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TABLE OF CONTENTS

# In This Issue

*Volume 4 · Issue 2 · May – August 2026*

FRONT MATTER

Copyright, Disclaimer & License ..... *iii*
Editorial Board ..... *v*
Guidelines for Publication (Author's Guidelines) ..... *vii*

EDITORIAL

**Dengue fever in Sudan: its epidemiological transformation and control efforts over the past decade amidst the ongoing war** ..... 47
*Elfatih M. Malik, Mubark Karsani*
DOI 10.69993/2026.4.2.en1

ORIGINAL ARTICLES

**Assessment of Platelet Count and Platelet Distribution Width among Sudanese Patients with Dengue Fever in Kassala State, Sudan** ..... 51
*Amna I. A. Idris*
DOI 10.69993/2026.4.2.en2

**Knowledge, Attitudes, Safety Awareness and Practice of Laser Dentistry Among Sudanese Dentists: A Cross-Sectional Survey** ..... 59
*Nasr M. A. Elsheikh, Fatima A. E. Abdallah, et al.*
DOI 10.69993/2026.4.2.en3

**Phytochemical Screening and Antibacterial Activity of Yemeni *Moringa oleifera* Leaves Extract** ..... 69
*Mohammed Hassan, Abubakr A. Hammad, et al.*
DOI 10.69993/2026.4.2.en4

**Hematological and Micronutrient Profile among Sudanese Schoolchildren in Egypt and Sudan** ..... 77
*Albara Ahmed, Mustafa B. M. Ahmed, et al.*
DOI 10.69993/2026.4.2.en5

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EDITORIAL

# Dengue fever in Sudan: its epidemiological transformation and control efforts over the past decade amidst the ongoing war

**Elfatih M. Malik**[1] and **Mubark Karsani**[2]

Invited
25 August 2026

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31 August 2026

## Editorial

Dengue is an arboviral disease caused by dengue virus (DENV) and transmitted by *Aedes* mosquitoes (mainly *Aedes aegypti* and *Aedes albopictus*). The virus belongs to the genus *Orthoflavivirus* (Family: Flaviviridae) and is classified into four distinct but antigenically related serotypes (DENV-1–DENV-4). Although infection with DENV is asymptomatic in most infected persons, the disease can be more profound and can manifest itself as “dengue without warning signs”, “dengue with warning signs”, and “severe dengue”[1]. According to the World Health Organization (WHO), more than 14 million cases and more than 11,000 deaths were reported in 2024 worldwide[2]. The disease is currently reported from more than 100 countries, with the highest burden in the Region of the Americas, which accounted for more than 90% of cases in 2024, followed by Asia and Africa[2]. The increase in cases and the spread were attributed to rapid urbanization, water shortage, poor waste management, displacement, and the growing climate sensitivity of transmission[2]. In 2024, the overall reported cases in Sudan exceeded 10,000, with eight deaths[2]. The relatively low number of reported dengue-associated deaths in Sudan should be interpreted cautiously, as fragmented surveillance systems, limited laboratory confirmation, and underreporting may contribute to underestimation of dengue mortality[3,4].

Dengue in Sudan was regarded primarily as a disease of the eastern region of Sudan, particularly in big cities such as Port Sudan and Kassala. However, over the past decade, dengue has come to represent a nationwide public health challenge affecting diverse ecological and socioeconomic settings across Sudan with marked spatiotemporal variation. The disease expanded from eastern Sudan and is reported in all regions of Sudan. Dengue transmission is now established in at least 11 Sudanese States, including Khartoum State, with a pooled DENV seroprevalence of 27% reported in a 2020 meta-analysis[4]. In 2023, a major outbreak was reported from North Kordofan, South Kordofan and Red Sea, and followed in 2024–2025 by outbreaks in River Nile, Gezira, Khartoum, and Kassala[5].

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The virological map of dengue in Sudan has undergone substantial changes over time. Earlier outbreaks were primarily associated with DENV-2 and DENV-3, including the identification of the Cosmopolitan genotype of DENV-2 in Kassala and genotype III of DENV-3 during the 2019 eastern Sudan outbreak[6]. Subsequently, molecular surveillance documented the first detection of DENV-4 in Sudan during outbreaks in Port Sudan and Kassala in 2019[7], indicating increasing viral diversity; this followed a large outbreak of chikungunya in 2018. More recently, the 2023 Kassala outbreak demonstrated simultaneous circulation of all four dengue virus serotypes[8]. These findings illustrate a shift from sporadic circulation of a limited number of serotypes toward a complex, hyperendemic epidemiological pattern characterized by multiple serotypes.

Dengue in Sudan is transmitted mainly by *Aedes aegypti* and is linked to poor water storage practices and weak control measures[9]. The emergence of an invasive and highly competent dengue vector, *Aedes albopictus*, may be contributing to expanded transmission dynamics and increasing epidemic potential in both urban and rural areas[10].

Diagnosis and clinical management of dengue remain major challenges in Sudan because the disease shares clinical features with other endemic febrile illnesses such as malaria, chikungunya, yellow fever, and other arboviral infections[11]. Several studies have documented dengue–malaria co-infection, highlighting the risk of misdiagnosis and delayed treatment when clinicians stop investigating after identifying a single pathogen[12,13]. Laboratory confirmation remains limited by insufficient molecular diagnostic capacity, shortages of reagents, and weak public health laboratory infrastructure[14,15]. Clinically, Sudanese outbreaks have reported high frequencies of hemorrhagic manifestations, dengue hemorrhagic fever, dengue shock syndrome, thrombocytopenia, and severe disease[16,17]. Recent surveys among Sudanese physicians also revealed important knowledge gaps regarding recognition of severe dengue, less common symptoms, and effective management and vector control practices[18]. These challenges underscore the need for enhanced diagnostic capabilities, clinician training, and integrated laboratory surveillance capable of identifying multiple circulating arboviruses simultaneously[14], guided throughout by the recently published WHO guidelines[1].

In this issue of the *Journal* we publish **Assessment of Platelet Count and Platelet Distribution Width (PDW) among Sudanese Patients with Dengue Fever in Kassala State, Sudan**, a comparative analytical cross-sectional, laboratory-based study. It documents the degree of thrombocytopenia associated with dengue infection and its severity, and supports platelet monitoring as part of the routine hematological assessment of suspected dengue patients.

The recent expansion and severity of dengue outbreaks in Sudan are closely linked to armed conflict, population displacement, and the collapse of public health infrastructure. Since the onset of the war in April 2023, millions of people have been displaced internally or across borders, often settling in overcrowded areas with inadequate water, sanitation, and healthcare services, reflecting how conflict-driven displacement creates conditions that facilitate the spread of vector-borne diseases[15,19,20].

Studies conducted during the conflict found significantly higher dengue infection rates among displaced populations, while reports from conflict-affected States highlighted barriers to healthcare access, insecurity, shortages of preventive supplies, and breakdowns of surveillance systems[15,21]. Humanitarian camps in Darfur have already experienced dengue outbreaks and dengue–malaria co-infections, illustrating how displacement facilitates disease transmission[12]. The destruction of health facilities, disruption of vector control programs, interruptions in disease surveillance, and reduced laboratory capacity have created ideal conditions for dengue expansion[14,15,19]. Consequently, dengue has become part of a broader complex emergency in which conflict amplifies environmental, social, and health system vulnerabilities that favor arboviral epidemics[15,19].

Control efforts in Sudan have largely focused on vector control, surveillance strengthening, public awareness campaigns, and promotion of integrated public health approaches. During the 2010 Port Sudan outbreak, coordinated house inspections, larval source reduction, entomological surveillance, and insecticide spraying significantly reduced *Aedes aegypti* indices and were associated with interruption of transmission[22]. Multiple studies have subsequently emphasized the importance of routine epidemiological and entomological surveillance, climate-based early warning systems, and community engagement to reduce mosquito breeding sites[10,23]. Recent investigations have highlighted the value of One Health approaches that integrate human, animal, and environmental surveillance, particularly given the concurrent circulation of multiple

arboviruses[14,21]. Community knowledge, attitudes, and practices studies conducted during the 2025 outbreak demonstrated generally high awareness but identified persistent misconceptions that require targeted educational interventions[24]. Nevertheless, persistent weaknesses in laboratory capacity, intersectoral coordination, funding, and health-system resilience continue to limit effective dengue control, particularly under ongoing conflict conditions[10,21,23].

In conclusion, dengue in Sudan has evolved from a localized endemic disease into a nationwide complex public health threat characterized by expanding geographic distribution, co-circulation of multiple serotypes, and recurrent outbreaks. Global climate change, concomitant with the ongoing conflict, has further accelerated transmission by disrupting surveillance, vector control, laboratory services, and access to healthcare. A national strategic plan for dengue and arboviruses is highly needed now more than ever. The plan should consider adopting a One Health approach integrating vector control and case management with malaria as the co-existence of *Aedes aegypti* and *Anopheles stephensi* (the invasive vector) and co-infection between dengue and malaria is common in Sudan.

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ORIGINAL ARTICLE

# Assessment of Platelet Count and Platelet Distribution Width among Sudanese Patients with Dengue Fever in Kassala State, Sudan

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## Abstract

STRUCTURED SUMMARY · 4 SECTIONS

**BACKGROUND**

*Dengue fever is a mosquito-borne viral infection and a significant public health concern in Sudan, including Kassala State, where outbreaks recur. The infection is often associated with a decline in platelet count and changes in platelet distribution width (PDW), both used as candidate diagnostic and prognostic markers of disease severity. This study assessed platelet count and PDW among Sudanese patients with dengue fever in Kassala State and compared these parameters with those of healthy controls.*

**METHODS**

*This comparative analytical cross-sectional, laboratory-based study included 50 dengue fever patients and 50 age- and sex-matched apparently healthy controls from the same source population. Platelet count and PDW were measured using a Sysmex KX-21N hematology analyzer.*

**RESULTS**

*The mean age of patients was 38.5 ± 9.8 years, and 68.0% were male. Disease severity was classified as mild (32.0%), moderate (38.0%), and severe (30.0%). Platelet count was significantly lower in dengue patients than in controls (95.5 ± 59.5 vs 250.9 ± 70.1 $\times 10^9$/L, $P < 0.001$), while PDW was significantly higher in dengue patients than in controls (24.8 ± 5.7% vs 20.6 ± 5.5%, $P < 0.001$). No significant correlation was observed between age and either platelet count or PDW. However, platelet count decreased, and PDW increased significantly as disease severity increased ($P < 0.001$).*

**CONCLUSION**

*Platelet count was significantly lower in dengue patients than in healthy controls and decreased further with increasing disease severity, while PDW was significantly higher than in controls and increased further with increasing severity.*

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KEYWORDS
Dengue fever · Hematology analyzer · Platelet count · Platelet distribution width · Sudan · Thrombocytopenia

## Introduction

Dengue fever is the most rapidly spreading mosquito-borne viral infection worldwide, with a more than 30-fold rise in incidence over the past decade and expansion into new geographic regions.[1–4] More than 100 countries are now considered endemic, and the World Health Organization (WHO) estimated a record 14 million cases and over 9,000 deaths globally in 2024 alone.[5,6] Although dengue was historically uncommon in Africa, large outbreaks have occurred across the continent since the year 2000, and the virus is increasingly recognized as an emerging public health threat in the region.[7] In Sudan, recurrent outbreaks have been documented, particularly in the Red Sea State, underscoring the need for region-specific hematological data to guide clinical management.[8] Cross-sectional serosurveys have further confirmed active dengue transmission and identified associated risk factors among residents of eastern Sudan, including Kassala and neighboring El-Gadarif state.[9,10]

Dengue virus is transmitted primarily by *Aedes* mosquitoes that breed readily in small collections of stagnant water; infection typically presents as an acute febrile illness that can progress, in a minority of cases, to severe disease with plasma leakage or hemorrhagic manifestations.[2,5] Kassala State, in eastern Sudan bordering Eritrea, combines a hot climate, seasonal rainfall, and population movement that favor sustained vector transmission, making it one of the areas repeatedly affected by dengue outbreaks.[8]

Dengue virus (DENV) comprises four antigenically related but genetically distinct serotypes (DENV-1–DENV-4); secondary infection with a different serotype carries a higher risk of severe disease through antibody-dependent enhancement.[1] Local surveillance in Kassala has confirmed simultaneous circulation of all four DENV serotypes during a recent outbreak at Kassala Teaching Hospital,[11] while an earlier assessment of the 2019 Kassala outbreak identified important gaps in surveillance and laboratory response capacity that continue to hamper timely case detection in the state.[12]

Dengue virus infection frequently causes thrombocytopenia, and platelet indices derived from the routine complete blood count, platelet count, mean platelet volume, plateletcrit (PCT), and platelet distribution width (PDW) have attracted growing interest as diagnostic and prognostic tools.[13,14]

Platelet distribution width reflects variability in platelet size and is regarded as a marker of platelet activation and turnover; several authors have proposed it as a low-cost adjunct to platelet count for assessing dengue severity.[14] The mechanisms underlying dengue-associated thrombocytopenia are multifactorial and include direct viral suppression of bone-marrow megakaryocytes, increased peripheral platelet destruction through immune-mediated mechanisms, and platelet consumption secondary to endothelial activation and mild coagulopathy.[13,15] As platelets are cleared and replaced during infection, the circulating population becomes more heterogeneous in size, a change that can, in principle, be captured by platelet distribution width. Because platelet indices are generated automatically with every complete blood count at negligible additional cost, they are attractive candidate biomarkers for resource-limited settings such as Kassala State, provided that their diagnostic performance is locally validated rather than extrapolated from other populations.[14]

Previous studies have reported inconsistent findings regarding PDW in dengue infection. Wayez et al. observed progressively higher PDW values with more severe thrombocytopenia,[16] and Mukker and Kiran reported significant PDW alterations among patients with markedly reduced platelet counts.[17] Zeb et al. similarly described altered platelet indices among Pakistani dengue patients.[18] Elsewhere, Khatri et al. and Nehara et al. reported significantly higher PDW among Indian dengue patients with thrombocytopenia compared with those without,[19,20] while Navya et al. and Kantharaj found platelet indices useful for guiding clinical decisions, including transfusion requirements, in dengue-positive patients.[21,22] In Sudan, Bashir et al. reported that low platelet count, low mean platelet volume, and high PDW could serve as probable indicators of dengue infection in Red Sea State,[8] and a subsequent Sudanese study similarly documented pronounced thrombocytopenia and bleeding manifestations among dengue patients in Port Sudan,[23] while a more recent study assessed these indices in Khartoum State.[24] However, no prior study conducted in Kassala State has directly compared platelet count and PDW between dengue patients and healthy controls, or

examined these parameters across clinical severity categories, in this specific population.

The present study therefore aimed to assess platelet count and PDW among Sudanese patients with dengue fever in Kassala State and to compare these parameters with those of healthy individuals, in order to establish locally relevant reference data rather than assuming that findings from other Sudanese states apply directly to Kassala.

## Materials and Methods

### Study Design and Setting

This was a comparative analytical cross-sectional, laboratory-based study conducted at Kassala Teaching Hospital, Kassala State, Sudan. Data were collected between September and October 2025.

### Study Population and Sampling

Participants comprised Sudanese patients aged ≥18 years with a clinical diagnosis of dengue fever (cases, per WHO criteria),[25] recruited from among outpatients and inpatients presenting to Kassala Teaching Hospital during the study period, and age- and sex-matched apparently healthy controls with no history of dengue fever or other hematological disorders, recruited by convenience sampling from hospital staff, patient companions, and community volunteers drawn from the same catchment area and time frame as the cases. Age- and sex-matching between cases and controls was performed by frequency matching: controls were recruited so that the overall age-group and sex distribution of the control set matched that of the cases, without pairing individual controls to individual cases.

#### Sample Design

The minimum sample size required for the study was calculated using the formula for comparison of two independent means:

$$n = 2(Z\alpha/2+Z\beta)^2(\sigma^2/d^2)$$

where $Z\alpha/2 = 1.96$, $Z\beta = 0.84$ (80% power), $\sigma$ is the control-group standard deviation of platelet count reported in a recent Sudanese case-control study of dengue-associated thrombocytopenia (Ahmed et al., 2025),[24] and d is the minimum clinically important difference in platelet count. A value of $d = 40 \times 10^9$/L was chosen because it approximates one standard deviation of the reference control-group platelet count and was therefore considered the smallest difference likely to be clinically meaningful; this calculation was based on platelet count alone.

Using $\sigma \approx 38.4 \times 10^9$/L, the minimum calculated sample size was 15 per group; this was increased to 50 per group to allow for possible data exclusion.

### Inclusion and Exclusion Criteria

For cases, Sudanese patients aged 18 years and above who were diagnosed with dengue fever and who agreed to take part in the study were included. Individuals with bleeding disorders, thrombocytopenia unrelated to dengue infection, chronic diseases (diabetes mellitus, hypertension, cardiovascular, hepatic, or renal disease), pregnant women, and patients who had received a platelet transfusion within the preceding six months were excluded from both the case and control groups. Both patients and control subjects were asked to sign informed consent documents after due explanation.

### Sample Collection and Laboratory Methods

A total of 100 participants were enrolled: 50 cases and 50 controls by convenience sampling.

Dengue fever was diagnosed clinically based on the WHO case definition (fever plus two or more of the following: nausea/vomiting, rash, headache, myalgia/arthralgia, positive tourniquet test, leukopenia), and confirmed by NS1 antigen/IgM serology.[25] Three milliliters of venous blood were then collected under sterile conditions from each participant into EDTA anticoagulant tubes and processed promptly, or stored at −20 °C when immediate analysis was not possible. Platelet count and PDW were measured using an automated Sysmex KX-21N hematology analyzer, which determines platelet number and size distribution by electrical impedance sensing across a calibrated aperture. The manufacturer's reference ranges were 150–400 $\times 10^9$/L for platelet count and 8.3–56.6% for PDW.

### Quality Control

Internal quality control was performed daily using commercial control blood samples run on the Sysmex KX-21N analyzer before testing of study samples, in accordance with the manufacturer's recommendations. Results falling outside the manufacturer-defined control range triggered recalibration before analysis resumed.

### Data and Sample Collection

Data collection encompassed both the laboratory blood sampling procedure described above and questionnaire-

based data. Demographic data (age, sex, residence) and clinical information, including clinician-assessed disease severity (mild, moderate, and severe) based on WHO dengue case assessment criteria, were collected using a structured questionnaire administered to each participant.[25]

### Statistical Analysis

Data were analyzed using SPSS version 26. The normality of continuous variables was assessed and homogeneity of variance was checked prior to parametric testing; Spearman's correlation was used in place of Pearson's correlation where these assumptions were not met. Continuous variables were summarized as mean ± standard deviation and compared between cases and controls using the independent-samples t-test. The age and sex distributions of cases and controls were compared (Table 1a) to confirm that matching was achieved at the group level.

Comparison of platelet count and PDW across the three severity categories was performed using one-way ANOVA, with Bonferroni-corrected pairwise post-hoc comparisons between severity groups where the overall ANOVA was significant. Categorical variables were expressed as frequencies and percentages. Pearson's (or Spearman's, as applicable) correlation coefficient was used to assess the relationship between age and platelet parameters. A P-value ≤ 0.05 was considered statistically significant.

### Ethical Considerations

Ethical approval was obtained from the Research Ethics Committee of Kassala Teaching Hospital, in coordination with the Kassala State Ministry of Health. Written informed consent was obtained from all participants after the study objectives and any risks associated with sampling were explained. All participant information was kept confidential.

## Results

A total of 100 participants (50 dengue fever patients and 50 healthy controls) were analyzed. Among the patients, the mean age was 38.5 ± 9.8 years; the largest proportion were aged 31–40 years (32.0%), followed by 20–30 years (28.0%), 41–50 years (24.0%), and 51–60 years (16.0%). Sixty-eight percent (n = 34) of patients were male, and 32.0% (n = 16) were female. Regarding disease severity, 38.0% (n = 19) had moderate disease, 32.0% (n = 16) had mild disease, and 30.0% (n = 15) had severe disease (Table 1).

**Table 1. Demographic and Clinical Characteristics of Dengue Fever Patients (n = 50).**

| Characteristic | Category | n | % |
|---|---|---|---|
| Age (years) | 20–30 | 14 | 28.0 |
| | 31–40 | 16 | 32.0 |
| | 41–50 | 12 | 24.0 |
| | 51–60 | 8 | 16.0 |
| Sex | Male | 34 | 68.0 |
| | Female | 16 | 32.0 |
| Disease severity | Mild | 16 | 32.0 |
| | Moderate | 19 | 38.0 |
| | Severe | 15 | 30.0 |

**Table 1a. Demographic Comparison between Dengue Fever Patients and Healthy Controls (n = 50 each).**

| Characteristic | Category | Cases (n) | Controls (n) | P-value |
|---|---|---|---|---|
| Age (years) | 20–30 | 14 | 12 | 0.966 |
| | 31–40 | 16 | 17 | |
| | 41–50 | 12 | 10 | |
| | 51–60 | 8 | 7 | |
| Sex | Male | 34 | 32 | 0.943 |
| | Female | 16 | 17 | |

Comparison of platelet parameters between dengue fever patients and healthy controls showed a significant reduction in platelet count among patients (95.5 ± 59.5 vs 250.9 ± 70.1 $\times 10^9$/L, P < 0.001), while PDW values were also significantly higher among dengue patients than controls (24.8 ± 5.7% vs 20.6 ± 5.5%, P < 0.001).

The mean difference in platelet count between dengue patients and controls was −155.4 $\times 10^9$/L (95% CI: −180.9 to −129.9; independent-samples t-test, P < 0.001). The mean difference in PDW was 4.2% (95% CI: 2.0 to 6.4; P < 0.001), consistent with a significant between-group difference. Confidence intervals were calculated from the reported means, standard deviations, and group sizes using the standard formula for the difference between two independent means (Table 2).

**Table 2. Comparison of Platelet Count and PDW between Dengue Fever Patients and Healthy Controls.**

| Parameter | Dengue patients (n = 50) | Healthy controls (n = 50) | P-value |
|---|---|---|---|
| Platelet count ($\times 10^9$/L) | 95.5 ± 59.5 | 250.9 ± 70.1 | < 0.001 |
| PDW (%) | 24.8 ± 5.7 | 20.6 ± 5.5 | < 0.001 |

The mean platelet count decreased progressively from mild to severe dengue (167.6 ± 28.2, 87.3 ± 22.4, and 28.9 ± 11.1 $\times 10^9$/L, respectively), while PDW increased significantly with disease severity (21.2 ± 3.5%, 24.2 ± 4.6%, and 29.3 ± 5.9%, respectively). One-way ANOVA confirmed a significant overall difference in platelet count across severity groups ($F(2,47) = 156.4$, $P < 0.001$) and in PDW ($F(2,47) = 11.6$, $P < 0.001$). Bonferroni-corrected pairwise comparisons ($\alpha = 0.0167$) showed that platelet count differed significantly between every pair of severity groups (mild vs moderate: mean difference 80.3 $\times 10^9$/L, 95% CI 62.4 to 98.2, $P < 0.001$; moderate vs severe: 58.4 $\times 10^9$/L, 95% CI 46.3 to 70.5, $P < 0.001$; mild vs severe: 138.7 $\times 10^9$/L, 95% CI 122.8 to 154.6, $P < 0.001$), confirming a stepwise decline across all three categories. For PDW, pairwise comparisons showed significant differences between mild and severe dengue (mean difference −8.1%, 95% CI −11.7 to −4.5, $P < 0.001$) and between moderate and severe dengue (−5.1%, 95% CI −8.9 to −1.3, $P = 0.011$), while the difference between mild and moderate dengue did not reach the Bonferroni-corrected threshold (−3.0%, 95% CI −5.8 to −0.2, $P = 0.036$), indicating that the rise in PDW was most pronounced at the transition to severe disease (Table 3).

**Table 3. Comparison of Platelet Count and PDW between Dengue Fever Patients According to Disease Severity**

| Parameter | Mild Dengue (n = 16) | Moderate Dengue (n = 19) | Severe Dengue (n = 15) | P-value |
|---|---|---|---|---|
| Platelet count ($\times 10^9$/L) | 167.6 ± 28.2 | 87.3 ± 22.4 | 28.9 ± 11.1 | < 0.001 |
| PDW (%) | 21.2 ± 3.5 | 24.2 ± 4.6 | 29.3 ± 5.9 | < 0.001 |

No significant correlation was found between age and platelet count ($r = -0.137$, $P = 0.342$) or between age and PDW ($r = -0.031$, $P = 0.832$) among dengue fever patients (Table 4).

**Table 4. Correlation between Age and Platelet Parameters among Dengue Fever Patients.**

| Parameter | r | P-value |
|---|---|---|
| Age vs platelet count | −0.137 | 0.342 |
| Age vs PDW | −0.031 | 0.832 |

## Discussion

This study assessed platelet count and PDW among Sudanese patients with dengue fever in Kassala State and compared these parameters with healthy controls. The findings confirm that dengue infection was associated with a marked and statistically significant reduction in platelet count, consistent with the well-established role of thrombocytopenia as a hallmark of dengue infection and a criterion used in clinical severity assessment.[8,16–18] Most patients in this study were male and aged between 31 and 40 years; male predominance has similarly been reported in other dengue series, but as occupational exposure was not directly measured in this study, differential outdoor exposure to mosquito vectors among men remains only a plausible hypothesis rather than a demonstrated explanation. Disease severity was distributed relatively evenly across mild, moderate, and severe categories in this sample, although the convenience sampling approach means this distribution should not be assumed to represent the true clinical spectrum of dengue presentation in Kassala State more broadly. Consistent with platelet count, PDW was also significantly higher among dengue patients than healthy controls in this study, in agreement with reports by Wayez et al.[16] and Mukker and Kiran,[17] who described significant elevations in PDW with worsening thrombocytopenia, and with Bashir et al.,[8] who proposed high PDW as a probable indicator of dengue infection in Red Sea State, Sudan. This finding is also broadly consistent with the more recent Khartoum State study by Ahmed et al.,[24] reinforcing PDW's potential value as a low-cost adjunct marker of dengue infection across different Sudanese settings. Similarly, Zeb et al. reported measurable alterations in platelet indices among dengue patients in Pakistan,[18] and larger hospital-based cohorts by Asha et al. and Antony Gnana Shahila and Jothilingam have likewise shown significant differences in platelet indices between dengue patients and controls, though the specific parameters affected varied across studies.[26,27] Some variation in effect size across these studies may still relate to differences in the day of illness at which blood was sampled relative to the onset of fever, the proportion of patients with severe versus mild disease, sample size, and possible platelet clumping or analyzer-related variability.

In the present study, platelet count decreased significantly with increasing disease severity, whereas PDW increased progressively from mild to severe dengue cases ($P < 0.001$). Our findings are consistent with those of Wayez et al.,[16] who reported increased PDW in dengue patients with thrombocytopenia and highlighted the clinical value of platelet indices during disease progression. Similarly, Bashir et al.[8] found significantly higher PDW and lower platelet counts in Sudanese dengue patients compared with healthy controls.

These findings suggest that both platelet count and PDW were consistently and significantly altered among dengue patients compared with controls in this population, supporting a potential complementary role for PDW alongside platelet count in dengue assessment in similar endemic settings. However, because this study did not evaluate sensitivity, specificity, or predictive values, these findings describe an association rather than an established diagnostic performance for either parameter.

The absence of a significant correlation between age and either platelet count or PDW indicates that age alone does not predict the degree of thrombocytopenia or platelet size variability in this cohort, suggesting that other factors such as viral load, infecting serotype, timing of presentation, or host immune response may play a more prominent role and warrant further investigation. Because platelet count declined consistently and significantly in this cohort, it remains a simple, inexpensive, and widely available parameter that was associated with dengue infection in this population.

## Conclusion

Platelet count was significantly reduced among Sudanese dengue fever patients in Kassala State compared with healthy controls and decreased further with increasing disease severity, supporting an association between thrombocytopenia and dengue infection and severity in this population. PDW was also significantly higher in dengue patients than in healthy controls overall, and increased further with increasing dengue severity. These findings should be interpreted as cross-sectional associations rather than evidence of diagnostic or prognostic accuracy, and platelet count and PDW should continue to be monitored as part of the routine hematological assessment of suspected dengue patients pending further validation.

## Future Research

Future studies should adopt multicenter, prospective designs with larger and randomly selected samples across different regions of Sudan to improve generalizability and allow assessment of platelet count and PDW trends across the course of illness. Incorporating dengue serotype identification and viral load quantification would help clarify their contribution to the variability in platelet indices observed across studies, while formal evaluation of the sensitivity, specificity, and predictive value of platelet count and PDW is needed before these parameters can be recommended as diagnostic or prognostic markers of dengue severity.

## Limitations

This study was conducted at a single center with a relatively small sample size, which may limit the generalizability of the findings to other regions of Sudan. Participants were recruited by convenience rather than random sampling, which may have introduced selection bias and further limits generalizability of the findings. The comparative analytical cross-sectional design and single-timepoint sampling did not allow assessment of changes in platelet parameters throughout the course of illness or according to the day of illness, and platelet count and PDW were measured concurrently with dengue status rather than before disease onset, so a causal or temporal interpretation of the association is not possible.

## Conflict of Interest

No conflict of interest.

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# Knowledge, Attitudes, Safety Awareness and Practice of Laser Dentistry Among Sudanese Dentists: A Cross-Sectional Survey

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## Abstract

STRUCTURED SUMMARY · 4 SECTIONS

**BACKGROUND**

Laser technology is increasingly integrated into dental practice worldwide, yet adoption in low-resource settings often lags behind favorable attitudes. No previous study has characterized Sudanese dentists' knowledge, attitudes, safety awareness and practice of laser dentistry.

**METHODS**

In this cross-sectional study, 413 Sudanese dentists completed a structured, self-administered online questionnaire between August 2023 and January 2024 through non-probability convenience sampling. Composite Knowledge (0–8), Attitude (4–20) and Safety-awareness (0–4) scores were derived; internal consistency was assessed using Cronbach's alpha. Associations were tested with chi-square/Fisher's exact and Mann–Whitney U/Kruskal–Wallis tests, and independent correlates of laser use by multivariable logistic regression ($p < 0.05$). Reporting followed STROBE.

**RESULTS**

Half of respondents (219, 53.0%) recognized that lasers treat both hard and soft tissue. Interest was high (346, 83.8%), yet only 77 (18.6%) used lasers. The commonest barrier (n = 353) was inadequate hands-on experience (245, 69.4%), followed by cost (90, 25.5%). Awareness of ocular (280, 67.8%) and cutaneous (263, 63.7%) hazards was moderate, but plume-inhalation awareness was low (133, 32.2%). Internal consistency was good to excellent (alpha 0.87–0.89). Independent correlates of laser use were prior safety training (aOR 19.9, 95% CI 7.2–55.1), higher knowledge score (aOR 1.55 per point, 1.23–1.96) and private-sector practice (aOR 2.88, 1.16–7.20); gender and qualification were not.

**CONCLUSION**

Positive attitudes were not matched by practical experience or comprehensive safety knowledge, particularly regarding plume inhalation. Structured undergraduate and postgraduate training with explicit plume-safety content is recommended.

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Lasers · Dentistry · Health knowledge, attitudes, practice · Dental education · Occupational

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## Introduction

Light Amplification by Stimulated Emission of Radiation (laser) became a working reality in 1960, when Maiman produced a coherent, monochromatic beam from a ruby crystal, building on Einstein's earlier description of stimulated emission.[1,2] Different laser wavelengths interact differently with dental hard and soft tissue,[3] and the technology has since diversified into several wavelength-specific systems: carbon dioxide, neodymium-doped yttrium aluminum garnet (Nd:YAG), erbium-based, and diode lasers.[4–9]

Laser-based approaches are now described across nearly every discipline of dentistry, spanning minimally invasive cavity preparation, caries detection, soft-tissue surgery, periodontal debridement, tooth whitening, and photobiomodulation for wound healing and pain control.[4–9] The evidence for some applications remains mixed: systematic reviews report inconsistent improvement in clinical attachment level when lasers are added to non-surgical periodontal therapy,[10] with comparable uncertainty for laser-assisted periodontal surgery,[11] a reminder that adoption should be guided by outcome evidence rather than novelty.

Despite this expanding evidence base, translation into everyday practice has been uneven outside high-income settings. Cross-sectional surveys from Saudi Arabia consistently show that familiarity outpaces use; comparable awareness–practice gaps have been reported from India and Pakistan, most often attributed to cost and insufficient hands-on training.[12–21] Within Sudan, and across the wider region, comparable survey data on laser dentistry remain scarce.[22,23] To our knowledge, no previous survey has examined Sudanese dentists' knowledge, attitudes and practice with regard to laser dentistry.

This gap is not merely academic. Curricular and policy decisions are difficult to make rationally without baseline data on practitioners' current knowledge, attitudes and self-reported barriers, and the absence of such data carries direct implications for patient safety, for the efficient use of scarce training and equipment resources in the Sudanese health system, and for alignment with international laser-safety standards.

The present study was therefore designed to assess Sudanese dentists' knowledge and awareness of laser applications, their attitudes toward safety and efficacy, and their current practice patterns and perceived barriers, and, extending beyond previous descriptive surveys, to identify the factors independently associated with laser use.

## Materials and Methods

### Study Design and Period

A cross-sectional, questionnaire-based study was conducted among dentists in Sudan between August 2023 and January 2024.

### Setting and Participants

The target population comprised licensed dentists practicing in Sudan across government hospitals, private clinics, and academic institutions, from early-career practitioners to experienced specialists. Any licensed dentist practicing in Sudan who was willing to give informed consent was eligible; those who declined or did not complete the questionnaire were excluded.

### Sampling and Sample Size

The questionnaire was distributed electronically as a self-administered Google Forms link. The sampling technique was non-probability convenience sampling, in which dentists were reached through professional networks and social media. Because recruitment relied on an openly shared link, the total number of dentists who were invited or who opened the form was not captured; a formal response rate and denominator, therefore, cannot be computed, and this is acknowledged as a limitation.

The minimum required sample size was estimated using the standard formula for a single proportion, $n = Z^2pq / d^2$, where $Z = 1.96$ (the standard normal value for a 95% confidence level), $p = 0.5$ (the proportion assumed to give the maximum required sample in the absence of a prior estimate), $q = 1 - p = 0.5$, and $d = 0.05$ (the accepted margin of error). This yields $n = (1.96^2 \times 0.5 \times 0.5) / 0.05^2 = 384$, so a minimum of approximately 385 respondents was required. A total of 413 dentists provided complete responses and were included in the analysis, exceeding the calculated target.

### Questionnaire

The questionnaire was developed for this study to assess the constructs specified in the study objectives and was informed by the content of previous surveys of dental professionals' knowledge, attitudes and practices regarding laser dentistry.[12–21] Items were organized a priori according to the construct they were intended to measure. Demographic and professional items described participant characteristics; knowledge items assessed factual recognition of laser applications and types; attitude items assessed perceptions of laser safety and effectiveness; practice items assessed current use, interest, barriers to non-use, and support for undergraduate education; and safety-awareness items assessed recognition of ocular, cutaneous, dental-tissue and plume-related hazards. The analytic knowledge domain comprised eight factual items: one item on hard- and soft-tissue use, six items on applicability across dental specialties, and one item on identification of clinically used laser types. The attitude domain comprised four five-point Likert items addressing perceived benefits of laser treatment. The safety-awareness domain comprised four hazard-recognition items. The instrument was reviewed by three subject-matter experts for content and face validity and was piloted on 30 dentists, whose responses were not included in the final analysis. Internal consistency was acceptable at pilot (Cronbach's alpha 0.86) and good to excellent in the final sample (0.87 to 0.89 across the composite scales).

### Variables and Scoring

Three composite scores were derived. The Knowledge score (range 0–8) summed correct responses to the eight factual knowledge items; the Safety-awareness score (range 0–4) summed affirmative recognition of the four specified hazards; and the Attitude score (range 4–20) summed the four five-point Likert items (coded 1 = strongly disagree to 5 = strongly agree). For descriptive interpretation, Knowledge and Safety-awareness scores were converted to percentages of the maximum obtainable score and classified using the commonly applied Bloom cut-off points: < 60% = poor, 60–79% = moderate, and ≥ 80% = good.[24–26] These thresholds are used in KAP and health-professional survey research as pragmatic performance bands, with ≥ 80% representing a high level of mastery or adequacy, 60–79% an intermediate level, and < 60% an insufficient level. They are not presented as empirically validated clinical thresholds for laser dentistry. The same percentage bands were applied descriptively to the Attitude score, with < 60% interpreted as less positive, 60–79% as moderately positive, and ≥ 80% as positive; attitude was also retained as a continuous score for analysis. The categorization was used for descriptive presentation only, whereas the continuous composite scores were retained for inferential analyses. Current practice was a single binary item and was analyzed as a proportion rather than as a scale.

### Statistical Analysis

Data were cleaned before analysis: two biologically impossible age entries (3 and 4 years) were treated as missing for age summaries, and inconsistently coded experience schemes and duplicate specialty labels were harmonized. Categorical variables were summarized as frequencies and percentages, and continuous variables as mean ± SD. Internal consistency was assessed with Cronbach's alpha. Associations between categorical variables were tested with the Pearson chi-square test (Fisher's exact test where more than 20% of cells had expected counts below 5); differences in ordinal composite scores across groups were tested with the Mann–Whitney U test (two groups) or the Kruskal–Wallis test (more than two groups), as the scores were non-normally distributed. Factors independently associated with current laser use were identified using multivariable binary logistic regression, with results reported as adjusted odds ratios (aOR) with 95% confidence intervals; model calibration was assessed with the Hosmer–Lemeshow test. Analyses were performed in SPSS and Python, and two-sided $p < 0.05$ was considered statistically significant.

### Ethics

Ethical clearance was obtained from the Alfajr College Research Ethics Committee under reference number AFC-REC-2023-07-15, dated 13 July 2023. Electronic informed consent was obtained from all participants, who were informed of the study's purpose, assured of confidentiality, and free to withdraw at any stage. All data were anonymized and stored securely.

## Results

### Participant Characteristics

A total of 413 dentists completed the questionnaire. The sample was predominantly female (268, 64.9%), with a mean age of approximately 32.6 ± 6.9 years. Most held a Bachelor of Dental Surgery (BDS) qualification (246,

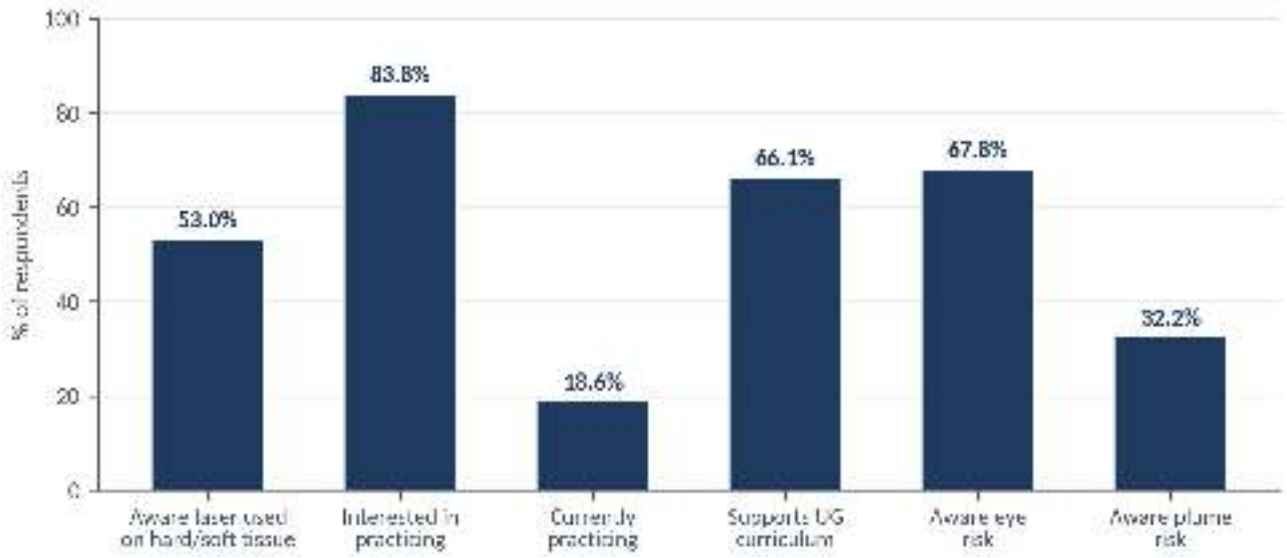
59.6%) and worked as general practitioners (167, 40.4%); 266 (64.4%) had fewer than five years' experience. Respondents were distributed across government hospitals (154, 37.3%), private clinics (104, 25.2%), mixed settings (125, 30.3%) and academic institutions (30, 7.3%). Full characteristics are shown in Table 1, and the study's key knowledge, attitude and practice indicators are summarized in Figure 1.

**Table 1. Demographic and professional characteristics of the surveyed Sudanese dentists (N = 413).**

| Characteristic | Category | n (%) |
|---|---|---|
| Gender | Female | 268 (64.9%) |
| | Male | 145 (35.1%) |
| Qualification | BDS | 246 (59.6%) |
| | MSc (Master's) | 125 (30.3%) |
| | MD | 35 (8.5%) |
| | PhD | 7 (1.7%) |
| Professional status | General practitioner | 167 (40.4%) |
| | Dental intern | 135 (32.7%) |
| | Specialist | 77 (18.6%) |
| | Consultant | 34 (8.2%) |
| Experience | < 5 years | 266 (64.4%) |
| | 5–10 years | 78 (18.9%) |
| | > 10 years | 69 (16.7%) |
| Setting | Government hospital | 154 (37.3%) |
| | Private clinic | 104 (25.2%) |
| | Both | 125 (30.3%) |
| | Academic | 30 (7.3%) |

*Age (≥ 18 y): mean approximately 32.6 ± 6.9 years. Two biologically impossible age entries (3 and 4 years) were excluded from the age summary (see Strengths and Limitations).*

**Figure 1. Key knowledge, attitude and practice indicators among the surveyed Sudanese dentists (N = 413).**




*Values are the percentage of respondents endorsing each item: recognition that lasers treat hard and soft tissue, interest in practicing laser dentistry, current laser use, support for including laser education in the undergraduate curriculum, and awareness of ocular and laser-plume hazards.*

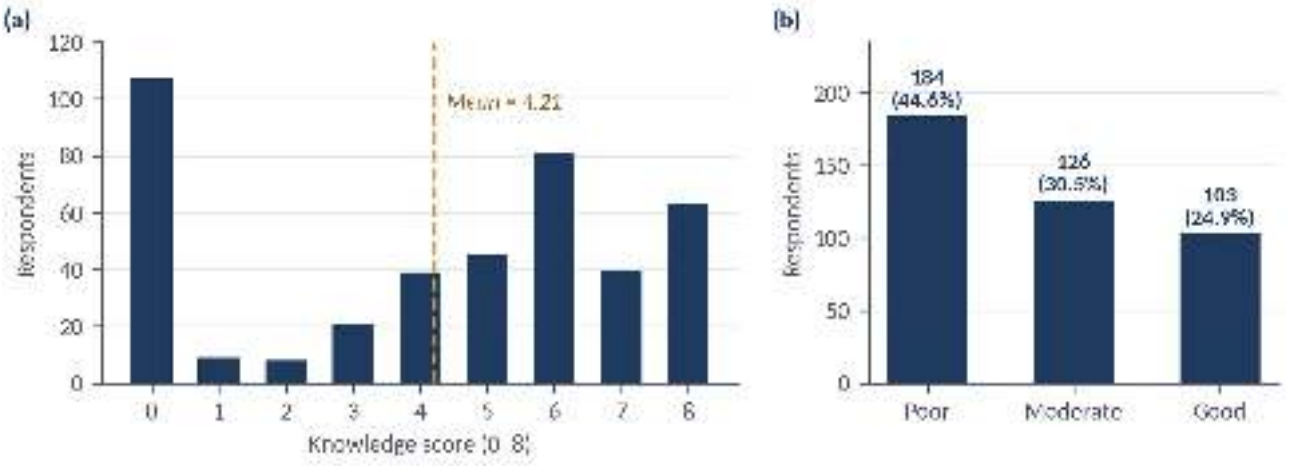
## Knowledge

Just over half of respondents (219, 53.0%) correctly identified that lasers can treat both hard and soft dental tissue, while 38.5% were uncertain. Perceived applicability varied by specialty (Table 2), being highest for periodontology (70.7%) and oral surgery (70.0%) and lowest for orthodontics (38.3%) and prosthodontics (40.7%). Fewer than half of respondents (42.1%) could identify the laser types used clinically (Table 2). The mean Knowledge score was 4.21 ± 2.94 of 8; by Bloom's classification, 44.6% were categorized as Poor, 30.5% as Moderate and 24.9% as Good (Figure 2).

**Table 2. Proportion of dentists who considered lasers applicable, by knowledge item and dental domain (N = 413).**

| Item | Yes (%) |
|---|---|
| Laser used on hard and soft tissue | 53.0 |
| Periodontology | 70.7 |
| Oral surgery | 70.0 |
| Endodontics | 59.6 |
| Pedodontics | 46.7 |
| Orthodontics | 38.3 |
| Prosthodontics | 40.7 |
| Can identify laser types | 42.1 |

Figure 2. **Distribution of the composite Knowledge score across the sample (N = 413).**




*Panel (a) shows the frequency of each score from 0 to 8, with the dashed line marking the sample mean (4.21); panel (b) shows respondents classified by Bloom's cut-offs into Poor (< 60%), Moderate (60–79%) and Good (≥ 80%) knowledge categories, with counts and percentages.*

### Attitudes

Attitudes were considerably more positive than actual practice. Most respondents agreed that laser treatment is associated with reduced pain, less bleeding, shorter treatment time with faster healing, and more conservative caries removal than conventional handpieces. The mean Attitude score was 16.67 ± 3.19 of 20, with the large majority (86%) classified as having a positive (Good) attitude.

### Practice, Interest and Barriers

Interest in practicing laser dentistry was high (346, 83.8%), yet only 77 (18.6%) were currently using lasers. Among respondents who named a device (n = 81), the diode laser predominated (57, 70.4%). The barriers item was a single-choice question answered by 353 respondents; the most frequently cited barrier was inadequate hands-on experience (245, 69.4%), followed by cost (90, 25.5%), patient acceptance (9, 2.5%) and difficulty convincing the patient (9, 2.5%). These four categories account for the full set of responses (100%). Two-thirds of respondents (273, 66.1%) supported incorporating laser education into the undergraduate curriculum, while 5.6% were opposed.

### Safety Awareness

Awareness of laser-associated hazards was inconsistent. Most respondents recognized risk to the eyes (280, 67.8%) and skin (263, 63.7%), and just over half recognized thermal damage to adjacent tooth structure (224, 54.2%). Awareness of laser-plume inhalation risk was markedly lower (133, 32.2%), with 53.8% uncertain. Only 144 (34.9%) reported ever having received formal education on a laser safety protocol. The mean Safety-awareness score was 2.18 ± 1.62 out of 4.

### Reliability

Internal consistency was good to excellent: Cronbach's alpha was 0.89 for the Knowledge scale, 0.88 for the Attitude scale and 0.87 for the Safety-awareness scale (0.92 for the combined knowledge-plus-safety items). No item, if removed, materially improved the reliability of its scale.

### Factors Associated with Laser Use

In bivariate analysis, current laser use was strongly associated with prior safety training, practice setting, and knowledge and safety-awareness categories (all $p < 0.001$), and with interest in laser dentistry ($p < 0.001$), but not with gender ($p = 0.89$) or qualification ($p = 0.68$) (Table 3). Knowledge scores differed by qualification, experience and setting (Kruskal–Wallis, all $p < 0.001$); a difference by gender was also observed, but a subset of early-career respondents provided highly homogeneous response patterns (see Strengths and Limitations), so this particular difference should be interpreted with caution. In multivariable logistic regression (Table 4, Figure 3), prior safety training (aOR 19.9), a higher knowledge score (aOR 1.55 per point) and private-sector practice (aOR 2.88 vs government) were independently associated with laser use; gender, qualification and interest were not. The model was well calibrated (Hosmer–Lemeshow $p = 0.29$; McFadden pseudo-$R^2$ = 0.51).

**Table 3. Bivariate associations between participant characteristics and current laser use.**

| Predictor | Test | Chi-square | df | p value |
|---|---|---|---|---|
| Prior safety training | Pearson chi-square | 143.43 | 2 | < 0.001 |
| Practice setting | Pearson chi-square | 44.86 | 3 | < 0.001 |
| Knowledge category | Pearson chi-square | 103.40 | 2 | < 0.001 |
| Safety-awareness category | Pearson chi-square | 47.66 | 2 | < 0.001 |
| Interest in laser | Chi-square (corrected) | 11.73 | 1 | < 0.001 |
| Gender | Chi-square (corrected) | 0.02 | 1 | 0.89 (ns) |
| Qualification | Pearson chi-square | 1.52 | 3 | 0.68 (ns) |

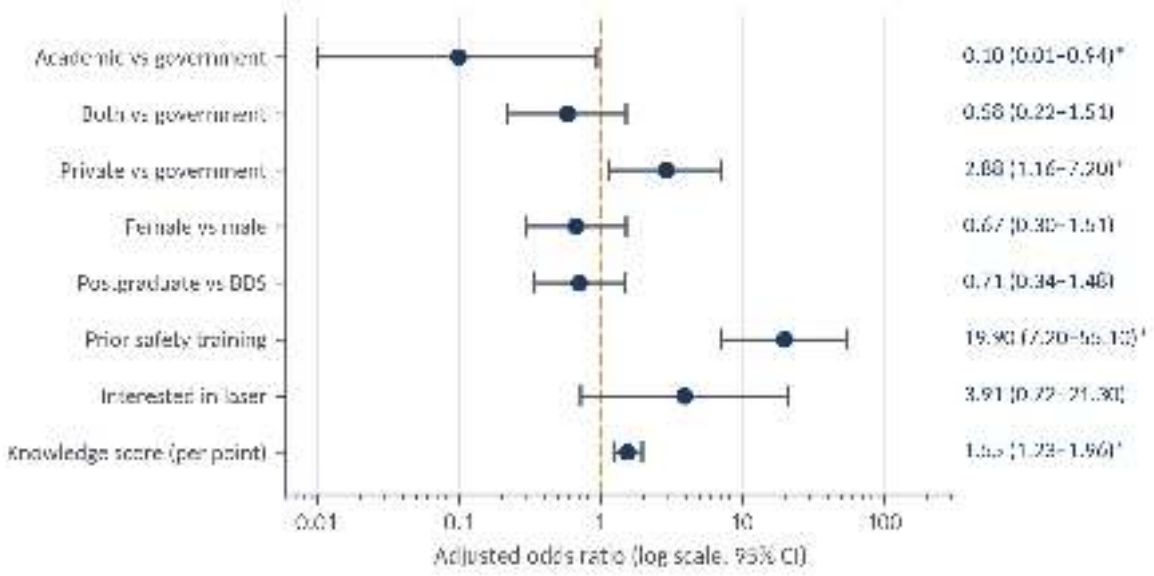
*Outcome: current laser use (yes/no). df, degrees of freedom; ns, not significant. Pearson chi-square statistics are reported for tables with more than two rows; for the 2 × 2 tables (interest, gender) the continuity-corrected (Yates) chi-square is reported. No table had more than 20% of cells with an expected count below 5, so Fisher's exact test was not required; where computed, Fisher's exact p values were consistent (interest p < 0.001; gender p = 0.89; qualification p = 0.68). All expected counts were adequate except a single small subgroup for qualification (PhD, n = 7), for which the result should be read with that caveat.*

**Table 4. Multivariable logistic regression identifying factors independently associated with current laser use (N = 413).**

| Predictor | aOR | 95% CI | p value |
|---|---|---|---|
| Prior safety training (yes vs no) | 19.9 | 7.2–55.1 | < 0.001 |
| Knowledge score (per +1 point) | 1.55 | 1.23–1.96 | < 0.001 |
| Private vs government setting | 2.88 | 1.16–7.20 | 0.023 |
| Both vs government setting | 0.58 | 0.22–1.51 | 0.26 (ns) |
| Academic vs government setting | 0.10 | 0.01–0.94 | 0.044 |
| Interested in laser (yes vs no) | 3.91 | 0.72–21.3 | 0.11 (ns) |
| Female vs male | 0.67 | 0.30–1.51 | 0.33 (ns) |
| Postgraduate vs BDS | 0.71 | 0.34–1.48 | 0.36 (ns) |

*aOR, adjusted odds ratio; CI, confidence interval; ns, not significant. Reference categories: government setting, male, and BDS qualification. All predictors were entered simultaneously (N = 413). Model fit: McFadden pseudo-$R^2$ = 0.51; Hosmer–Lemeshow chi-square = 8.53, df = 7, p = 0.29. The academic-setting estimate rests on a small cell (n = 30) and has a wide confidence interval; it should be interpreted with caution as an unstable estimate.*

**Figure 3. Forest plot of adjusted odds ratios (with 95% confidence intervals) from the multivariable logistic regression model of factors independently associated with current laser use (N = 413).**




*Reference categories: government setting, male, BDS. The dashed reference line is set at an odds ratio of 1; an asterisk denotes p < 0.05. Values correspond to Table 4.*

## Discussion

This study characterizes Sudanese dentists' knowledge, attitudes, safety awareness and practice regarding laser dentistry, and its central finding mirrors a pattern reported repeatedly elsewhere: enthusiasm for laser technology considerably outpaces its clinical use. In our sample, 346 (83.8%) expressed interest yet only 77 (18.6%) actually practiced, a gap comparable to that reported from Al-Kharj, Saudi Arabia, and from Coimbatore, Tanjore and across Pakistan.[12,16,17,20] Taken together, this suggests that the awareness–practice gap

is a structural feature of dental systems with limited postgraduate laser-training infrastructure rather than a country-specific phenomenon.

The reasons Sudanese dentists gave for not practicing, principally inadequate hands-on experience and then cost, closely parallel barriers reported in comparable settings.[12,19,20] Notably, our multivariable analysis reframes this descriptive picture: independent of other factors, it was prior safety training and higher knowledge, not demographic characteristics, that were associated with laser use. This is consistent with a training-limited rather than an attitude-limited adoption pathway and strengthens the case that equipment subsidy alone is unlikely to close the gap. This association should, however, be interpreted cautiously: the cross-sectional design cannot establish direction. It is at least as plausible that dentists who already use lasers subsequently obtain safety training as the reverse, so the relationship is likely bidirectional, and the estimates should be read as associations rather than causal effects. Cost may also remain an important barrier at the system level (for example, in equipment acquisition and maintenance) even though it did not emerge as an independent individual-level predictor of use.

Awareness of safety risks was uneven, and this may be the most clinically consequential finding. While most respondents recognized ocular and cutaneous hazards, only 133 (32.2%) identified laser-plume inhalation as a risk. Laser-generated plume can contain fine and ultrafine particulate matter and biological material, including cellular material and microorganisms, and occupational exposure is therefore an established safety concern requiring appropriate control measures.[27,28] The low recognition of plume risk in this sample may therefore represent an important occupational-health knowledge gap. It warrants targeted educational attention, including explicit instruction on plume evacuation and local exhaust ventilation and appropriate respiratory and eye protection within laser-safety and infection-control training.[27,28]

Two-thirds of respondents supported incorporating laser education into the undergraduate curriculum, consistent with recommendations emerging from the Saudi, Indian and Pakistani literature.[13,20,21] This convergence across otherwise different education systems, healthcare structures and training infrastructures strengthens the case that curriculum reform, rather than isolated continuing-education courses, may be the more durable long-term intervention. For Sudan and comparable low-resource settings, the practical implication is a phased strategy: introduce didactic laser content and simulation in the pre-clinical years; add supervised hands-on modules (soft-tissue diode applications first, given their lower cost and wide applicability) in the clinical years; prioritize accessible, low-cost postgraduate short courses; and make plume-safety instruction explicit throughout. Diode systems are already in documented clinical use in Sudan, including for the management of dentine hypersensitivity,[29] which makes them a practical entry point for such training. In the interim, shared-equipment arrangements between institutions and structured referral pathways could bridge the equipment-access gap. Finally, the low perceived applicability of lasers in orthodontics and prosthodontics may reflect genuinely narrower indications in those disciplines as much as unfamiliarity with emerging evidence, and disentangling the two would be a useful focus for future work.

## *Strengths and Limitations*

**Strengths.** This is the first characterization of laser-dentistry knowledge, attitudes and practice among Sudanese dentists; the sample is reasonably large (N = 413) and spans career stages and practice settings; the composite scales showed good-to-excellent internal consistency; and the analysis extends beyond description to identify factors independently associated with laser use.

**Limitations.** First, the self-administered online design yields a self-selected sample, and because the response rate and denominator are not established, selection and non-response bias cannot be quantified; findings may over-represent dentists interested in laser technology. Second, all measures were self-reported and subject to social-desirability and recall bias, with potential information (misclassification) bias in items relying on respondent recall. Third, the cross-sectional design precludes causal inference, particularly for the training–use association, and residual confounding by unmeasured factors cannot be excluded. Fourth, data-quality checks identified two biologically impossible age entries (3 and 4 years), which were excluded; while isolated, these indicate that some self-reported fields require cautious interpretation. Fifth, a subset of early-career respondents provided highly homogeneous ('don't know' or 'neutral') response patterns; although these appear to be genuine independent submissions,

such straight-lining can attenuate or exaggerate group differences, and the observed gender difference in knowledge should therefore be read with caution. Finally, although the instrument underwent expert review and piloting, more extensive psychometric validation would strengthen future work; the findings pertain to dentists reachable through online professional networks and may not generalize to all dentists practicing in Sudan.

### Conclusion

Sudanese dentists displayed generally positive attitudes toward laser dentistry and recognized many of its clinical advantages, but this enthusiasm was not matched by practical experience, specialty-specific knowledge, or comprehensive safety awareness, particularly regarding laser-plume inhalation. Independent of demographic factors, prior safety training and a higher knowledge score were the strongest correlates of laser use. This pattern parallels findings from Saudi Arabia, India and Pakistan, suggesting a shared structural gap between attitude and practice. Closing it will likely require coordinated curricular reform, accessible hands-on postgraduate training, and explicit plume-safety education, rather than equipment subsidy alone.

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### Conflict of Interest

All authors declare that they have no competing interests.

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### Availability of Data

The dataset analyzed is available from the corresponding author on reasonable request.

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ORIGINAL ARTICLE

# Phytochemical Screening and Antibacterial Activity of Yemeni *Moringa oleifera* Leaves Extract

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## Abstract

STRUCTURED SUMMARY · 4 SECTIONS

**BACKGROUND**

*Moringa oleifera* is a valuable medicinal plant widely cultivated in tropical and subtropical regions. Its leaves are abundant in vitamins, minerals, and essential phytochemicals with documented pharmacological activities. Previous studies have reported diverse therapeutic properties, including antibacterial, anti-diabetic, anti-inflammatory, antioxidant, cardiovascular, anti-ulcer, anti-allergic, and wound-healing properties. This study specifically focuses on evaluating the phytochemical composition and antibacterial potential of the methanolic extract of *Moringa oleifera* leaves cultivated in the Fuwah area of Al-Mukalla, Yemen, addressing a regional knowledge gap.

**METHODS**

Leaves were collected from Al-Mukalla, Yemen, authenticated, dried, and extracted by methanol using cold maceration. Phytochemical screening was conducted following standard protocols. *In vitro* antibacterial activity was assessed using the agar well diffusion method against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella* spp., *Proteus* spp., *Escherichia coli*) bacterial strains.

**RESULTS**

Phytochemical analysis confirmed the presence of saponins, tannins, alkaloids, flavonoids, and phenols. The methanolic extract exhibited clear concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones of 13 mm, 17 mm, and 22 mm at 125, 250, and 500 mg/mL, respectively. No inhibitory effect was observed against any of the tested Gram-negative bacteria.

**CONCLUSION**

The methanolic extract of Yemeni *Moringa oleifera* leaves contains diverse bioactive compounds and demonstrates potent antibacterial activity against Gram-positive *S. aureus* with no clear effect on the tested Gram-negative strains.

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KEYWORDS
Antibacterial activity · *Moringa oleifera* · Phytochemical screening · Gram-positive bacteria · Gram-negative bacteria

## Introduction

Medicinal plants have historically served and continue to serve as primary sources of drugs used in traditional, community, and modern medicines.[1] *Moringa oleifera*, a significant health-promoting plant within the *Moringaceae* family, is widely cultivated globally and has been recognized for its extensive nutritional and commercial uses.[2,3] Traditionally, *Moringa oleifera* leaves have been used to address various health issues, including asthma, diabetes, hypertension, diarrhea, fever, cough, spasms, hepatomegaly and splenomegaly, infections, ulcers, inflammation, and wound healing.[4,5]

A study on *Moringa oleifera* leaves, cultivated in Eastern Libya in 2021, which also employed methanol extraction, found significant antibacterial activity, markedly against Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and marked effects against Gram-negative bacteria (*Escherichia coli*).[2] The phytochemical screening in that study detected carbohydrates, alkaloids, and phenols. Similarly, fresh *Moringa oleifera* leaves were examined using methanol extraction and demonstrated inhibitory activity against Gram-positive bacteria and marked activity against Gram-negative bacteria (*Escherichia coli*), with phytochemical screening identifying carbohydrates, alkaloids, and phenols.[1] These studies provide a baseline for comparison with our methanolic extract from Yemeni origin.

Furthermore, a study on extraction and comparative evaluation conducted in Yemen investigated the phytochemical properties of *Moringa oleifera* seed oil and identified dominant fatty acids, notably lauric acid, myristic acid, and oleic acid.[6] However, investigations specifically addressing the antibacterial properties of Yemeni *Moringa oleifera* leaves remain limited.

Antimicrobial resistance is a major, expanding worldwide issue that requires urgent attention. There are rising rates of multidrug-resistant infections globally, and specifically in Yemen where healthcare infrastructure faces significant challenges.[2-7] Fighting dangerous bacteria, particularly drug-resistant pathogens, necessitates the development of novel, potent antibacterial therapies, including botanical medicines.[2]

According to searches in Google Scholar and PubMed databases, few previous studies have investigated the phytochemical screening and antibacterial activity of *Moringa oleifera* leaves in Yemen. Therefore, the current study aims to identify the phytochemical composition of the methanolic extract of *Moringa oleifera* leaves obtained by maceration and to assess its antibacterial activity using the agar well diffusion method, in accordance with previously published research.[2–6]

## Materials and Methods

This is an experimental laboratory-based study that assessed the phytochemical composition of the methanolic extract of *Moringa oleifera* leaves obtained by maceration and collected from Al-Mukalla, Yemen.

### Plant Material

Fresh leaves of *Moringa oleifera* were collected from the Fuwah area in Al-Mukalla, Yemen (Figure 1). Identification and authentication were performed by a senior botanist at the Pharmacy Department, College of Medicine and Health Sciences, Hadhramout University. The leaves were then washed, air-dried at room temperature, ground into a fine powder using a mortar and pestle, and stored in 100 g aliquots.

**Figure 1. Fresh *Moringa oleifera* leaves at the collection site, Fuwah area, Al-Mukalla, Yemen.**

## Chemicals, Reagents, Equipment, and Instruments

### Equipment and Instruments

The equipment and instruments used in this study included an autoclave, water bath, Buchner funnel, sensitive balance, incubator, Petri dishes, filter papers, micro-tips, beakers, conical flasks, flame, test tubes, measuring cylinder, micropipette, round-bottom flask, wire loop, containers, gloves, sterile cotton swab, mortar, and pestle.

### Chemicals and Reagents

The chemicals and reagents used in this study included methanol, distilled water, sodium hydroxide (NaOH), ferric chloride ($FeCl_3$), Dragendorff's reagent, and Mueller–Hinton agar (MHA).

## Microorganisms

Clinical bacterial isolates were obtained from the maintained microorganism collection at Ibn Sina General Hospital, Al-Mukalla, Hadhramout, Yemen. The clinical bacterial isolates included the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.

The isolates were subcultured on Mueller–Hinton agar and maintained at 4 °C until use. The identities of the isolate strains were confirmed using standard biochemical tests. Before antibacterial testing, each strain was standardized to 0.5 McFarland turbidity (≈ $1.5 \times 10^8$ CFU/mL) to ensure reproducible inoculum density.

## Processing and Extraction of Plant Material

Around 100 g of the powdered samples were cold-macerated in 1 L of analytical-grade methanol for five days.[8] The resulting extract was dried and stored in sterile, airtight containers at 4 °C until further analysis. Extraction yield was calculated as the percentage of dried extract relative to the initial dry weight.

## Phytochemical Screening

Qualitative phytochemical screening was performed according to standard protocols to detect the presence of major classes of phytoconstituents such as saponins, tannins, alkaloids, flavonoids, and phenolic compounds in the *Moringa oleifera* leaf extract.[9]

### Test for Saponins (Froth Test)

One gram of the extract was weighed into a conical flask, 10 mL of distilled water was added, and the mixture was boiled for five minutes and then filtered. Subsequently, 2.5 mL of the filtrate was transferred to a test tube containing 10 mL of distilled water. The mixture was shaken vigorously for 30 seconds and allowed to stand for 30 minutes. The formation of stable honeycomb froth (≥ 1 cm height) indicated the presence of saponins.[9]

### Test for Tannins (Ferric Chloride Test)

Three to four drops of 10% ferric chloride solution were added to a portion of the extract diluted with distilled water. The appearance of a green color indicated the presence of tannins.

### Test for Alkaloids (Dragendorff's Test)

One milliliter of Dragendorff's reagent was added to 1 mL of extract in a test tube. The formation of a cloudy orange or reddish-brown precipitate indicated the presence of alkaloids.[10]

### Test for Flavonoids (Alkaline Reagent Test)

One milliliter of extract was added to 1 mL of NaOH solution and gently shaken. The appearance of yellow or red coloration indicated the presence of flavonoids.[10]

### Test for Phenols (Ferric Chloride Test)

One milliliter of distilled water was mixed with 2 mL of extract and warmed to 45–50 °C. Then, 2 mL of 3% ferric chloride solution ($FeCl_3$) was added. The development of dark green or bluish-black coloration indicated the presence of phenolic compounds.[11] All tests were performed in triplicate to ensure consistency of observations.

## In Vitro Antibacterial Activity Assay

The antibacterial activity of the methanolic leaf extract of *Moringa oleifera* was evaluated using the agar well diffusion method following CLSI-adapted procedures.[1,12] The bacterial strains isolated were sub-cultured on Mueller–Hinton agar (MHA) and incubated at 37 °C for 18–24 hours to obtain fresh cultures.

### Agar Well Diffusion Method

Mueller–Hinton agar (MHA) was prepared by dissolving 38 g of agar powder in 1 L of distilled water. The mixture was heated with continuous stirring over a flame until completely dissolved. It was then autoclaved at 121 °C for 15 minutes, followed by a 15-minute warming period inside the autoclave. The medium was allowed to cool for several minutes, poured into sterile Petri dishes, and left to dry.

### Inoculation and Preparation

A sterile cotton swab was used to inoculate the bacterial suspension onto the surface of the agar. Using a sterile test tube, four 10 mm diameter cups were cut into each

plate, and the agar discs were removed. Using an automatic microliter pipette, two alternate cups were filled with 0.2 mL of the extract sample at different concentrations (500, 250, and 125 mg/mL). The remaining two cups received the respective solvent as a negative control and the standard antibiotic ceftriaxone 400 µg/mL as a positive control.

### Incubation and Measurement

The plates were allowed to stand at room temperature for two hours to facilitate diffusion. They were then incubated in an upright position at 37 °C for 18–24 hours. Two replicates were carried out for every extract concentration against each test organism. Following incubation, the diameters of growth inhibition zones were measured in millimeters using a digital caliper, averaged, and recorded as mean values.[1,12]

### Data Analysis

Antibacterial activity was interpreted based on inhibition zone diameters. Extracts producing inhibition zones ≥ 10 mm were considered active. Results were summarized descriptively due to the qualitative nature of phytochemical screening and the limited number of replicates.

## Results

### Extraction Yield

Fresh leaves of *Moringa oleifera* equivalent to 100 g dry weight yielded 13.15 g of crude methanolic extract, corresponding to an extraction yield of 13.15%. The extract was dark green, viscous, and readily soluble in methanol.

### Phytochemical Screening

Qualitative phytochemical analysis of the methanolic extract revealed the presence of all five tested phytochemical groups. Table 1 summarizes the results, with relative intensities indicated according to the strength of the observed reactions. As shown in Table 1, the methanolic extract of Yemeni *Moringa oleifera* leaves contains saponins, tannins, alkaloids, flavonoids, and phenolic compounds, with tannins, alkaloids, and flavonoids showing strong positive reactions. These findings indicate a diverse phytochemical profile with multiple classes of bioactive compounds.

**Table 1. Qualitative phytochemical composition of the methanolic extract of *Moringa oleifera* leaves.**

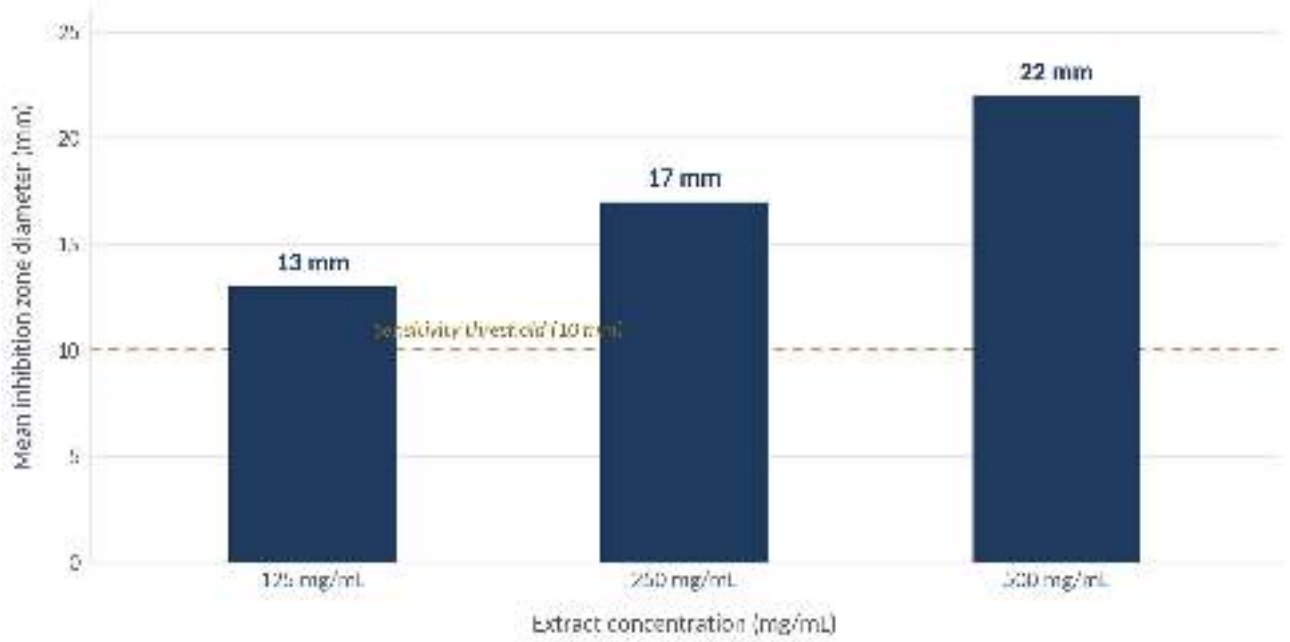
| Phytochemical class | Test performed | Intensity* |
|---|---|---|
| Saponins | Froth test | + |
| Tannins | Ferric chloride test | ++ |
| Alkaloids | Dragendorff's test | ++ |
| Flavonoids | Alkaline reagent test | ++ |
| Phenolic compounds | Ferric chloride test | + |

** Intensity was based on the observed strength of each reaction: + = weak to moderate presence; ++ = strong presence.*

### Antibacterial Activity

The antibacterial activity of the methanolic extract of *Moringa oleifera* leaves at concentrations of 125, 250, and 500 mg/mL was evaluated against the Gram-positive bacterium *Staphylococcus aureus* and three Gram-negative bacterial strains: *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. Methanol was used as a negative control, while ceftriaxone (400 µg/mL) was used as the positive control. Results are expressed as mean inhibition zone diameters (mm) obtained from duplicate experiments. The results demonstrated that the methanolic extract exhibited clear, concentration-dependent antibacterial activity exclusively against *Staphylococcus aureus*. The inhibition zone diameters increased progressively with increasing extract concentration (Figure 2). All tested concentrations (125–500 mg/mL) produced inhibition zones exceeding the 10 mm sensitivity threshold, indicating susceptibility. Mean inhibition zone diameters increased progressively with extract concentration: **125 mg/mL:** 13 mm, **250 mg/mL:** 17 mm, and **500 mg/mL:** 22 mm. This pattern demonstrates a consistent dose-response relationship.

Figure 2. **Mean inhibition zone diameters of *Moringa oleifera* methanolic leaf extract against *Staphylococcus aureus* (125–500 mg/mL).**




*Values are means of duplicate experiments. The dashed line marks the 10 mm sensitivity threshold applied in this study.*

In contrast, no inhibitory effect was observed against any of the Gram-negative bacterial strains tested (*E. coli*, *Klebsiella* spp., *Proteus* spp.), even at the highest concentration of 500 mg/mL. The positive control ceftriaxone produced expected inhibition zones against all strains. The negative control, methanol, showed no inhibition, confirming that the observed antibacterial activity was attributable to the extract components rather than to the solvent.

## Discussion

This study investigated the phytochemical composition and antibacterial activity of the methanolic extract from *Moringa oleifera* leaves cultivated in Fuwah, Al-Mukalla, Yemen. The methanolic extract had a sticky, dark-green appearance and an extraction yield of 13.15%. The extraction yield obtained in this study is comparable to the 14.68% yield reported in a recent Nepali study (2023) using a similar methodology.[13]

### *Phytochemical Composition*

Phytochemical screening confirmed the presence of saponins, tannins, alkaloids, flavonoids, and phenolic compounds in the methanolic extract. These findings are consistent with previous studies, although some variations in the detected phytochemical constituents have been reported across different geographical regions.[1,2,13]

The Libyan study published in 2021 using methanol extraction revealed the presence of alkaloids, phenols, and flavonoids but did not detect tannins or saponins.[2] Similarly, a study published in 2020 identified alkaloids, tannins, and phenols but did not report the presence of flavonoids.[1] In contrast, the more recent Nepali study (2023) and the present study both detected all five phytochemical classes. These findings suggest that extraction methodology, plant maturity, or geographical factors may influence the phytochemical profiles of *Moringa oleifera*.[13] The consistency between the present findings and those of the 2023 Nepali study reinforces the reproducibility of phytochemical profiles and suggests that *Moringa oleifera* from different geographical regions may share several core phytochemical characteristics.

The presence of these phytochemical compounds is noteworthy due to their well-documented therapeutic properties. Saponins exhibit antimicrobial and immunomodulatory activities, primarily through membrane permeabilization.[14] Tannins exhibit astringent and antimicrobial effects through multiple mechanisms, including protein precipitation and enzyme inhibition.[15] Alkaloids display a range of pharmacological activities, including the ability to intercalate with bacterial DNA.[16,17] Flavonoids function as potent antioxidants and possess anti-inflammatory and antimicrobial properties through mechanisms such as membrane disruption and enzyme inhibition.[18,19]

Phenols act as effective antimicrobial agents by disrupting bacterial cell membranes and denaturing proteins.[20] The identification of these phytochemical constituents in Yemeni *Moringa oleifera* leaves supports the potential biological activity of the plant and provides a basis for further investigation of its possible pharmaceutical applications.

## Antibacterial Activity

The evaluation of antibacterial activity revealed that the methanolic extract of Yemeni *Moringa oleifera* leaves exhibited strong, concentration-dependent antibacterial activity against *Staphylococcus aureus*, with inhibition zones ranging from 13 to 22 mm. Notably, even the lowest concentration tested (125 mg/mL) produced an inhibition zone exceeding the 10 mm sensitivity criterion used in this study. This activity can be compared with findings from previous studies, as summarized in Table 2.

Table 2. **Comparative antibacterial activity of *Moringa oleifera* extracts against *S. aureus*.**

| Study | Extract type | Concentration (mg/mL) | Mean inhibition zone (mm) |
|---|---|---|---|
| Current study | Methanol | 125 | 13 |
| | | 250 | 17 |
| | | 500 | 22 |
| Gupta et al. 2023[13] | Ethanol | 200 | 9 |
| | | 400 | 15 |
| | | 600 | 18 |
| | | 800 | 22 |
| Bagheri et al. 2020[1] | Methanol | 50 | 9 |

Under the conditions tested, the methanolic extract in the present study produced greater inhibition than that reported in the 2023 Nepali study using ethanolic extraction.[13] At 125 mg/mL, the present study produced a 13 mm inhibition zone, whereas the previous study reported only 9 mm at 200 mg/mL. At 250 mg/mL, the present extract produced a 17 mm inhibition zone compared with 15 mm at 400 mg/mL in the previous study. At 500 mg/mL, the present extract produced a 22 mm inhibition zone, which was greater than the 18 mm zone reported at 600 mg/mL and comparable to the 22 mm zone reported at 800 mg/mL.

The complete absence of antibacterial activity against the Gram-negative bacteria tested (*E. coli*, *Klebsiella* spp., *Proteus* spp.) is consistent with previous studies demonstrating that *Moringa oleifera* extracts exhibit greater antibacterial activity against Gram-positive bacteria.[1,2,13] This difference in susceptibility may be related, at least in part, to structural differences between the cell envelopes of Gram-positive and Gram-negative bacteria.

## Conclusion

The present study demonstrated that the methanolic extract of *Moringa oleifera* leaves cultivated in Yemen contains a wide range of active phytochemical compounds, including saponins, tannins, alkaloids, flavonoids, and phenolic compounds. These constituents may contribute to the plant's biological properties, particularly its antibacterial activity. The extract exhibited significant, concentration-dependent inhibitory effects against *Staphylococcus aureus*, with inhibition zones ranging from 13–22 mm at concentrations of 125–500 mg/mL. However, no activity was observed against the Gram-negative bacteria tested (*Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.). The observed difference in susceptibility may be attributed to structural differences in bacterial cell walls. This study contributes to the growing body of evidence supporting the antibacterial potential of *Moringa oleifera* and provides preliminary phytochemical and antibacterial characterization of *Moringa oleifera* leaves cultivated in Yemen. The findings address a regional knowledge gap and provide a foundation for further investigations into the chemical composition, mechanisms of action, and therapeutic potential of Yemeni *Moringa oleifera*.

## Limitations of the Study

A major limitation of the present study is that the phytochemical analysis was limited to qualitative screening of major classes of phytochemicals. While this approach provides preliminary evidence of the presence of potentially bioactive constituents in the *Moringa*

*oleifera* leaf extract, it does not allow for the identification or quantification of individual compounds or their concentrations.

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### *Conflict of Interest*

The authors declare that they have no conflicts of interest related to this study.

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### *Authors' Contributions*

**Mohammed Hassan** supervised the study and **Abubakr A. Hammad** co-supervised it. **Aseela Altaffi** assisted with the microbiological laboratory work. **Abdullah Khanbri** coordinated the student research group and prepared the abstract. **Ahmed Baothman** prepared the introduction. **Mohammad Al-Sadi**, **Qusai Barajeh** and **Saleh Mgower** prepared the materials and methods. **Fatima Al-Jaberi** and **Hania Al-Sebaii** prepared the processing and extraction section. **Abrar Al-Hoson** and **Nojoud Bin Makhashin** prepared the results. **Somia Bashan** and **Somia Bajry** prepared the discussion. **Mohammad Al-Maqdi**, **Taha Al-Habshi** and **Saleh Basleb** prepared the conclusion, data analysis and limitations. **Al-Anood Al-Shamasi** and **Salem Saeed** wrote, organized and formatted the manuscript. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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ORIGINAL ARTICLE

# Hematological and Micronutrient Profile among Sudanese Schoolchildren in Egypt and Sudan

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## Abstract

STRUCTURED SUMMARY · 4 SECTIONS

**BACKGROUND**

Iron and vitamin B12 deficiencies are among the common health problems of school-age children worldwide, affecting physical growth and academic performance. The Sudanese conflict has displaced large numbers of families to neighboring countries, with substantial changes in living conditions and diet. This study compared the hematological and micronutrient profiles of Sudanese schoolchildren in Egypt and Sudan.

**METHODS**

A cross-sectional analytical study was conducted between May and July 2025 in one school in Giza, Egypt, and one in Karrari, Khartoum. Of 224 targeted students, 120 were assessed (70 Egypt, 50 Sudan; response rate 53.6%). Complete blood count was performed on a Sysmex KX-21, thin films were examined microscopically, and serum iron and vitamin B12 were measured on an AutoLumo A1000. Groups were compared by independent-samples t-test and re-analyzed by ANCOVA with age and gender as covariates.

**RESULTS**

Unadjusted differences were significant for MCH ($p = 0.020$), RDW ($p = 0.003$), platelet count ($p < 0.001$), total WBC ($p = 0.045$), lymphocytes, monocytes, eosinophils and serum iron (all $p < 0.001$). Hemoglobin, MCV, MCHC, neutrophils and vitamin B12 did not differ. After adjustment for age and gender, all retained significance except MCH (adjusted $p = 0.080$) and total WBC (adjusted $p = 0.056$). Blood films showed no significant morphological changes in either group.

**CONCLUSION**

The two groups differed in several hematological and micronutrient parameters, most differences persisting after adjustment. Overt anemia was not prevalent in either group. The findings support regular hematological screening of displaced Sudanese children.

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Anemia · Iron · Vitamin B12 · Schoolchildren · Sudan

## Introduction

Micronutrient deficiency in the body is a common nutritional problem worldwide affecting people of different ages, genders, and backgrounds. When the body lacks enough hemoglobin, the biomolecule responsible for carrying oxygen to body tissues, oxygen-carrying capacity is reduced, leading to physical and mental symptoms that affect daily activities [1]. Red blood cells (RBCs) play a vital role in normal human body function, basically through their role in transporting oxygen and carbon dioxide [2]. Clinical symptoms vary depending on the severity and underlying cause. Common symptoms include fatigue, weakness, shortness of breath, dizziness, and pale skin. Because these symptoms can be subtle or mistaken for other conditions, anemia is sometimes known as a "silent" disorder [3]. Types of anemia include: vitamin deficiency, resulting from a deficiency in essential nutrients (e.g., vitamin B12 or folate), anemia of chronic disease, and hereditary forms like sickle cell and thalassemia [4].

Vitamin B12, or cobalamin, is a water-soluble vitamin that plays a vital role in hematopoiesis and maintaining healthy nerve cells [5]. Vitamin B12 is also necessary for overall energy levels, brain function, and disease prevention [5]. B12 deficiency may lead to megaloblastic anemia, characterized by abnormally large and dysfunctional erythrocytes [6,7]. B12 absorption requires intrinsic factor produced by stomach parietal cells. Certain medical conditions such as pernicious anemia or gastrointestinal abnormalities can interfere with B12 absorption and lead to deficiency [8].

Iron is an essential nutrient that plays a vital role in maintaining body health. It is a key component of hemoglobin responsible for oxygen transport. Without enough iron, the body cannot produce enough healthy RBCs, leading to iron deficiency anemia [9]. Iron is also involved in energy production, immune system function, hormone synthesis, and cognitive development [10]. There are two types of iron: heme iron (found in animal products) and non-heme iron (found in plant-based sources) [10]. Consuming non-heme iron with vitamin C-rich foods enhances its absorption [10]. Iron supports numerous physiological functions in the human body [9].

Globally, anemia in school-aged children remains a serious public health problem, affecting growth, development, and educational performance [11]. The military conflict in Sudan has resulted in the displacement of large populations to internal regions and neighboring countries such as Egypt [12,13]. Children are among the groups most affected by this displacement.[14] This study aimed to assess and compare the hematological and micronutrient profiles of Sudanese schoolchildren residing in Giza, Egypt, and Karrari, Sudan.

## Materials and Methods

### *Study Design and Study Area*

The study followed a cross-sectional analytical design conducted among Sudanese schoolchildren in two schools located in Egypt and Sudan, in the period between May and July 2025. The two research localities were selected because they were mostly populated by Sudanese families during the military conflict that erupted in Sudan in April 2023; one school in each country was selected. The school in Giza Governorate, Egypt, had attracted Sudanese families who chose to emigrate to Egypt and had their children enrolled in schools in that locality. Karrari Locality in Khartoum was selected to provide a comparable educational and health-screening baseline for children remaining in Sudan during the study period. The two research localities provided comparable educational and health-screening environments for Sudanese schoolchildren during the period of the study.

The study population was schoolchildren aged 8–16 years, enrolled in the selected schools, who were present during the data-collection period. Children with known chronic illnesses, acquired or inherited hematologic disorders, who received recent blood transfusion, or who had acute infections at the time of sampling were excluded from the study.

### *Sample Size*

The following equation was used to calculate the sample size:

$n = Z^2 \times PQ / e^2$

Where n indicates the minimum required sample size, Z is the desired confidence level taken as 1.96 for a 95% confidence level, P represents the expected prevalence of iron deficiency anemia among Sudanese schoolchildren taken as 17.8% based on previous study conducted in

River Nile State, Sudan, by Osman et al. [1], Q is the complement of P =1-P, and e is the margin of error taken as 0.05.

$n = 1.96^2 \times 0.178 \times 0.822 / 0.05^2 = 224.8$, rounded to 224.

The sample was divided equally between Egypt and Sudan.

### Quality Control and Pre-analytical Standardization

Both laboratory sites used identical analytical platforms: the Sysmex KX-21 automated hematology analyzer for CBC and the AutoLumo A1000 analyzer for serum iron and vitamin B12 testing. Standard calibration protocols and daily internal quality control (IQC) runs were executed per manufacturer guidelines [15,16]. Blood samples were collected during standard morning school hours, transported in cold boxes, and processed on the day of collection following standardized standard operating procedures [17].

### Sampling Technique

A simple random sampling approach was used in both countries. Each school administration provided an updated list of enrolled students within the eligible age range. Using this list as the sampling frame, names of the participants were selected through a lottery process to ensure unbiased representation.

### Data Collection Methods and Techniques

Data collection took place during scheduled school hours by trained research personnel, who obtained the data using a structured questionnaire through direct interview. The questionnaire included students' demographic data, including gender and educational level. These data were later incorporated with students' hematologic data.

### Sample Collection and Handling

Five milliliters of venous blood were collected from each participant; a tourniquet was applied to the upper arm, and a blood sample was collected under aseptic conditions following standard clinical procedures [17]; 2 mL were placed in EDTA tubes for hematological evaluation (complete blood count); the remaining volume was used to obtain serum for the evaluation of serum iron and vitamin B12. Samples were transported in temperature-controlled containers to accredited laboratories in each country for analysis.

### Laboratory Equipment and Analytical Methods

### Hematological Analysis

Complete blood count (CBC) was analyzed using the cell counter full automated hematology analyzer (Sysmex KX-21, Sysmex, Japan), an automated device used to measure hemoglobin levels, red cell indices (MCV, MCH, MCHC), and white cell count, among other blood indices that are calculated directly or indirectly.

### CBC Principle

The cell counter "Coulter principle" depends on detecting and measuring changes in electric resistance or impedance produced by a cell suspended in a diluent (an electrically conductive liquid) passing through a small aperture.

### Blood Morphology

A drop of venous EDTA anticoagulated blood was placed onto a clean glass slide, then spread to create a thin film. After air-drying, the film was fixed and stained using Giemsa stain. The rainbow area in the stained slides was then examined microscopically under oil immersion to evaluate blood cell morphology and identify cellular abnormalities.

### Serum Iron Assay

Serum Iron Assay: Venous blood was collected under aseptic conditions; serum was obtained by centrifugation at 3000 rpm for 5 minutes and separated into plain tubes. Serum iron concentration was measured spectrophotometrically using the AutoLumo A1000 automated analyzer (Autobio Diagnostics Co., Ltd., Zhengzhou, China; Cat. No. A29102, Package Insert Version 2.1) [15]. Briefly, 20 µL of serum was aspirated; iron bound to transferrin was released in a (Fe3+) to ferrous (Fe2+) state and reacted with a chromogenic substrate to form a colored complex. Absorbance was measured automatically over an analytical range of 10.0–1000.0 µg/dL [15].

Serum Vitamin B12 Assay: Serum vitamin B12 levels were quantitatively determined using a competitive chemiluminescence microparticle immunoassay (CLIA) on the AutoLumo A1000 analyzer (Autobio Diagnostics Co., Ltd., Zhengzhou, China; Cat. No. A29204, Package Insert Version 2.3) [16]. A 50 µL serum sample was incubated with magnetic microparticles coated with intrinsic factor and enzyme-labeled vitamin B12 conjugate. After competitive binding and automated washing, a chemiluminescent substrate was added, and emitted light was measured by a photomultiplier tube.

The assay had a calibration linearity range (measuring range) of 50.0–2000.0 pg/mL [16].

### Data Analysis

Continuous variables were summarized as mean ± standard deviation (SD) with 95% confidence intervals (95% CI), and categorical variables as frequencies and percentages. The distribution of continuous variables was assessed before analysis, and between-group comparisons were performed using the independent-samples t-test, with Welch's correction applied where variances were unequal. Effect size was evaluated using Cohen's d [18]. Because the two groups differed at baseline in age and gender, each outcome was re-analyzed by analysis of covariance (ANCOVA) with country of residence as the fixed factor and age and gender as covariates; adjusted mean differences, 95% CIs and p-values are reported. A two-sided p-value below 0.05 was considered statistically significant. All analyses were performed using SPSS version 26.

### Ethical Considerations

Primary ethical approval for this cross-sectional study was granted by the Research Ethics Committee at Al-Fajr College for Science and Technology, Khartoum, Sudan. Field data collection and venous blood sampling, were conducted under official administrative permission and institutional authorization from the participating schools' authorities. Participation was entirely voluntary. Written informed consent was obtained from the parents or legal guardians of all participating minors before blood collection, and verbal assent was obtained from participating children. All specimens were handled following standard clinical guidelines, and participant data were anonymized using unique identification codes. All study procedures adhered to the ethical principles outlined in the Declaration of Helsinki [19].

## Results

### Participant Characteristics

The study targeted 224 students, who were divided equally between Egypt and Sudan, with 112 students in each. However, due to conflict-related access limitations in Sudan and cost constraints in both sites, only 50 students residing in Sudan (Sudan group) could be assessed, giving a 44.6% response rate. While 70 students residing in Egypt (Egypt group), with a 62.5% response rate, were successfully assessed. The final achieved sample size was therefore 120 participants, giving a total response rate of 53.6%. The variables collected in the study included students' educational level, which was comparable between the two groups, with the intermediate level slightly higher in the Sudan group than in the Egypt group (68.0% and 64.3%, respectively). Regarding gender, the proportion of male students was higher in the Egypt group than in the Sudan group (57.1% and 40.0%, respectively).

### Red Cell Parameters

The mean hemoglobin level, MCV, and MCHC showed no statistically significant differences between the Egypt group and the Sudan group (12.13 ± 1.79 g/dL vs. 12.16 ± 0.99 g/dL, p = 0.906; 83.78 ± 9.54 fL vs. 84.38 ± 5.03 fL, p = 0.656; and 32.35 ± 1.35 g/dL vs. 32.64 ± 0.99 g/dL, p = 0.181, respectively). MCH and RDW showed a significant decrease in the Egypt group compared to the Sudan group in the unadjusted analysis (27.61 ± 3.48 pg vs. 28.68 ± 1.33 pg, p = 0.020; and 14.05 ± 1.86% vs. 14.88 ± 1.10%, p = 0.003, respectively) (Table 1). After adjustment for age and gender (ANCOVA), RDW maintained its statistical significance (adjusted p = 0.005), whereas MCH lost statistical significance (adjusted p = 0.080) (Table 2).

### Platelets and White Blood Cells

Platelet count (PLT) was significantly higher in the Egypt group compared to the Sudan group (289.26 ± 108.16 $\times 10^3/\mu L$ vs. 214.80 ± 49.10 $\times 10^3/\mu L$, p < 0.001; adjusted p < 0.001). Regarding the white blood cell profile, except for neutrophils (p = 0.151, adjusted p = 0.242), total and differential counts showed significant differences between the two groups. The Egypt group demonstrated significantly higher levels for TWBC (6.09 ± 2.05 $\times 10^3/\mu L$ vs. 5.42 ± 1.54 $\times 10^3/\mu L$, p = 0.045; adjusted p = 0.056), lymphocytes (40.17 ± 10.70% vs. 33.60 ± 7.76%, p < 0.001; adjusted p = 0.001), and monocytes (8.89 ± 5.59% vs. 4.80 ± 1.39%, p < 0.001; adjusted p < 0.001), while eosinophils were significantly lower in the Egypt group (1.50 ± 0.72% vs. 2.94 ± 1.38%, p < 0.001; adjusted p < 0.001) (Table 1 and Table 2).

### Blood Film Morphology

Peripheral blood film examination showed no significant morphological changes in either group.

**Table 1. Participant characteristics and hematological and micronutrient profile of the two groups (N = 120).**

| Parameter | Egypt Group (n = 70) Mean ± SD | Sudan Group (n = 50) Mean ± SD | Mean Difference (95% CI) | t | Cohen's d | p-value |
|---|---|---|---|---|---|---|
| **Demographics** | | | | | | |
| Age (years) | 12.21 ± 2.76 | 10.48 ± 3.41 | 1.73 (0.59 to 2.88) | 2.97 | 0.57 | 0.004* |
| **Complete Blood Count** | | | | | | |
| Hemoglobin (g/dL) | 12.13 ± 1.79 | 12.16 ± 0.99 | −0.03 (−0.53 to 0.47) | −0.12 | −0.02 | 0.906 |
| MCV (fL) | 83.78 ± 9.54 | 84.38 ± 5.03 | −0.60 (−3.23 to 2.03) | −0.45 | −0.08 | 0.656 |
| MCH (pg) | 27.61 ± 3.48 | 28.68 ± 1.33 | −1.08 (−1.97 to −0.18) | −2.36 | −0.39 | 0.020* |
| MCHC (g/dL) | 32.35 ± 1.35 | 32.64 ± 0.99 | −0.29 (−0.71 to 0.13) | −1.35 | −0.24 | 0.181 |
| RDW (%) | 14.05 ± 1.86 | 14.88 ± 1.10 | −0.84 (−1.37 to −0.30) | −3.08 | −0.53 | 0.003* |
| Platelet count ($\times 10^3/\mu L$) | 289.26 ± 108.16 | 214.80 ± 49.10 | 74.46 (45.69 to 103.22) | 5.07 | 0.84 | < 0.001* |
| Total WBC ($\times 10^3/\mu L$) | 6.09 ± 2.05 | 5.42 ± 1.54 | 0.66 (0.02 to 1.31) | 2.03 | 0.36 | 0.045* |
| **Differential White Cell Count (%)** | | | | | | |
| Neutrophils | 46.34 ± 11.40 | 44.01 ± 6.06 | 2.32 (−0.83 to 5.48) | 1.44 | 0.24 | 0.151 |
| Lymphocytes | 40.17 ± 10.70 | 33.60 ± 7.76 | 6.57 (3.27 to 9.88) | 3.90 | 0.69 | < 0.001* |
| Monocytes | 8.89 ± 5.59 | 4.80 ± 1.39 | 4.09 (2.72 to 5.45) | 5.86 | 0.93 | < 0.001* |
| Eosinophils | 1.50 ± 0.72 | 2.94 ± 1.38 | −1.44 (−1.86 to −1.02) | −6.77 | −1.38 | < 0.001* |
| **Micronutrients** | | | | | | |
| Serum iron (µg/dL) | 116.99 ± 70.53 | 77.94 ± 10.99 | 39.05 (22.13 to 55.97) | 4.52 | 0.72 | < 0.001* |
| Vitamin B12 (pg/mL) | 466.56 ± 273.35 | 469.80 ± 89.05 | −3.24 (−71.87 to 65.39) | −0.09 | −0.01 | 0.927 |

*SD, standard deviation; CI, confidence interval; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; WBC, white blood cell. Comparisons by independent-samples t-test with Welch's correction where variances were unequal. * p < 0.05.*

### *Micronutrient Profile*

Serum iron levels were significantly higher in the Egypt group compared to the Sudan group (116.99 ± 70.53 µg/dL vs. 77.94 ± 10.99 µg/dL, p < 0.001; adjusted p < 0.001). In contrast, serum vitamin B12 levels showed no statistically significant difference between the two groups (466.56 ± 273.35 pg/mL vs. 469.80 ± 89.05 pg/mL, p = 0.927; adjusted p = 0.685) (Table 1 and Table 2).

**Table 2. Unadjusted versus age- and gender-adjusted multivariable analysis (ANCOVA).**

| Outcome Parameter | Unadjusted Mean Difference (Egypt − Sudan) | Adjusted Effect (β) (Egypt vs Sudan) | Adjusted 95% CI | Adjusted p-value |
|---|---|---|---|---|
| Hemoglobin (g/dL) | −0.03 | −0.05 | −0.62 to 0.53 | 0.872 |
| MCV (fL) | −0.60 | −0.83 | −3.89 to 2.24 | 0.594 |
| MCH (pg) | −1.08 | −0.94 | −2.01 to 0.12 | 0.080 |
| MCHC (g/dL) | −0.29 | −0.18 | −0.64 to 0.28 | 0.436 |
| RDW (%) | −0.84 | −0.88 | −1.48 to −0.28 | 0.005* |
| Platelet count ($\times 10^3/\mu L$) | +74.46 | +70.80 | 36.98 to 104.61 | < 0.001* |
| Total WBC ($\times 10^3/\mu L$) | +0.66 | +0.68 | −0.02 to 1.38 | 0.056 |
| Neutrophils (%) | +2.32 | +2.12 | −1.45 to 5.70 | 0.242 |
| Lymphocytes (%) | +6.57 | +6.18 | 2.51 to 9.86 | 0.001* |
| Monocytes (%) | +4.09 | +4.60 | 3.03 to 6.18 | < 0.001* |
| Eosinophils (%) | −1.44 | −1.49 | −1.89 to −1.09 | < 0.001* |
| Serum iron (µg/dL) | +39.05 | +37.92 | 17.23 to 58.61 | < 0.001* |
| Vitamin B12 (pg/mL) | −3.24 | −17.01 | −99.86 to 65.85 | 0.685 |

*β, adjusted mean difference from analysis of covariance with country of residence as the fixed factor and age and gender as covariates; CI, confidence interval. Positive values indicate higher means in the Egypt group. * p < 0.05.*

## Discussion

The study was conducted in the duration between May and July 2025 on Sudanese primary and intermediate school children, 70 from Giza Governorate (Egypt group) and 50 from Karrari Locality (Sudan group) of the same age group, to assess their hematological and micronutrient parameters.

The present study demonstrated a statistically insignificant difference in hemoglobin levels between

the Egypt group and Sudan group (12.13 g/dl and 12.16 g/dl, P-value 0.906). This finding was consistent with Mohammed Saeed Elsamani Eltayeb et al (2016) [20], who reported low mean hemoglobin levels of 11.7g/dl among Sudanese children (mean age 12.3 years), and data also consistent with Tariq E. Elmissbah (2024) [21], who concluded a decreased mean hemoglobin of 12.0 among primary school students aged between 6–14 y in Khartoum, Sudan. These findings indicate that overt anemia was not prevalent among the studied population of displaced Sudanese children in Giza during the study period.

The study reported an insignificant decrease in MCV among the Egypt group (83.78 fL) compared to Sudan (84.38 fL), P-value (0.656). This finding was consistent with Jasmina Pluncevic Gligoroska et al (2019) [22], who reported a Mean Cell Volume (MCV) of 84.45 fL among children under 12 years of age. Data are also consistent with Shuchismita Behera et al (2016) [23], who reported an MCV of 84.3 fL among school-aged children (6-12y) in Odisha, India. The study reported a significant decrease in MCH in unadjusted analysis among Egypt group (27.61 pg) compared to Sudan (28.68 pg), P-value (0.020). However, after multivariable adjustment for age and gender (ANCOVA), this difference was no longer statistically significant (adjusted P-value 0.080). This finding was in line with Tariq E. Elmissbah (2024) [21] who reported low MCH of 27.0 pg among Sudanese primary school students, and with Jasmina Pluncevic Gligoroska et al (2019) [22] who reported MCH of 27.6 pg among children in Macedonia which represent the lower limit of the normal range, and data also consistent with I Achouri et al (2015) [24], who reported significant decreased MCH (26.6).

The study reported an insignificant decrease in MCHC among the Egypt group (32.35 g/dl) compared to Sudan (32.64 g/dl), P-value (0.181). This finding was consistent with Shuchismita Behera et al [23] who reported low MCHC (32.0) among children in Odisha, India, and Tariq E. Elmissbah (2024) [21] who concluded low mean MCHC of 32.5 among primary school students aged between 6–14 y in Khartoum, Sudan, and Jasmina Pluncevic Gligoroska et al (2019) [22] who reported low MCHC was 32.7 among children in Macedonia.

RDW showed a significant decrease among Egypt group (14.05%) compared to Sudan (14.88%), P-value (0.003), this finding was in line with Mabrouk et al (2022) [25], who reported a low RDW mean among school aged from 6–12 year in Beni- Suef, Egypt (mean 13.3). These results reinforce the use of RDW as an early hematological marker for detecting nutritional deficiencies before overt anemia develops, and the data also show that many studies have not investigated RDW compared to other RBC indices, especially among this population.

The study concluded a significant increase in platelet count (PLT) mean among the Egypt group (289.26 $\times 10^3/\mu L$) compared to Sudan (214.80 $\times 10^3/\mu L$), p-value (<0.001); this finding was in line with Oberoi Tania et al (2016) [26], who reported an increase in platelet count in 24.5% of children in Odisha, India. The data were also consistent with Amna Gouda Mabrouk et al (2022) [25], who reported an increase in platelet count among anemic children compared to the non-anemic children in Beni- Suef, Egypt (272,100 and 264,700), respectively.

The study reported a significant increase in TWBCs in the unadjusted analysis among the Egypt group ($6.09 \pm 2.05 \times 10^3/\mu L$) compared to Sudan ($5.42 \pm 1.54 \times 10^3/\mu L$), P-value (0.045). However, after adjustment for age and gender (ANCOVA), this difference retained a borderline trend toward significance (adjusted P-value 0.056). This finding was in line with Ahmed et al (2015) [27], who reported a significant difference in TWBC count.

The study reported a significant increase in lymphocyte count among the Egypt group (40.17%) compared to Sudan (33.60%), P-value (<0.001). This finding was in line with Butsabong Lerkvaleekul et al [28], who described a significant increase in lymphocytes (CD8 T-lymphocytes, CD19+ B-cells, and NK cells in children aged 5–10 years in school in Thailand.

The study reported a significant increase in Monocyte count among the Egypt group (8.89%) compared to Sudan (4.80%), P-value (<0.001).

Most of the observed differences persisted after multivariable adjustment for age and gender (Table 2), although MCH (adjusted p = 0.080) and TWBC (adjusted p = 0.056) no longer reached significance. Country of residence therefore appears to be independently associated with several, but not all, of the hematological variations observed.

The study reported a significant decrease in Eosinophil count among the Egypt group (1.50%) compared to Sudan (2.94%), P-value (<0.001). The study reported a significant increase in Serum iron among the Egypt group (116.99 µg/dL) compared to the Sudan group

(77.94 µg/dL), P-value (<0.001). However, both means were within the normal reference range, so this difference is unlikely to indicate iron deficiency in either group. Although fewer studies targeted serum iron levels compared to ferritin, iron depletion is a slow, multi-stage mechanism that depends on the rate of blood/iron loss; it takes months to years to completely deplete the body's iron stores. Serum iron, described as one of the last iron markers, drops during the progression, which may clarify the increased level in the Egypt group compared to Sudan, in addition to the wide range of normal serum iron among children (both groups were within the normal range). The study reported an insignificant decrease in serum B12 among the Egypt group (466.56 pg/mL) compared to Sudan (469.80 pg/mL), P-value (0.927). This finding was in line with Gil Prieto R et al. (2008) [29], who reported serum vitamin B12 percentiles in Spanish schoolchildren aged 13–15 years, with median values comparable to the means observed in both of our groups.

### Limitations

Assessing full minerals and vitamins could offer a comprehensive view of hematological status, including nutritional parameters; in the study, some investigations were not assessed, such as folate, vitamin C, and ferritin, due to limited resources.

Furthermore, a key limitation of this study is the multi-center analytical design, as specimens were analyzed in separate laboratories in Sudan and Egypt. Although identical instrument models (Sysmex KX-21 and AutoLumo A1000) and standardized operational protocols were utilized, inter-laboratory variations such as subtle differences in reagent lot numbers, calibration frequency, storage temperature, precise time-to-analysis, and internal/external quality assurance parameters could not be fully controlled. Additionally, pre-analytical variables including the exact time of blood sampling within that window and participant fasting status were not systematically standardized, which represents a potential source of variance, as serum iron is subject to appreciable within-individual biological variation and is influenced by recent dietary intake and time of collection [30].

The two groups also differed significantly in age (12.21 ± 2.76 vs 10.48 ± 3.41 years, p = 0.004) and in gender distribution. Although both were adjusted for by ANCOVA, residual confounding cannot be excluded. Recruitment was limited to a single school in each country and the achieved response rate was 53.6%, so the findings may not be generalizable to all displaced Sudanese schoolchildren. Dietary, socioeconomic and parasitological data were not collected, and ferritin, transferrin saturation and C-reactive protein were not measured, so iron status could not be fully characterized. Finally, the cross-sectional design permits the description of associations but does not allow causal inference.

### Conclusion

The study showed hematological and micronutrient differences between the two groups, particularly a significant decrease in MCH, RDW, and eosinophils, but showed significant increased Platelets, TWBCs, Lymphocytes, monocytes, and serum iron. No significant morphological changes were observed, and vitamin B12, hemoglobin, MCV and MCHC remained comparable between the two groups.

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### Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### Use of Artificial Intelligence

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, version GPT-4o) between June 2026 and August 2026 solely for language editing, improving readability, and English grammar refinement. The tool was not used to collect, analyze, or interpret raw biological data, nor to generate scientific conclusions. Following the use of this service, the authors reviewed, edited, and validated all content and take full responsibility for the integrity and accuracy of the published work.

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### *Availability of Data*

The data supporting the findings of the present study are accessible through the corresponding author for research and humanitarian purposes.

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