006***
History of blood transfusions					
No	315	157(49.8)	Reference		
Yes	100	75(75.0)	3.01(1.82-2.62)	2.03(1.16-3.56)	**0.012***
Having cats in the neighbor					
No	158	79(50.0)	Reference		
Yes	257	153(59.5)	1.49(1.00-2.22)	1.36(0.89-2.08)	**0.155**
Having rodents(rats) in the house					
No	214	102(47.7)	Reference		
Yes	201	130(64.7)	2.01(1.35-2.98)	1.51(0.97-2.35)	**0.065**

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Raw meat Consumption					
No	177	95(53.6)	Reference		
Yes	238	137(57.5)	1.17(0.79-1.73)	1.00(0.65-1.55)	**0.976**
Shawarma Consumption					
No	109	65(59.6)	Reference		
Yes	306	167(54.6)	0.81(0.52-1.26)	1.16(0.68-1.98)	**0.574**
Raw milk consumption					
No	186	97(52.1)	Reference		
Yes	229	135(58.9)	1.37(0.92-2.62)	1.12(0.71-1.75)	**0.615**
Awareness of toxoplasmosis transmission of routes					
Yes	117	63(52.5)	Reference		
No	298	169(57.1)	1.12(0.73-1.72)	1.02(0.61-1.69)	**0.928**

DISCUSSION

This study is among the few in Yemen that investigate the prevalence of *T. gondii* infection among a significant clinical category of toxoplasmosis in immunocompetent hosts—pregnant women in Ibb Governorate. The overall seroprevalence of anti-*T. gondii* antibodies found in this study was 55.9%. The current findings align with previous studies conducted in Yemen, which reported seroprevalence rates ranging from 45.4% among pregnant women in northern Yemen (9) to 46.2% in Taiz (10) and 64.3% in Aden, southern Yemen (11).

Comparing our findings with those from other countries, the seroprevalence reported in this study (55.9%) is consistent with that in Egypt (57.9%) (12). However, higher seroprevalence rates have been reported in several countries, including Brazil (71%) (13), Lebanon (82.6%) (14), Ethiopia (85.3%) (15), and Ghana (92.5%) (16). In contrast, lower seroprevalence rates have been reported in Hodiedah Governorate (14.4%) (17), Sana'a City (18.7%) (18), Dhamar Governorate (21.2%) (8), Palestine (17.6%) (19), Saudi Arabia (24.1%) (20), and Turkey (30.1%) (21).

Globally, the variation in the seroprevalence of *T. gondii* infection can be attributed to 3.4% of the pregnant women tested positive for IgM antibodies alone various factors, such as differences in diagnostic methods with varying sensitivities and the prevalence of consuming raw or undercooked contaminated meat (13, 22, 23).

According to the current study, pregnant women with IgM antibodies alone had detectable levels of 3.37%, those with both anti-*T. gondii* IgM and IgG antibodies had 7.5%, and those with IgG antibodies alone had 45.0%. These findings indicate a lower seroprevalence of anti-*T. gondii* IgM antibodies alone, which may suggest early infection with toxoplasmosis during pregnancy. Early infection has been associated with spontaneous abortion, and it has been suggested that IgM seropositivity is associated with this risk (9, 24, 25).

On the other hand, the higher seroprevalence of anti-*T. gondii* IgG antibodies alone may indicate chronic infection. This higher prevalence has been reported in previous studies among pregnant women in Aden Governorate (11). Similarly, other studies conducted in Saudi Arabia (26), Egypt (27), Sudan (28), and Ethiopia (29) have reported comparable findings.

The current investigation also documented that domestic cats are a major contributing factor to *T. gondii* contamination (9, 11). These findings are consistent with the results reported in our study. Bivariate analysis revealed a statistically significant association between the age of pregnant women and the seroprevalence of *T. gondii* infection, with higher seroprevalence observed in women aged ≥25 years. This evidence is consistent with prior studies conducted in Taiz Governorate (10), Sana'a City (9), and Turkey (21). In Yemen, older women are often more involved in agricultural activities and animal rearing, which may increase their exposure to *T. gondii.* This could help explain the observed association.

The bivariate analysis also revealed a statistically significant association between giving birth to a deformed baby and *T. gondii* infection. However, this finding contrasts with a study conducted in Dhamar Governorate (8). This association may be attributed to fetal death resulting from disruptions in nutrient and oxygen transport due to vascular lesions formed in the placenta as a consequence of *T. gondii* infection, leading to lethal hypoxic damage to the fetus (30).

This association may suggest a potential link, though causality cannot be established.

Additionally, the present study documented a history of abortion as a significant risk factor for human toxoplasmosis, aligning with findings from Dhamar Governorate (8) and Taiz Governorate (25). There is a suggestion that *T. gondii* may be an etiological factor in miscarriage, particularly in cases where fetal losses exceed three (30).

Risk factors discussed in our study highlighted that rearing cats in the home was a significant risk factor for seropositivity. This finding is consistent with studies conducted in Dhamar Governorate (8) and Aden Governorate (11). Additionally, a history of blood transfusion was identified as a significantly associated risk factor for infection. Significant associations between blood transfusions and toxoplasmosis have also been reported in further studies conducted in Myanmar (32) and Mexico (33), which aligns with our findings. However, other studies, including those in Dhamar (8) and Ethiopia (34), reported no significant association between blood transfusions and toxoplasmosis, contradicting our results.

Currently, screening for *T. gondii* infection is not routinely performed in blood banks (35). The parasite is present in the blood in the form of tachyzoites, which are the invasive stage and can disseminate to other organs via the bloodstream (36). Therefore, the presence of *T. gondii*-infected blood poses a potential source of infection for pregnant women receiving transfusions, as indicated by our survey.

Blood transfusion is a worthwhile topic that might be improved by taking policy consequences into account, such as the creation and application of uniform screening procedures for *T. gondii* in blood banks. Transfusion safety could be increased by implementing such techniques as leukoreduction or targeted serological testing, especially for high-risk populations like expectant mothers. (37, 38).

In the present study, no significant association was found between seropositivity and the consumption of raw or undercooked meat (including shawarma). This finding is consistent with studies conducted in Dhamar Governorate (8), Khartoum, Sudan (39), and Turkey (21). However, it contrasts with a survey conducted in northern Brazil, where a significant association was observed between seropositivity for anti-*T. gondii* and the consumption of undercooked meat (13). Additionally, residence and gestational age showed no significant association with the seroprevalence of anti-*T. gondii* antibodies, which aligns with similar findings reported in Dhamar Governorate (8).

Limitations

This study has various limitations that should be recognized. First, data on potential risk factors were gathered via self-reported questionnaires, which may have introduced self-reporting bias and impacted the accuracy of the reported exposures. Second, the serological findings were not verified by polymerase chain reaction (PCR) testing, which may have provided a more precise diagnosis of *Toxoplasma gondii* infection and discriminated between active and prior infections. In addition, there was insufficient follow-up to track the progression or potential outcomes of infection during pregnancy, limiting the ability to assess long-term maternal and fetal consequences.

CONCLUSION

The study revealed a high prevalence of *Toxoplasma gondii* infection, indicating a significant public health concern for pregnant women and their offspring in Yemen. The findings highlight the importance of developing standard screening programs for toxoplasmosis during pregnancy in Yemen, and it is suggested that prenatal care services include routine screening and health education.

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Conflict of Interest

The author declares that there is no conflict of interest.

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OPEN ACCESS Original Article

Nutritional Deficiencies in Celiac Disease: A Cross-Sectional Study of Iron and Vitamin B12 Status in Diyala, Iraq

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ABSTRACT

Background: Celiac disease (CD) is an autoimmune disorder triggered by gluten ingestion, leading to chronic inflammation and small intestinal damage. The prevalence of CD in Iraq, particularly in Diyala province, has significantly increased in recent years. Nutritional deficiencies are common among CD patients due to impaired nutrient absorption resulting from intestinal damage .
Objective: This study aimed to characterize iron and vitamin B12 status in Diyala's CD population.
Methods: This cross-sectional study assessed iron and vitamin B12 status in 90 CD patients and 30 healthy controls at AL Shams Medical Labs, Diyala, between October 2024 and May 2025.
Results: The mean age of CD patients was 48.03 ± 1.84 years, while controls had a mean age of 36.33 ± 2.32 years. The results showed a significant increase in anti-tissue transglutaminase antibodies (tTG-IgA and tTG-IgG) and I-FABP levels in CD patients compared to healthy controls. Additionally, serum iron and ferritin levels were significantly lower, while total iron-binding capacity (TIBC) was significantly higher in CD patients ($P < 0.05$). Although mean vitamin B12 levels were lower in the CD patient group, this difference was not statistically significant ($P = 0.114$).
Conclusion: Overall, CD significantly affects iron metabolism, whereas vitamin B12 levels appear less consistently impacted, emphasizing the need for targeted nutritional monitoring.

Keywords: Celiac Disease; I-FABP, Nutritional Deficiencies; Iron; Vitamin B12.

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INTRODUCTION

Celiac disease (CD) is a chronic autoimmune enteropathy triggered by gluten consumption in genetically predisposed individuals. Because the only effective treatment is permanent adherence to a gluten-free regimen, the challenge of managing the condition becomes apparent, along with the necessity to evaluate health-related quality of life in CD patients [1]. Globally, its prevalence is estimated at around 1% [2], but in many developing countries, including Iraq, the disease remains underdiagnosed and poorly managed. Limited awareness among healthcare providers, lack of screening programs, and similarities with other gastrointestinal disorders pose significant challenges to timely diagnosis and treatment in Iraq. Since CD is associated with injury to the small intestinal mucosa, its typical presentation primarily involves gastrointestinal nutrient malabsorption. Common symptoms include persistent diarrhea, abdominal bloating and discomfort, weight loss, and growth reduction in infants [3,4].

Nevertheless, individuals with CD often face various extraintestinal manifestations and disorders, such as persistent fatigue, depression, osteoporosis, anxiety, impaired fertility and sex desire, especially among women [3]. Although CD typically involves a wide range of symptoms, some individuals remain without noticeable symptoms, even when intestinal mucosal injury is present [5]. Those individuals are at an increased risk of complications, as they often fail to perceive the clinical progression of CD and are generally less responsive to the therapy [6].

Iron deficiency anemia (IDA) remains the most prevalent extra-intestinal manifestation, affecting approximately 40% of CD patients at diagnosis, while vitamin B_{12} deficiency occurs in 41% of untreated cases [7,8]. These deficiencies stem not only from malabsorption due to mucosal damage but also from chronic inflammation-mediated mechanisms, including hepcidin dysregulation and cytokine-driven iron sequestration [8].

Recent studies in Middle Eastern populations reveal distinct patterns of micronutrient depletion. In Iraq, CD patients with Marsh III histological severity demonstrated a 59% prevalence of low ferritin levels and significant hemoglobin reductions, particularly among those with poor dietary compliance [9]. This geographical variation highlights the interplay between genetic predisposition, dietary habits, and environmental factors in shaping nutritional outcomes. Despite histological recovery post-GFD, up to 33% of pediatric patients continue showing iron deficiency, and 21.1% exhibit vitamin D insufficiency, suggesting persistent enterocyte dysfunction or inadequate dietary compensation [10]. The nutritional landscape in Iraq presents unique challenges for CD management. Traditional Iraqi diets heavily reliant on wheat-based staples may complicate GFD adherence, while limited access to fortified gluten-free products exacerbates deficiency risks. This study therefore aims to characterize iron and vitamin B_{12} status in Diyala's CD population.

METHODOLOGY

This cross-sectional comparative study was conducted on celiac patients and healthy controls at Al Shams Medical Labs in Diyala City between 1 October 2024 and 10 May 2025. Diagnosis of celiac disease (CD) in patients was confirmed using serological tests for anti-tissue transglutaminase (anti-tTG) antibodies and intestinal fatty acid-binding protein (I-FABP). (I-FABP). Participants were excluded if they did not have confirmed celiac disease or had conditions that could confound iron or vitamin B_{12} levels, such as chronic kidney disease, liver disease, hematological disorders, or pernicious anemia. Individuals who had received blood transfusions or taken iron or B_{12} supplements within the past three months were also excluded. Additionally, pregnant or lactating women, patients with malignancies or severe chronic illnesses, and those who did not provide informed consent were not included in the study.

Blood Samples Collection

Nearly 4 mL of blood was collected from the CD patients and an equal volume from healthy controls under strictly aseptic conditions. The blood samples were then transferred into gel-containing tubes and left to clot at normal room temperature. Subsequently, they were centrifuged at 2000 rpm for 15 minutes, after which the resulting serum was aliquoted into 4 Eppendorf tubes. These tubes were then stored at −20°C to be analyzed later for the detection of iron, ferritin, total iron-binding capacity (TIBC) as well as vitamin B12.

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Serological Assessments

The tTG-IgA and tTG-IgG antibodies were detected using the Chorus® instrument (Diesse Diagnostica Senese, Italy), which employs an immunoenzymatic method based on mono-test devices, following the manufacturer's instructions. Intestinal Fatty Acid-Binding Protein (I-FABP) levels were determined using a commercial ELISA kit (manufacturer: DIALAB, Austria), according to the enzyme-linked immunosorbent assay protocol provided by the manufacturer. Serum iron and TIBC levels were measured using the cobas® c 311 analyzer (Roche Diagnostics, Germany) following the manufacturer's protocol. Serum ferritin levels were evaluated using the cobas® E411 analyzer (Roche Diagnostics, Mannheim, Germany), which utilizes the electrochemiluminescence immunoassay (ECLIA) technique. Serum vitamin B_{12} levels were measured using the VIDAS® system (bioMérieux, France), which is based on an enzyme-linked fluorescent assay (ELFA) technique, following the manufacturer's instructions.

Ethical Approval

The study was approved by the Ethics Committee of the College of Medical and Health Techniques, University of Bilad Alrafidain (No. E-503/2024-8-15), and conducted in accordance with the Declaration of Helsinki. All participants provided informed written consent. Data were collected at Al Shams Medical Laboratories, licensed by the Iraqi Ministry of Health, from patients who voluntarily underwent diagnostic testing as part of routine clinical care.

Statistical Analysis

The data were analyzed using SPSS version 26.0. Descriptive statistics were presented as mean ± standard error of the mean. An independent t-test was employed to compare means between groups, with a p-value < 0.05 considered statistically significant.

RESULTS

Demographic characteristics of the study groups

A total of 120 individuals were enrolled in this study, comprising 90 patients diagnosed with celiac disease (CD) and 30 clinically healthy controls. The average age of CD patients was 48.03 ± 1.84 years, while that of the control group was 36.33 ± 2.32 years.

Serological Assessment of tTG-IgA, tTG-IgG, and I-FABP in Celiac Disease and Control Groups

The findings indicate a significant elevation in TTG-G and TTG-A antibody levels among celiac disease patients compared to healthy controls. Specifically, the TTG-G levels in patients (40.04±3.38 IU/mL) were markedly higher than in controls (8.89±1.09 IU/mL, P = 0.001). Likewise, TTG-A levels were significantly increased in patients (45.78±4.64 IU/mL) compared to controls (3.75±0.42 IU/mL, P = 0.001) (Table 1). In addition, serum levels of I-FABP were significantly higher among patients (5.10±2.33 ng/mL) as compared to control groups (1.50 ± 0.73 ng/mL, P = 0.001) (Figure 1).

Table 1: Serum levels of Tissue Transglutaminase Antibody Levels among Study Groups.

tTG Tests (Normal Range)	Group	N	Mean	Std. Error Mean	*P*-value
TTG-G **Negative: < 20 IU/mL** **Positive: ≥ 20 IU/mL**	Patients	90	40.0412	3.38725	0.001
	Controls	30	8.8967	1.09040	
TTG-A **Negative: < 9 IU/mL** **Equivocal:10-15 IU/mL** **Positive > 16 IU/mL**	Patients	90	45.7880	4.03553	0.001
	Controls	30	4.6407	0.62913	
***P*i value <i 0.05**				* i *Independent.-samples* i *T test*	

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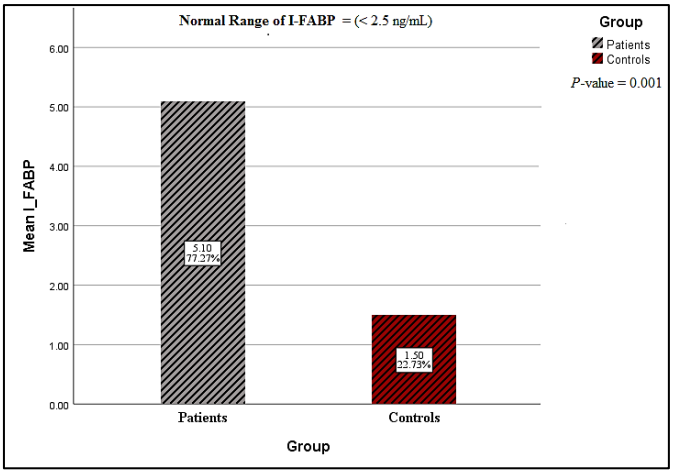

Figure 1: Serum Levels of I-FABP in Celiac Disease Patients Compared to Healthy Controls

Serological Assessment of Iron, TIBC, and Ferritin in CD Patients and Controls

The results of this study demonstrate significant alterations in iron metabolism in patients with celiac disease. Specifically, iron levels were significantly lower in the patient group (10.96±0.63 µg/dL) compared to healthy controls (17.86±1.05 µg/dL), indicating a potential deficiency in iron status (*P*=0.002). Similarly, ferritin levels were significantly reduced in patients (31.73±6.06 µg/L) compared to controls (82.76±5.19 µg/L), reflecting diminished iron reserves (*P*=0.001). Additionally, TIBC was significantly elevated in the patient group (76.44±3.22 µg/L) compared to controls (45.86±1.63µg/L) (*P*=0.001), suggesting increased iron-binding capacity, likely due to impaired iron utilization (Table 2).

Table 2: Serum levels of Iron, Ferritin, and TIBC Levels among Study Groups.

Iron Panel (Normal Range)	Group	N	Mean	Std. Error Mean	*P*-value
IRON	Patients	90	10.9662	0.63201	0.002
(10 -30 µg/dL)	Controls	30	17.8667	1.05671	
FERRITIN	Patients	90	31.7388	6.06057	0.001
(20 -300 µg/L)	Controls	30	82.7667	5.19298	
TIBC	Patients	90	76.4417	3.22072	0.001
(10 -70 µg/dL)	Controls	30	45.8667	1.63351	
***P* value < 0.05**				* *Independent-samples T test*	

Serological Assessment of Vitamin B_{12} in CD Patients and Controls

The mean vitamin B_{12} levels were insignificantly lower in the celiac disease (CD) patient (267.17±10.11 pg/mL, *P* = 0.002) compared to the control group (295.06±14.25 pg/mL) (*P* > 0.05), indicating a potential association between reduced vitamin B_{12} levels and CD (Figure 2).

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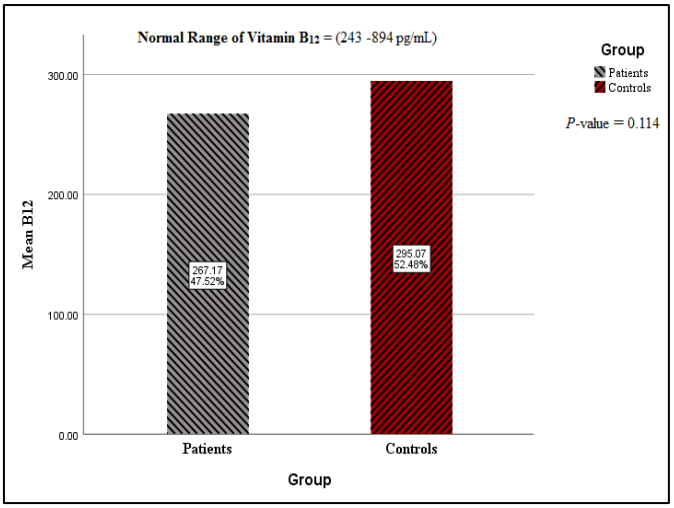

Figure 2: Serum levels of Vitamin B_{12} Levels in Celiac Disease Patients Compared to Healthy Controls.

DISCUSSION

Celiac disease (CD) is an autoimmune condition marked by the body's immune response to gluten, causing inflammation and harm in the small intestine. The diagnosis and monitoring of CD often involve serological tests, including anti-tissue transglutaminase (anti-tTG) IgA and IgG antibodies as well as intestinal fatty acid-binding protein (I-FABP). The current results revealed a statistically significant increase in serum levels of anti-tissue transglutaminase antibodies and I-FABP among CD patient groups. These findings are consistent with numerous studies conducted in various regions of the world [11–14]. Serological testing is widely recognized as a reliable, non-invasive standard for the diagnosis and follow-up of celiac disease. The mechanism behind this elevation in tTG-IgG and tTG-IgA levels involves an immune response triggered by gluten ingestion in genetically predisposed individuals. Gluten peptides cross the intestinal barrier and are deamidated by tissue transglutaminase 2 (TG2), enhancing their affinity for HLA-DQ2 or DQ8 molecules on antigen-presenting cells (APCs). This leads to activation of gluten-specific T cells, which stimulate B cells to produce anti-TG2 antibodies [15].

Fatty acid-binding proteins (FABPs) are low-molecular-weight (14–15 kDa) intracellular proteins that play a role in the metabolism of cholesterol and phospholipids, facilitate the transport of long-chain fatty acids, and help regulate lipid homeostasis [16]. The intestinal type (I-FABP) is exclusively produced in the small intestinal tract and encoded by the FABP2 gene located on chromosome 4 [17]. Intestinal fatty acid-binding proteins (I-FABPs) are distributed throughout the intestine, with highest expression in the jejunum, and are more concentrated in enterocytes located at the villous tip compared to those in the crypts. In celiac disease, elevated levels of intestinal fatty acid-binding protein (I-FABP) are due to enterocyte damage caused by the immune response to gluten. Specifically, the gluten-tTG (transglutaminase) complex triggers inflammation and damage to the villi of the small intestine, causing enterocytes to release I-FABP into the bloodstream. This damage and the subsequent release of I-FABP are reflected in the increased serum levels observed in untreated celiac patients [18,19].

Key observations of this study include lower iron levels, reduced ferritin concentrations, and elevated Total Iron-Binding Capacity (TIBC) in celiac disease (CD) patients compared to healthy controls. Similar findings were reported in an Iraqi study by Hasan *et al*. [9], which found an increase in TIBC and a decrease in iron and ferritin levels among CD patients. Another recent study conducted in Basrah, Iraq, indicated a highly significant decrease ($p \leq 0.001$) in serum iron levels in both age categories of CD patients compared to the healthy group [20].

The findings of this study are also consistent with global data around the world, which found similar results. Several international studies have reported significant alterations in iron metabolism in patients with celiac disease [21–24].

Iron deficiency anemia is a common complication of celiac disease, affecting up to half of newly diagnosed patients [25]. This condition arises primarily due to impaired nutrient absorption resulting from villous atrophy in the small intestine. The proximal duodenum, where most iron absorption occurs, is particularly affected by celiac disease [21]. Ferritin reflects body iron stores. Lower ferritin levels indicate diminished reserves and are often seen alongside iron deficiency anemia (IDA) [8, 26]. In our study, significantly reduced ferritin levels suggest compromised iron storage capacity among celiac patients. This aligns with previous studies indicating that only about 50% of patients recover their iron stores even after adhering to a gluten-free diet (GFD) [8, 25].

In celiac disease, elevated TIBC is mainly a compensatory response to iron deficiency resulting from impaired iron absorption. The aberrant expression of transferrin receptors (TfR) in enterocytes reflects this iron-starved state and further supports the biological demand for iron, prompting increased transferrin (and thus TIBC) production by the liver [24, 27].

Vitamin B_{12} is a water-soluble molecule with a complex chemical structure. Higher animals cannot synthesize vitamin B_{12} because they lack the genes required for cobalamin (vitamin B_{12}) production. In contrast, bacteria, yeasts, and certain algae are capable of synthesizing vitamin B_{12}. Interestingly, some of these bacteria inhabit the upper gastrointestinal tract of herbivores, which explains why herbivorous animals typically do not exhibit vitamin B_{12} deficiency despite having minimal dietary intake [28]. This study reveals an insignificant decrease in vitamin B_{12} levels in patients with celiac disease (CD) compared to healthy controls. This finding aligns with previous research indicating that vitamin B_{12} deficiency is not very common in CD patients, with prevalence ranging from 5% to 12% [7, 29–32]. Similarly, in a recent study conducted in the United kingdom, McGrogan et al. [33] found that vitamin B_{12} was normal among children with celiac disease. On the other hand, a recent study conducted in Baghdad, Iraq, revealed that vitamin B_{12} was significantly decreased among patients with CD [34].

Vitamin B_{12} deficiency in celiac disease (CD) is relatively uncommon compared to other micronutrient deficiencies. This is primarily because the terminal ileum (where B_{12} is absorbed) is typically unaffected in CD. Additionally, many gluten-free products are fortified with B_{12}, and patients often receive supplementation after diagnosis. However, B_{12} deficiency can still occur in CD patients with severe or widespread intestinal damage, slow mucosal healing (especially in adults), poor dietary intake, or coexisting conditions such as autoimmune atrophic gastritis [35]. Therefore, while B_{12} levels are often normal, persistent deficiency should prompt evaluation for additional causes beyond CD alone.

CONCLUSION

CD is an autoimmune disorder that mainly targets the small intestine, triggered by gluten ingestion in genetically predisposed individuals. Its onset involves a complex immune reaction to gluten proteins. This cross-sectional study highlights the presence of nutritional deficiencies, particularly in iron status, among patients with celiac disease (CD) in Diyala City. While a significant reduction in serum iron levels was observed, vitamin B12 levels showed only a minor and statistically insignificant decrease. However, clinical monitoring remains advisable.

Conflict of Interest

The authors declare that no conflict of interest.

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OPEN ACCESS Original Article

Comparative Assessment of Heavy Metals and pH in Shisha Water and Blood of Smokers

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ABSTRACT

Background: Shisha smoking is an emerging public health concern that poses significant health risks, particularly among young adults. Smokers can be predisposed to various toxicants, including heavy metals.
Objective: This study assessed the level of heavy metal content and pH in shisha water and blood of smokers.
Methods: A cross-sectional study design comprised of 150 subjects categorized into five groups of thirty each: shisha smokers, cigarette smokers, both shisha and cigarette smokers, secondhand smokers, and non-smokers, to enable a comprehensive comparison of different levels and types of tobacco smoke exposure in relation to heavy metal bioaccumulation in the body. Blood samples were collected for the measurement of the heavy metals using atomic absorption spectrophotometry. Shisha water samples were collected before smoking (pre-smoking water) and after smoking (post-smoking water) five consecutive smoking sessions without replacing the water, and pH was determined using a pH meter. Statistical analysis was done using SPSS.
Results: The results showed significantly ($p<0.001$) elevated levels of lead, cadmium, and zinc in post-smoking water compared to pre-smoking water. Serum Pb and Cd levels were significantly elevated in all smokers compared to non-smokers, while serum zinc levels were significantly reduced. Shisha smokers exhibited lower levels of Pb, Cd, and Zn than cigarette smokers. Secondhand shisha smokers exhibited higher serum Pb, Cd, and Zn levels compared to the primary shisha smokers. The pH of the smoked shisha water shifted from 6.5 to 3.4 after smoking five consecutive shisha sessions.
Conclusion: The study established that the water used in shisha traps or filters significant amounts of heavy metals such as lead, cadmium, and zinc in shisha smoke. The acidic pH of the shisha water correlates with higher levels of the dissolved metals, supporting the conclusion that water serves as a partial filter.

Keyword: Heavy metals, pH, Shisha Smoking, Waterpipe.

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INTRODUCTION

Shisha (waterpipe) smoking involves the use of charcoal to heat flavored tobacco, with the resultant smoke being inhaled after passing through a chamber filled with water [1]. Its use has increased globally, particularly among adolescents and young adults. Reports indicate that more than 100 million people around the globe smoke shisha every day [2], with the highest prevalence among those aged 15 to 30 years [3]. In Mediterranean regions and the Middle East, prevalence ranges between 20% and 70%, with specific rates of 34.2% in Lebanon, 32.9% in the West Bank [4], and approximately 9% to 15% among 13–15-year-olds in some Middle Eastern countries [5].

In Western countries such as the United States and the United Kingdom, the prevalence ranges from 9% to 12% among young adults [4]. In Nigeria, the use of shisha has become a common activity in a variety of social settings such as nightclubs, hotels, parties, bars, and lounges, largely due to ineffective tobacco legislation that fails to curb shisha consumption [6]. The National Health Survey (NDHS) estimates around 0.2% of adults aged 15-59 smoke shisha (Tobacco Control Data Initiative, 2023); region-specific studies among youth and urban populations revealed much higher rates: 3% to 7% among secondary and university students, about 7.1% among nightclub patrons in Ibadan [7], and nearly 18.6% among young adults in Lagos [8].

The World Health Organization reports that a single shisha session is equivalent to smoking 100 to 200 cigarettes [9]. Studies indicate that shisha smoke contains over 4,900 chemical substances [10], with more than 250 identified as toxic or hazardous by the National Toxicology Program [11]. Among these 250 harmful substances, 69 are carcinogens, such as nicotine, PAHs, carbon monoxide, heavy metals (e.g., cadmium, lead, arsenic), volatile organic compounds like benzene and naphthalene, and aldehydes, including formaldehyde and acrolein [12]. Unlike cigarettes, shisha sessions often last longer and involve deeper inhalation, which can lead to the systemic accumulation of toxic elements such as heavy metals, including lead, cadmium, arsenic, and chromium [13]. Chronic exposure to these heavy metals could be linked to genotoxic and carcinogenic effects and organ damage, especially in the heart, liver, kidneys, pancreas, bones, and brain [14, 15]. Several studies have shown elevated levels of heavy metals (Pb, Cd, As, Mn, Co, Cu, Ni, and Zn) in the blood of tobacco smokers compared to nonsmokers [16,17,18].

Despite the widespread belief among shisha users that the water in the waterpipe device serves to filter and reduce the smoke's toxicity, this assumption is not substantiated by scientific evidence [19]. This misconception has contributed to the growing perception among shisha users that shisha could be a safer alternative to cigarette smoking [20, 21]. Researchers have indicated that shisha water does not effectively filter out harmful substances such as nicotine, carbon monoxide, and heavy metals, but it cools the smoke, enabling a deeper inhalation during smoking [22]. A study by Akeel et al. [16] demonstrated that the water filtration mechanism in shisha does not effectively eliminate heavy metals from the smoke. These findings showed that the concentration of metals such as bismuth (Bi), chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V), and uranium (U) in the water was significantly smaller than in the smoke. Akeel and colleagues [16] also established that only about 3% (±1%) of the total metal content was filtered or trapped during the water bubbling phase.

The water used in shisha smoking may trap or absorb certain chemicals of the smoke, potentially leading to alterations in its pH following the smoking session. Improper disposal of this water could contribute to environmental contamination, which may be harmful to terrestrial and aquatic life. In Nigeria, many studies have focused on social prevalence, with limited attention given to biochemical or environmental health implications. There have been conflicting reports regarding the health risks associated with shisha smoking, particularly due to the passage of the smoke through water before inhalation.

To address this gap, this present study assessed the levels of heavy metals (zinc, lead, and cadmium) and pH values in shisha water and blood of smokers, suggesting whether the passing of the smoke through water eliminates or filters a significantly large portion of the heavy metals contained in the fresh samples of tobacco.

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METHODOLOGY

Study Area and Study Population

This research was conducted in Yenagoa, located in Bayelsa State, Nigeria. The study population included both male and female participants aged between 16 and 35 years. The participants are known frequent shisha and cigarette smokers who visit socio-economic settings like lounges, bars, ghettos, and nightclubs and have a history of regular shisha and cigarette smoking for at least twice a week for shisha smokers and 5 sticks daily for a period of two years.

Study Design

The study is a cross-sectional study conducted between March 2024 and December 2024. A purposive stratified sampling technique was used to select the study participants. A total of one hundred and fifty blood samples were collected from subjects comprising thirty shisha smokers (smoking at least 2 shisha heads twice per week), thirty cigarette smokers (smoking at least five sticks per day), thirty both shisha and cigarette smokers, thirty secondhand smokers (non-active smokers who work in nightclubs, bars, and lounges for at least two years in a closed space), and thirty apparently healthy non-smokers aged 16-36 years old. Also, a total of 20 water samples were collected, comprising 10 pre-smoking water samples and 10 water samples after smoking. Each water sample was collected after five consecutive smoking sessions of shisha head without replacing the water. The shisha bowl contained 750 ml of water, which was maintained without replacement during the five smoking sessions.

Selection Criteria

Inclusion Criteria

Apparently healthy males and females between the ages of 16 and 35 years who are known shisha smokers (smoking at least twice a week), exclusive cigarette smokers (smoking at least five sticks per day), both smokers (those who smoke both shisha and cigarettes for at least two years), secondhand shisha smokers (individuals that work in nightclubs, bars, and lounges in a closed environment for 2 years), and apparently healthy non-smokers who consented to the study.

Exclusion Criteria

People with a known chronic metabolic disease, those with a history of alcohol abuse, those who are habitual users of hard drugs, and those who did not consent to the study were excluded from the study. Also excluded were subjects who work in heavy metal-exposed environments like petrol stations, welding workshops, painting factories, and municipal waste incineration areas.

Ethical Approval

Approval for the study was secured from the Research and Ethics Committee of the Bayelsa State Ministry of Health (Approval No: BSHREC/Vol. 1/24/03/04). All participants signed a written informed consent form following a comprehensive briefing on the study procedures.

Sample Size

Sample size was determined using Cochran formula:

$$n = \frac{Z^2 \times p(1-p)}{(e)^2}$$

n= Sample size
Z= Confidence level at 95% (1.96)
p= Prevalence rate of 7.1% in Lagos, Nigeria (0.071) [7]
e= Error probability at 5% (0.05)

10% attrition factor = 10% of 101= 10.1
An initial sample size of 90 subjects was obtained after considering a 10% attrition factor. The subjects were grouped into 30 shisha smokers, 30 both shisha and cigarette smokers, and 30 secondhand shisha smokers. Additionally, 30 exclusive cigarette smokers and 30 apparently healthy non-smokers were recruited as control groups, making a total of 150 participants in the study.

Blood Sample Collection and Preparation

Blood samples were collected and prepared in accordance with World Health Organization guideline 2020. Five milliliters of venous blood were collected from the waterpipe smokers, cigarette smokers, both smokers, secondhand smokers, and the non-smokers and dispensed into a plain sample container. The samples were left at room temperature for one hour to allow complete clotting, after which the clots were gently dislodged and centrifuged at 1000 rpm for 10 minutes. The serum

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was separated from the cells and dispensed into a well-labeled plain sample bottle and then frozen at -20°C in a deep freezer until the time for analysis within one week of collection. The serum obtained was used for the determination of the heavy metals (cadmium, lead, and zinc).

Shisha Water Collection

Shisha water collection was in accordance with Akeel et al. [16]. Shisha water samples were obtained from ten shisha smoking spots in Hamilton Garden in Yenagoa, Bayelsa State. A total of twenty shisha water samples were collected, comprising ten before smoking (pre-smoking water) and ten water samples after smoking (post-smoking) for the analysis of heavy metals like cadmium (Cd), lead (Pb), and zinc (Zn). Each water sample was collected before smoking and after five consecutive smoking sessions without replacing the water. The water samples were collected into a one-liter glass container, and 1 ml of HNO_3 was added to it. Before adding the water samples into the glass container, deionized water was used to carefully rinse the container to eliminate potential contaminants that could interfere with the analytical results. The water from the shisha vessel was filtered to remove charcoal fragments, kept at room temperature, and analyzed within 24 hours for heavy metal content using the APHA 4500 standard procedure. Atomic Absorption Spectrophotometry (APHA D31100) was employed for the metal analysis. For the determination of the pH profile of the water, a water sample was collected before smoking, and five (5) other samples were collected after the 1st, 2nd, 3rd, 4th, and 5th sessions, or shisha sessions.

To eliminate bias, all blood and water samples were labeled with coded identifiers, and the laboratory personnel were not informed of the exposure status (shisha smoker, cigarette smoker, both smokers, secondhand smokers, or control).

Shisha Water Sample Preparation

Shisha water preparation was done in accordance with the method described by Katurji et al. [13] Sample preparation involved acid digestion, where 5 mL of concentrated nitric acid (HNO_3) was added to 100 mL of the sample in a conical flask. The flask was then placed on a hot plate and heated at 85°C until the volume was reduced to about 25 mL, ensuring complete digestion. Once digestion with 20% nitric acid was achieved, the sample was filtered using a 0.45-micron membrane filter. The filtrate was transferred into a 100 mL volumetric flask, and deionized water was added to reach the final volume of 100 mL. This solution was used to determine the concentrations of lead (Pb), cadmium (Cd), and zinc (Zn) using an atomic absorption spectrophotometer. A blank (deionized water) was used to calibrate the instrument and confirm the absence of contamination. The prepared sample was then aspirated into the spectrophotometer, and absorbance readings were recorded.

Determination of pH of Shisha Water Samples

The pH of the shisha water was measured using a pH meter following the method outlined by Ben-Chioma et al. [24]. Principle**:** The measurement relies on a combined glass electrode composed of a reference electrode and a sensing electrode. The sensing component consists of a thin, semi-permeable glass membrane that is responsive to pH changes. This membrane separates the internal solution of known pH from the external sample solution. As a result, electrical potentials are generated on both sides of the membrane. The pH of the sample is determined by calculating the difference between these two potentials. Procedure**:** A standard pH 7.0 buffer solution was poured into a beaker, and the rinsed electrode was immersed into it. The pH meter was allowed to stabilize and display the reading. Subsequently, the water samples were placed in a clean beaker, and the same procedure was repeated. The pH values were then recorded accordingly.

Measurement of Cadmium, Lead, and Zinc in Shisha Water and Blood

Heavy metal determination was done using an Atomic Absorption Spectrophotometer (model: Agilent 55B SPECTRA) according to the method of APHA 4500 as described by Yousefinejad *et al.*, [25] with modifications. Principle**:** The Atomic Absorption Spectrophotometer (AAS) operates on the principle that when a sample is introduced into a flame, it is atomized. A light beam from the AAS passes through this flame, and the monochromator isolates the specific wavelength of interest. The detector then measures how much light is absorbed by the free atoms of the target element in the flame. Procedure**:** The instrument was first calibrated using a blank

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(deionized water) to eliminate contamination and set a baseline. Next, the acid-digested shisha water sample was introduced into the AAS, and the absorbance values were recorded for analysis.

Measurement of Cadmium, Lead, and Zinc in Blood

Heavy metals were analyzed employing an Agilent 55B SPECTRA Atomic Absorption Spectrophotometer, following the APHA 4500 protocol, as modified from the protocol outlined by Yousefinejad et al. [25]. Blood concentrations of lead, cadmium, and zinc were measured using a wet digestion method. In this procedure, 2 ml of blood were placed in a beaker, followed by the addition of 5 ml of concentrated nitric acid (HNO_3). The mixture was gently heated on a hot plate until it reduced in volume and brown nitric oxide fumes were visible. Heating continued until a light-colored, clear solution indicated complete digestion. The beaker walls and lid were rinsed with deionized, metal-free water, and the mixture was filtered using Whatman filter paper with a pore size of 0.2 mm. The filtrate was then transferred into a 50 ml volumetric flask and topped up with distilled water. The concentrations of Pb, Cd, and Zn were then measured by aspirating the solution into an atomic absorption spectrophotometer set to a wavelength of 217 nm.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) Version 23.0 (SPSS Inc., Chicago, IL, USA; Version 23.0) was used for all statistical analysis. Student's t-test and one-way ANOVA were used for comparing values of the measured biochemical parameters between the control and experimental groups. All post hoc testing was done using Tukey HSD and Games-Howell methods as applicable. Data were considered significant at $p<0.05$.

RESULTS

Figure 1 revealed the pH profile of shisha water based on the number of smoked shisha sessions. The pH value in the pre-smoking shisha water is 6.5. However, following the consumption of the first, second, third, and fourth shisha sessions, the pH shifted towards more acidic values of 4.2, 3.9, 3.5, and 3.4, respectively, and continued to stabilize at a value of 3.4 after the consumption of the 5th shisha session.

Table 1 showed the mean concentration of Pb increased from 0.52 ± 0.18 ppm (before smoking) to 0.81 ± 0.15 ppm (after smoking). The mean difference of 0.298 ppm was statistically significant ($p < 0.001$). The effect size for Pb was 1.75, which represents a very large effect, suggesting a significant accumulation of Pb in the water after smoking. The 95% confidence interval (CI) for the mean difference was 0.213-0.378 ppm. The mean Cd concentration increased from 0.34 ± 0.05 ppm to 0.64 ± 0.11 ppm (mean difference = 0.300 ppm; $p < 0.001$), with an effect size of 3.47, signifying a significant accumulation after smoking. The 95% CI ranged from 0.209 to 0.391 ppm. The concentration of Zn also increased from 0.30 ± 0.04 ppm (before smoking) to 0.55 ± 0.09 ppm (mean difference = 0.252 ppm; $p < 0.001$, Cohen's d = 3.34), with a 95% CI of 0.172-0.331 ppm, indicating large and significant accumulation of zinc in the water after smoking.

Table 2 revealed significantly elevated ($p<0.05$) levels of Pb and Cd in all categories of smokers compared to the non-smokers. While the serum concentration of Zn level was significantly ($p<0.05$) lower in all smokers compared to the non-smokers. Serum Pb levels in shisha smokers were significantly lower than cigarette smokers, both users, and secondhand smokers ($p = 0.001$). Serum Cd levels in shisha smokers were significantly lower than cigarette smokers, both users, and secondhand smokers ($p = 0.001$). Serum levels of Zn exhibited significantly lower levels in shisha smokers than in cigarette smokers, both smokers, and secondhand smokers ($p = 0.001$). Serum Pb, Cd, and Zn levels in the cigarette smokers were significantly higher ($p<0.05$) than in the shisha smokers. Secondhand smokers exhibited higher serum Pb, Cd, and Zn levels compared to shisha smokers. Both shisha and cigarette smokers exhibited significantly elevated ($p<0.05$) levels of Pb, Cd, and Zn compared to shisha smokers. No significant difference was observed between cigarette smokers and both shisha and cigarette smokers ($p > 0.05$).

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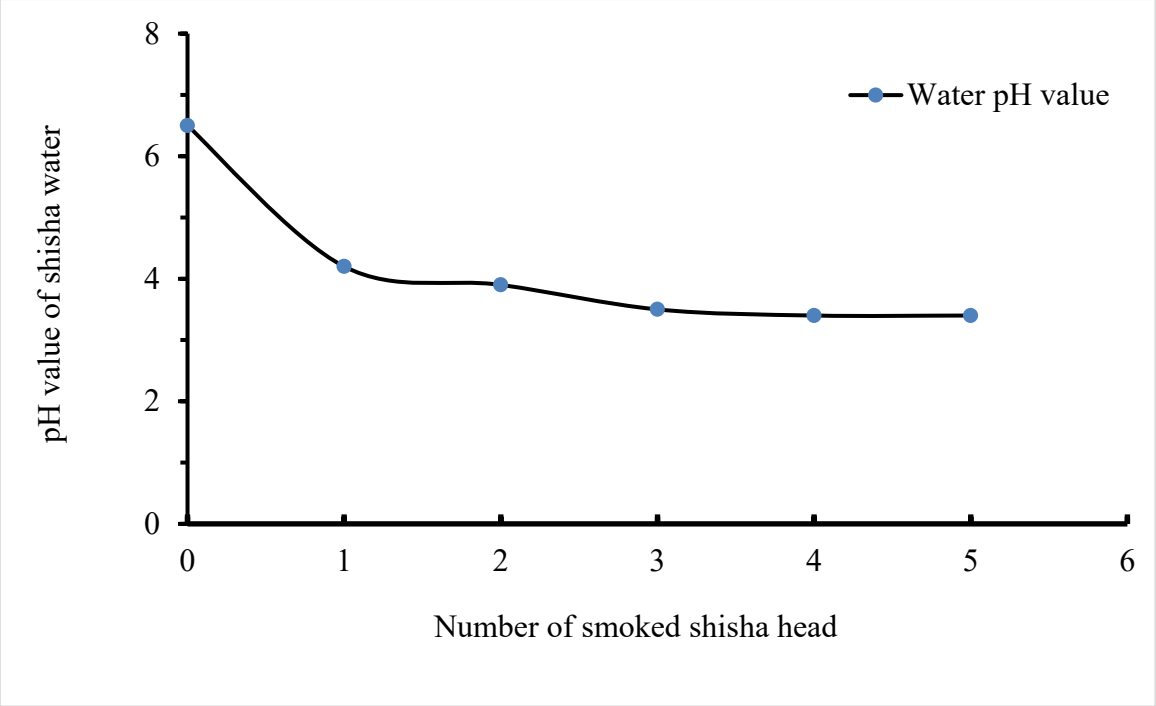

Figure 1: pH values of Shisha Water based on the Number of Smoked Shisha Session

Table 1: Comparison of Some Heavy Metal Concentration in Pre-smoking and Post-smoking Shisha Water.

	Mean ± SD			
Parameter	**Pre-Smoking (n = 10)**	**Post-Smoking (n = 10)**	***T-value***	***P-value***
Pb (ppm)	0.52 ± 0.18	0.81 ± 0.15	-8.96	0.000***
Cd (ppm)	0.34 ± 0.05	0.64 ± 0.11	-8.50	0.000***
Zn (ppm)	0.30 ± 0.04	0.55 ± 0.09	-8.17	0.000***

Key: n = total number, SD = standard deviation, t = t-test statistic, *p* = error probability, Pb = lead, Cd = cadmium, Zn = Zinc. * Significant difference observed, $p < 0.05$, ** Significant difference observed, $p < 0.01$, *** Significant difference observed, $p < 0.001$.

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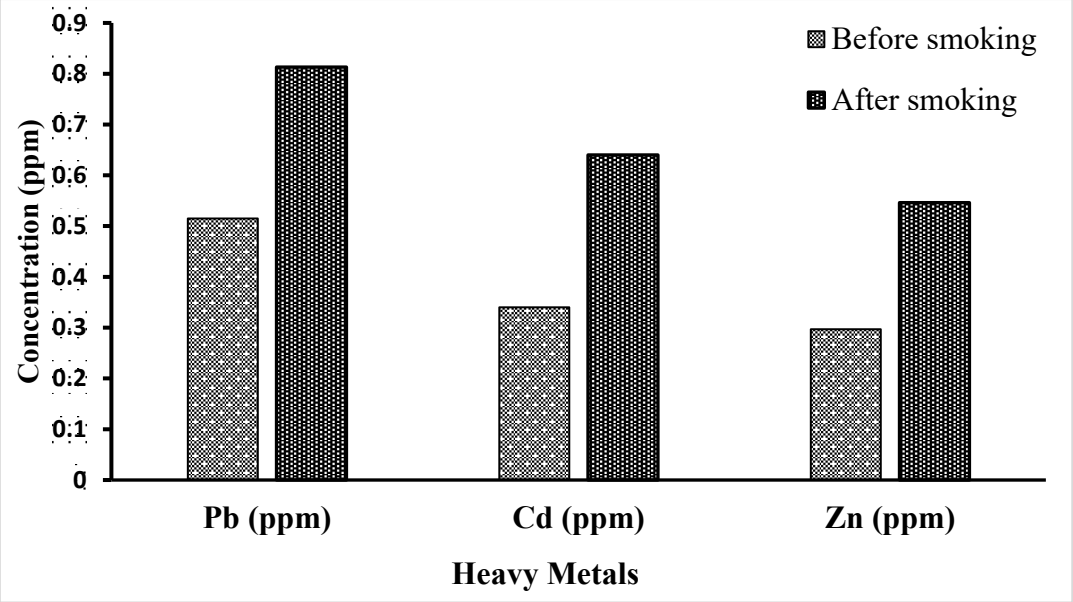

Figure 2: Heavy metal concentration in shisha water before and after smoking

Table 2: Serum Concentration of Heavy Metal Levels in the Study Population

Parameter	Mean ± SD Non-smokers (n = 30)	Shisha smokers (n = 30)	Cigarette smokers (n = 30)	Both Smokers (n = 30)	Second-Hand smokers (n = 30)	*F-value*	*p-value*
Pb (ppm)	0.29 ± 0.16α	0.55±0.16δ	0.82± 0.15	0.85 ± 0.14	0.79 ± 0.16	42.11	0.000***
Cd (ppm)	0.28 ± 0.14β	0.36±0.09γ	0.71± 0.18	0.74 ± 0.19	0.62 ± 0.19	28.37	0.000***
Zn (ppm)	0.68 ± 0.15 μ	0.24±0.19λ	0.54± 0.17	0.33 ± 0.15	0.60 ± 0.17	16.72	0.000***

Key: n = total number, SD = standard deviation, *F* = ANOVA statistic, p = error probability, Pb = lead, Cd = cadmium, Zn = Zinc. All *post hoc* testing were done using Turkey HSD and Games-Howell methods as applicable. *** Significant difference observed, $p < 0.001$. α Significant differences observed in Pb concentrations between non-smokers and each of the other pairs ($p = 0.000$, respectively). β Significant differences observed in Cd concentrations between non-smokers and each of the other groups ($p = 0.000$, respectively). γ Significant differences observed in Cd concentrations between shisha smokers and cigarette, both, and secondhand smokers ($p = 0.000$, respectively). μ Significant difference observed in Zn concentration between non-smokers and cigarette, combined, and secondhand smokers ($p = 0.000$, respectively). λ Significant differences observed in Zn concentrations between shisha smokers and each of cigarette smokers and both smokers ($p = 0.001$, $p = 0.000$, respectively).

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DISCUSSION

Shisha smoking is a rising social phenomenon globally. Shisha smoke contains high levels of harmful substances such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), particulate matter, volatile organic chemicals (VOCs), and heavy metals like aluminum, copper, manganese (Mn), cadmium (Cd), lead (Pb), arsenic (As), nickel (Ni), zinc (Zn), and cobalt (Co) that can cause many medical complications such as cancer, chronic obstructive pulmonary disease (COPD), reproductive disorders, cardiovascular disorders, and respiratory diseases [17, 18].

The water used in the shisha smoking attempt to purify or filter some of these chemicals during the bubbling phase of the smoke before inhalation [26, 27]. In this study, the levels of lead, cadmium, and zinc in smoked shisha water were significantly (p=0.000) higher than in the pre-smoking water. This implies that the metals in the shisha water are trapped or filtered during the bubbling phase of the smoke before it is inhaled. According to Qamar et al. [28], heavy metals and other chemical substances are emitted with shisha smoke in proportion to their concentration in the tobacco, and a small fraction of these substances becomes trapped in the water. This observation aligns with findings by Akeel et al. [20], who reported that only about 3% (±1%) of the total heavy metals are removed during the water filtration stage. This minimal reduction is insufficient to effectively shield users from heavy metal exposure. Similar studies by Qamar *et al.* [28] and Monzer and colleagues [29] indicated that the levels of heavy metals were found to be higher in post-smoking shisha water than pre-smoking water. Galal and colleagues [30] also documented that waterpipes provide minimal filtration of nicotine, further challenging the misconception that smoking through a waterpipe reduces the health risks associated with tobacco use. The result also revealed that the levels of zinc (0.30 ppm and 0.55 ppm) in the pre-smoking shisha water were lower than those of Pb (0.52 ppm and 0.81 ppm) and Cd (0.34 ppm and 0.64 ppm), respectively. This could be due to less volatility of zinc compared to other heavy metals like lead and cadmium [31].

The levels of lead (Pb) and cadmium (Cd) in the blood were found to be considerably higher among smokers compared to non-smokers. This is because the tobacco plant absorbs heavy metals from its growing environment, including soil, water, and fertilizers, which accumulate in the leaves. When tobacco is smoked, these metals are released into the air and inhaled, allowing them to enter the smoker's bloodstream directly [32, 33]. These findings are consistent with those of Ghaderi *et al.* [17], Sadiq *et al.* [34], and Khabour *et al.* [35], who also reported elevated levels of these toxic elements in the blood of smokers.

The concentration of zinc in the blood was considerably lower in smokers than in non-smokers, indicating that smoking may contribute to a zinc deficiency (hypozincemia), increasing the risk of oxidative damage. Although zinc is emitted during tobacco combustion, it is not as volatile or efficiently passed into mainstream smoke as cadmium and lead [36, 37]. Rodgman and Perfetti [31] stated that only a small proportion of zinc (0.4–2.7%) is transferred to smoke, compared to a much higher percentage for cadmium (7–22%). Low zinc levels can impair genetic stability and promote carcinogenesis, as well as heighten oxidative stress, as documented by Proudfoot et al. [38] and Bertini and colleagues [39]. The reduction in serum zinc among smokers may be due to increased cadmium levels, which stimulate the production of metallothioneins, which are proteins that bind both cadmium and zinc, thereby reducing zinc availability [36]. These findings are supported by findings from Al-Azzawy [40], Afridi et al. [41], and Dhia et al. [42], which documented decreased zinc levels in smokers. Similarly, Anetor et al. [43] found that Nigerian smokers had significantly higher cadmium and lower zinc in their blood than non-smokers.

Shisha smokers exhibited significantly lower serum levels of lead (Pb), cadmium (Cd), and zinc (Zn) compared to cigarette smokers. This is likely due to the water in the waterpipe, which acts as a filter during the bubbling of smoke, trapping some of the heavy metals and thereby decreasing the amount inhaled [16]. These findings support the works of Abbas et al. [44] and Saeed and Kazeem [45], which observed lower Pb and Cd concentrations in shisha users than in cigarette smokers. Similarly, Hani et al. [46] found reduced levels of zinc, magnesium, and cadmium in shisha smokers. However, these results contradict the work by Yousefinejad et al. [25], which reported higher levels of heavy metals in shisha

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smokers than in both cigarette smokers and non-smokers. Interestingly, secondhand smokers had higher serum concentrations of Pb, Cd, and Zn than shisha smokers, which could be due to their prolonged continuous exposure to shisha smoke in poorly ventilated environments like clubs and lounges [16]. This observation confirms the work by Shojaei and Rostami [32], who found elevated heavy metal levels in employees working at waterpipe cafes compared to shisha users.

There is a widespread belief that shisha water helps to cool the smoke and filter some of its harmful substances, including heavy metals and water-soluble toxins. In reality, continuous exposure to tobacco smoke alters both the chemical properties and pH of the water. If this water, with its acidic pH, is improperly disposed of, it can lead to environmental contamination and negatively affect soil, aquatic ecosystems, and wildlife. In this study, the pH value of the shisha water before smoking was found to be 6.5 (slightly acidic). Following the consumption of the 1st, 2nd, 3rd, and 4th shisha heads/sessions, the pH shifted towards more acidic values of 4.2, 3.9, 3.5, and 3.4, respectively, and remained stable at 3.4 after the consumption of the 5th shisha session. This acidification reflects the fact that shisha contains acid-forming gases such as carbon dioxide (CO_2), nitrogen oxides (NO_x), and sulfur dioxide (SO_2), which, when dissolved in water, form weak acids such as carbonic acid, sulfurous acid, and nitrous and nitric acids, respectively, hence lowering the pH of the water, making it more acidic. The acidic pH of the smoked water could be attributed to the accumulation and dissolution of combustion residues in shisha smoke, such as volatile organic compounds, polyaromatic hydrocarbons, tar, and nicotine, that become acidic when dissolved in water [47, 48]. Furthermore, the acids and metal ions present in smoke can overwhelm the normal buffering capacity of water (primarily the bicarbonate system), thereby reducing its ability to neutralize acids and resulting in a drop in pH [1, 16). This aligns with the findings by Al-Kazwini *et al.*, [40], who indicated a pH of shisha water from 6.0 to 3.6 after ten successive smoking sessions.

Limitations

This study identifies several notable limitations that affect the interpretation of its findings. The restricted sample size for water analysis limits the statistical power and generalizability of the results. A larger sample would have provided a more precise estimation of heavy metal concentrations and enhanced the reliability of the observed trends. The cross-sectional design of the study inhibits any conclusions about causality. While associations between shisha smoking and elevated levels of toxic metals can be acknowledged, the essential temporal relationship needed to establish cause and effect remains unresolved. Additionally, there is the possibility of confounding exposures such as environmental pollution, dietary habits, or occupational hazards, which may have impacted the identified metal levels. In the absence of thorough background exposure assessments to account for these factors, attributing the increased metal concentrations solely to shisha smoking may be an exaggeration.

CONCLUSION

The study established that the water used in shisha traps or filters a significant amount of heavy metals from the smoke, but the reduction is not sufficient to eliminate the associated health risks. The acidic pH of the shisha water observed in this study correlates with higher levels of the dissolved metals, supporting the findings that water serves as a partial filter. Unlike cigarette butts, the contaminated shisha water is not easily retrievable once discarded. Therefore, proper regulatory measures on water disposal and public smoking policies should be undertaken to avoid the environmental pollution.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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OPEN ACCESS Original Article

Plasma Dopamine Level as a Biomarker for Pain in Myofascial Temporomandibular Disorders

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ABSTRACT

Background: Myofascial Temporomandibular Disorders (M-TMD) are characterized by chronic pain and dysfunction of the jaw muscles, often linked to stress and neurochemical imbalances. Dopamine, a neurotransmitter involved in pain modulation, has been proposed as a potential biomarker for M-TMD-related pain. This study aimed to evaluate plasma dopamine levels in M-TMD patients compared to healthy controls and explore its role as a pain biomarker.

Objective: The primary objective was to assess plasma dopamine levels in M-TMD patients and correlate them with clinical pain symptoms.

Methods: A case-control study was conducted with 50 participants (25 M-TMD patients and 25 age- and sex-matched healthy controls). Blood samples were collected in EDTA tubes, centrifuged at 2000 g for 10 minutes, and plasma was stored at −60°C. Dopamine levels were measured in nM. Clinical data, including age, gender, symptoms, and OPG findings, were recorded. Statistical analysis included mean comparisons (t-test) and subgroup analysis by gender and age.

Results: Plasma dopamine levels were significantly higher in M-TMD patients (mean: 5.01 nM) compared to controls (mean: 2.53 nM; $p < 0.001$). Female M-TMD patients had slightly higher dopamine levels (mean: 5.12 nM) than males (mean: 4.89 nM), though not statistically significant ($p = 0.23$). Common symptoms in M-TMD patients included bruxism (44%), morning jaw stiffness (40%), and stress-related jaw tension (56%). All patients exhibited mild condylar flattening on OPG, and no significant correlation was found between dopamine levels and age ($r = 0.12$, $p = 0.34$).

Conclusion: Elevated plasma dopamine levels in M-TMD patients suggest its potential role as a biomarker for pain and stress-related pathophysiology. The findings support further investigation into dopaminergic pathways in M-TMD and personalized treatment strategies targeting neurochemical imbalances.

Keywords: Plasma dopamine, biomarker, myofascial temporomandibular disorders (M-TMD/TMD), chronic orofacial pain modulation, Neurochemical imbalance, DC/TMD (Diagnostic Criteria for TMD), HPLC-ECD, stress-related jaw dysfunction.

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INTRODUCTION

Myofascial temporomandibular disorders (M-TMD) represent a prevalent musculoskeletal condition characterized by chronic orofacial pain, limited mandibular function, and tenderness of the masticatory muscles (1). Despite their high prevalence, affecting approximately 5-12% of the global population (2), the underlying neurobiological mechanisms of M-TMD-related pain remain incompletely understood. Recent evidence suggests that central sensitization and neurochemical imbalances, particularly in dopaminergic pathways, may contribute significantly to pain perception and chronification in M-TMD (3).

The dopaminergic system plays a crucial role in pain modulation through its projections to key regions of the pain matrix, including the basal ganglia and anterior cingulate cortex (4). Clinical observations have noted elevated dopamine levels in other chronic pain conditions, such as fibromyalgia and migraine (5), suggesting a potential shared neurochemical pathway. However, the specific relationship between plasma dopamine levels and M-TMD pain has not been thoroughly investigated.

Emerging biomarker research highlights the potential of neurochemical indicators for objective pain assessment (6). Plasma dopamine measurements offer several advantages as a potential biomarker, including relative stability and clinical accessibility compared to cerebrospinal fluid measurements (7). Preliminary studies have demonstrated altered dopamine metabolism in TMD patients (8), but these findings require replication with standardized methodologies. Therefore, the current study aims to quantitatively compare plasma dopamine levels between M-TMD patients and healthy controls, examine the relationship between dopamine levels and clinical pain characteristics, and evaluate the diagnostic accuracy of plasma dopamine as a biomarker for M-TMD.

METHODOLOGY

Study Design

This case-control study was conducted at Al-Awlaki Laboratory, Sana'a, Yemen, between March 2023 and December 2024.

Sample Size

The study included a total of 50 participants, divided into two equal groups. Study group 25 patients diagnosed with M-TMD and 25 control group age- and sex-matched healthy individuals.

Participant Selection and Group Allocation

Inclusion Criteria

The M-TMD group (n=25) comprised patients aged 18-60 years, exhibiting persistent (>3 months) unilateral or bilateral masticatory muscle pain. Eligibility required palpation tenderness in ≥3 masticatory muscle sites and a minimum pain intensity of ≥4 on a 10-cm visual analog scale (VAS). The control group (n=25) consisted of age- and sex-matched healthy volunteers with no history of temporomandibular disorder symptoms, orofacial pain, or current use of analgesic or psychotropic medications.

Exclusion Criteria

Both groups excluded individuals with systemic rheumatic diseases, neurological disorders affecting pain perception, a history of TMJ surgery or trauma, and those who were pregnant or lactating.

Clinical Assessment and Biochemical Analysis Protocol

Clinical Assessment Protocol

All participants underwent comprehensive clinical evaluation beginning with a standardized DC/TMD clinical examination. Pressure pain threshold (PPT) measurements were then performed using a digital algometer (FDX 25, Wagner Instruments) at three designated masseter muscle sites, two temporalis muscle sites, and the TMJ lateral pole. Maximal mouth opening was quantified in millimeters, followed by detailed documentation of pain characteristics, including VAS scores (0-10), pain duration, and qualitative descriptors (dull, aching, or sharp character).

Blood Sampling and Biochemical Analysis

Sample Collection

Fasting venous blood samples (5 mL) were collected between 08:00 and 10:00 AM using pre-chilled 3 mL K_2EDTA Vacutainer tubes (Becton Dickinson). Samples were immediately placed on ice and processed within 30 minutes of collection.

Plasma Processing

Blood samples underwent centrifugation at 2000 × g for 10 minutes at 4°C (Eppendorf 5804R). Plasma was separated using sterile polypropylene pipettes, aliquoted into 1.5 mL protein LoBind tubes (Eppendorf), and stored at -60°C until analysis, with a maximum storage duration of 3 months.

Dopamine Quantification

Plasma dopamine levels were determined via high-performance liquid chromatography with electrochemical detection (HPLC-ECD; Waters 2465 system). Chromatographic conditions included an Atlantis T3 C18 column (3 µm, 2.1 × 100 mm), a mobile phase of 50 mM sodium phosphate buffer (pH 3.1) with 10% methanol, a flow rate of 0.4 mL/min, and a detection potential of +650 mV. The calibration curve (0.5-50 nM) demonstrated linearity ($r^2 > 0.99$), with intra- and inter-assay coefficients of variation <8% and a limit of detection of 0.2 nM.

Quality Control Measures

All samples were processed in duplicate with randomized analysis order to prevent batch effects. Internal standard (3,4-dihydroxybenzylamine) recovery rates of 85-115% were verified, and pooled plasma quality control samples underwent periodic analysis to ensure assay consistency.

Treatment Protocol

All enrolled M-TMD patients underwent a standardized multidisciplinary initial comprehensive evaluation protocol. This assessment included quantitative pain evaluation using the Visual Analogue Scale (VAS; 0-10 cm), pressure pain threshold (PPT) measurement via digital algometry at standardized masticatory muscle sites, and functional assessment of maximum unassisted mouth opening (mm) along with lateral and protrusive excursions (mm). The evaluation further incorporated psychosocial assessment through the DC/TMD Axis II questionnaire battery and a neurochemical counseling session explaining dopaminergic dysregulation's potential role in pain modulation.

A tiered pharmacotherapeutic intervention was implemented based on symptom severity. First-line therapy featured a muscle relaxation protocol using clonazepam (0.25-0.5 mg PO qHS, titrated to effect) with a maximum 4-week duration to prevent dependence, accompanied by monitoring for sedation and cognitive effects. Concurrently, an analgesic regimen employed ibuprofen (400 mg PO q8h PRN, maximum 2400 mg/day) with pantoprazole (20 mg daily) for gastroprotection when indicated, including renal function monitoring for chronic users. For refractory cases, adjunctive therapy involved dopaminergic modulation through sulpiride (25-50 mg PO BID, titrated based on plasma dopamine levels >5.5 nM), with ECG monitoring for QT prolongation and monthly prolactin level assessment.

Certified orofacial pain specialists administered a structured 6-week physical rehabilitation program. The manual therapy components encompassed intraoral and extraoral myofascial release techniques, strain-counterstrain applications for trigger points, and postural re-education with cervicothoracic stabilization. Complementing this, the therapeutic exercise protocol incorporated progressive mandibular range-of-motion exercises, isometric strengthening for masticatory muscles, and a home exercise program with daily compliance logs. Licensed clinical psychologists specializing in chronic pain delivered behavioral medicine intervention through cognitive-behavioral therapy (CBT).

The stress management components included diaphragmatic breathing training, progressive muscle relaxation protocols, and mindfulness-based stress reduction techniques. Simultaneously, sleep hygiene optimization featured stimulus control therapy, sleep restriction when indicated, and circadian rhythm stabilization.

A standardized monitoring protocol was implemented for follow-up and outcomes assessment at three timepoints: baseline (T0), 6-week interim (T1), and 12-week final (T2). Primary outcome measures targeted VAS pain reduction ≥30% and PPT improvement ≥15%, while secondary measures evaluated jaw function improvement (mm), plasma dopamine normalization (<4.0 nM), and stress scale reduction (PSS-10). Safety monitoring encompassed documentation of treatment-emergent adverse events, verification of medication compliance, and assessment of therapy adherence throughout the protocol duration.

Ethical Considerations

This study was approved by University of Science and Technology, Aden, Yemen (MEC/AD096). In addition, the study protocol was conducted in accordance with the ethical principles of the Declaration of Helsinki.

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Written informed consent was obtained from all participants prior to enrollment.

Statistical Analysis

Data were analyzed using SPSS v.27. Continuous variables were compared via one-way ANOVA with Tukey's post-hoc test. Categorical variables were assessed using chi-square test. Logistic regression was employed for subgroup analyses. Significance was set at $p<0.05$, with 95% confidence intervals (CI).

RESULTS

Demographic and Clinical Characteristics

The study population comprised 50 participants equally distributed between M-TMD patients (n=25) and healthy controls (n=25). Group matching was successful, with no significant differences (Figure 1):

- Age (M-TMD: 34.2 ± 8.7 years vs. control: 35.1 ± 9.3 years; p=0.712).
- Gender distribution (M-TMD: 64% female vs. control: 60% female; χ^2=0.083, p=0.773).
- BMI (M-TMD: 24.3 ± 3.1 kg/m^2 vs. control: 23.8 ± 2.8 kg/m^2; p=0.542).

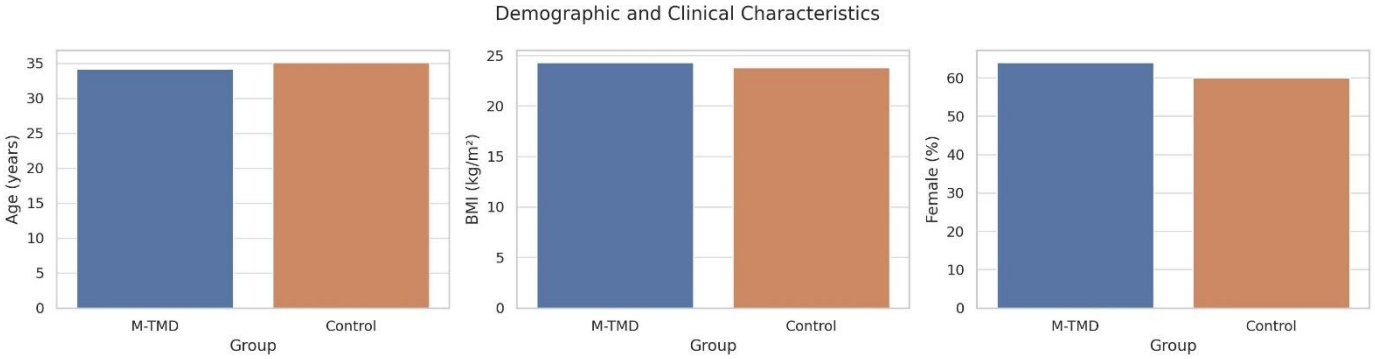

Figure 1: Demographic and clinical characteristic

Primary Outcome: Plasma Dopamine Levels

HPLC-ECD analysis revealed the following findings (Figure 2).

Group Comparison

- M-TMD patients exhibited 98% higher mean dopamine levels (5.01 ± 0.59 nM) versus controls (2.53 ± 0.29 nM).
- This difference was statistically significant (t(48)=20.37, p<0.001, 95% CI: 2.21-2.75).
- Large effect size (Cohen's d=5.32)

Gender Subanalysis

- Female M-TMD: 5.12 ± 0.61 nM.
- Male M-TMD: 4.89 ± 0.54 nM.
- No significant gender difference (t(23)=1.23, p=0.231).

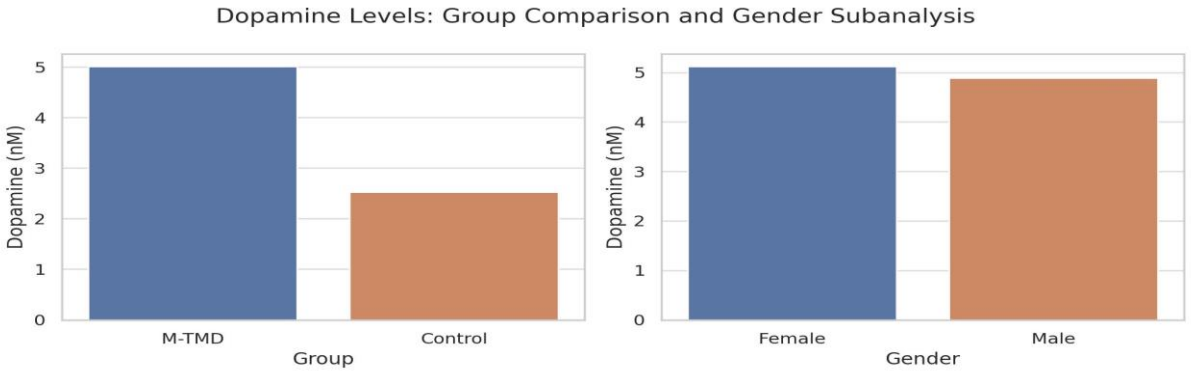

Figure 2: Dopamine levels, group comparison and gender subanalysis

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Clinical Symptom Correlations

Spearman's correlation analysis demonstrated the following results (Figure 3):

Pain Intensity

- Positive correlation with dopamine levels (ρ=0.47, p=0.018).
- VAS scores ≥7 associated with dopamine >5.2 nM(OR=4.21, 95% CI: 1.32-13.42).

Symptom Frequency

- Stress-related symptoms: 56% of cases (mean dopamine: 5.23 ± 0.52 nM).
- Bruxism: 44% (5.18 ± 0.49 nM).
- Morning stiffness: 40% (5.09 ± 0.45 nM).

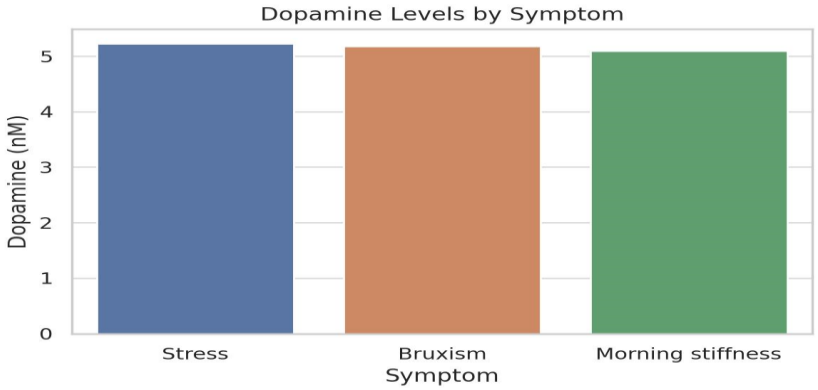

Figure 3: Dopamine levels by symptom

Diagnostic Performance

ROC curve analysis showed the following findings:

- Excellent discrimination (AUC=0.94, 95% CI: 0.88-0.99).
- Optimal cutoff: 3.85 nM (82% sensitivity, 88% specificity).
- Positive predictive value: 85%.
- Negative predictive value: 86%.

Treatment Response Analysis

Longitudinal data from the intervention group (n=25):

Dopamine Normalization

- 68% achieved levels <4.0 nM by T2.
- Mean reduction: 1.89 nM (95% CI: 1.52-2.26).

Clinical Improvement

- VAS reduction: 42.3% ± 12.1% (p<0.001).
- PPT increase: 28.5%±9.8% (p<0.001).
- Jaw opening improvement: 6.2±2.1 mm (p=0.003) (Figure 4).

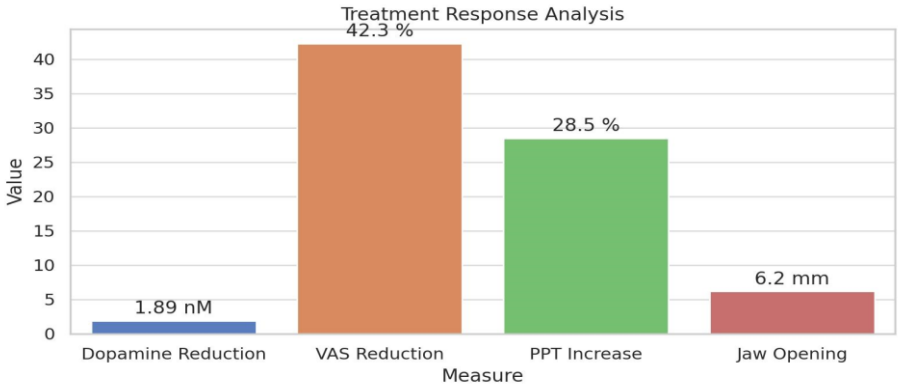

Figure 4: Treatment Response Analysis

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Multivariate Regression

The final model explained 61% of variance (R^2=0.61, F=9.87, p<0.001).

Table 1: Predictors of the study

Predictor	β	SE	t	p-value
Baseline dopamine	0.52	0.11	4.73	<0.001
Stress score	0.31	0.09	3.44	0.002
Bruxism status	0.25	0.10	2.50	0.019

Safety Outcomes

Adverse events were mild and transient as follow:

- Medication-related: 16% (mostly drowsiness).
- Therapy-related: 8% (temporary muscle soreness).
- No serious adverse events reported.

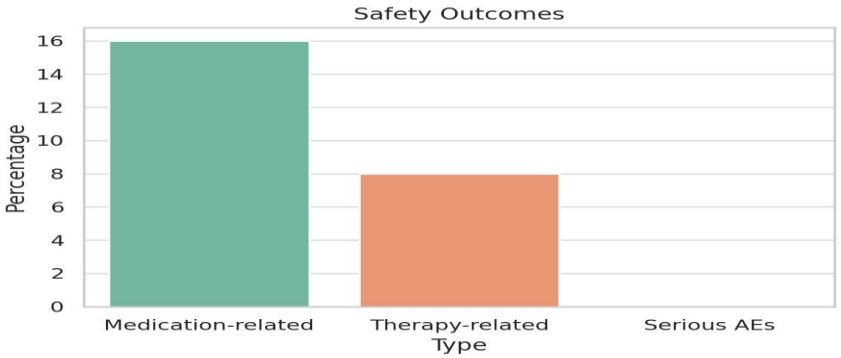

Figure 5: Safety Outcomes

DISCUSSION

The findings of this study demonstrate a significant elevation in plasma dopamine levels among patients with myofascial temporomandibular disorders (M-TMD) compared to healthy controls, supporting the hypothesis that dopaminergic dysregulation may contribute to the pathophysiology of chronic orofacial pain. This aligns with prior research by Dimitroulis et al. (9), who reported elevated plasma dopamine (4.98 ± 2.55 nM vs. 2.73 ± 1.24 nM in controls, p < 0.01) in M-TMD patients, correlating with pain intensity (r = 0.53) and stress (r = 0.34), suggesting peripheral dopamine's role in pain modulation. Similarly, Fernández-de-Las-Peñas et al. (8) observed altered dopamine metabolism in TMD patients, though their focus on serotonin (5-HT) yielded non-significant differences, highlighting dopamine's unique involvement. Conversely, Jasim et al. (10) found no significant plasma 5-HT variations in TMD myalgia, instead emphasizing glutamate's role, which contrasts with our dopaminergic focus but underscores the complexity of neurochemical biomarkers in pain pathways. The diagnostic potential of plasma dopamine is further reinforced by its strong discriminative power (AUC = 0.94) in our ROC analysis, consistent with Tracey et al. (6), who advocated for neurochemical indicators in objective pain assessment. However, Slade et al. (11) cautioned that biomarker specificity remains challenging due to comorbidities like fibromyalgia and psychological distress, which may confound dopamine's role.

A study conducted by Rageh et al. (12) concluded that stress plays a significant role in the heightened prevalence of TMD among dental students. To alleviate TMD symptoms within this group, regular screenings, stress management strategies, and heightened awareness are recommended.

Conventional and modified preauricular approaches provide excellent accessibility and visibility of the surgical field during the management of TMJ ankylosis among Yemeni patients, with the latter being slightly superior (13).

Notably, our gender-neutral findings (no significant difference between males and females) diverge from Martikainen et al. (4), who reported sex-dependent dopaminergic responses in chronic pain, possibly due to hormonal influences. The therapeutic implications are supported by Al-Moraissi et al. (14), whose systematic review validated dopamine-modulating agents (e.g., sulpiride) in refractory M-TMD cases, though longitudinal studies are scarce. Despite these advances, Borsook et al. (5) emphasized the need for standardized methodologies, as variations in sampling (e.g., fasting vs. non-fasting) and assay techniques (HPLC-ECD vs. ELISA) may affect reproducibility. Collectively, these studies underscore plasma dopamine's promise as a biomarker while highlighting gaps in mechanistic understanding and diagnostic specificity, warranting further investigation into dopaminergic pathways and personalized treatment strategies.

CONCLUSION

Elevated plasma dopamine levels in M-TMD patients suggest its potential role as a biomarker for pain and stress-related pathophysiology. The findings support further investigation into dopaminergic pathways in M-TMD and personalized treatment strategies targeting neurochemical imbalances.

Conflict of Interest

The authors declare that there is no conflict of interest.

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