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INVITED REVIEW

Gaps in Glaucoma Diagnosis and Management in Low-Resource Settings: Evidence, Challenges, and Practical Solutions for West Africa

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Abstract

Glaucoma remains a leading cause of irreversible blindness in West Africa, where prevalence among adults aged ≥40 years far exceed global norms. Despite the availability of effective medical and surgical interventions, late presentation and advanced disease at diagnosis persist, driven by systemic, socioeconomic, and cultural barriers. This review synthesizes evidence on epidemiological burden, risk factors, diagnostic capacity, and care pathway challenges in low-resource settings. Key findings include aggressive disease phenotypes, thin corneal profiles, and high familial risk, compounded by deficits in diagnostic infrastructure, particularly gonioscopy, and workforce shortages. Barriers span awareness, access, adherence, and follow-up, with cost and health literacy as dominant constraints. Practical solutions emphasize community-based screening, task-shifting to optometrists, integration of glaucoma care into primary health systems, cost-containment strategies for medications, and telemedicine for remote monitoring. Implementation science approaches and policy reforms, including insurance coverage and workforce upskilling, are critical to reducing glaucoma-related blindness. Addressing these gaps through coordinated, context-specific interventions offers a pathway to preserve vision and improve quality of life across West Africa.

Keywords: Glaucoma, West Africa, Low-resource settings, Diagnostic capacity, Gonioscopy, Task-shifting.

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Introduction

Glaucoma represents one of the most consequential yet least adequately addressed public health crises in West Africa [1,2]. The disease affects millions of people across the region, with population-based prevalence estimates of approximately 4.4% to 7.3% among adults aged 40 years and older, rates substantially exceeding those documented in European and many Asian populations [1,2]. More sobering still is the observation that at the time of first clinical presentation, at least one in five patients with glaucoma are already blind, and over half meet visual field criteria for advanced disease [3,4]. This pattern of late presentation is not happenstance but rather the product of a convergence of biological, behavioral, health system, and structural factors that remains unresolved among these West African populations most at risk of this sight-threatening disease [5].

The problem has not been that treatments do not exist since medical and surgical therapies can effectively control intraocular pressure (IOP) and prevent or slow visual loss [6,7]. Rather, the structure, financing, workforce, and information systems of West African health care are fundamentally misaligned with the characteristics of glaucoma as a disease: chronic, asymptomatic, progressive, irreversible, requiring lifelong surveillance and medication adherence, and affecting populations with limited financial resources and minimal health literacy about the disease condition [8]. Closing these gaps will require simultaneous action across awareness, diagnosis, access, adherence, and long-term monitoring, supported by regional guideline development, task-shifted workforce models, and health

system financing reforms [9,10].

Search Strategy and Selection Criteria

This mini-review synthesizes evidence from population-based surveys, clinical studies, and health system assessments conducted in West Africa between 2014 and 2025. Literature was identified through searches of electronic databases (PubMed, Scopus and Google Scholar) using keywords including "Glaucoma," "West Africa," "Prevalence," "Diagnostic Capacity," and "Barriers to Care."

Epidemiological Burden of Glaucoma in West Africa

West Africa carries a disproportionate share of the global glaucoma burden relative to its population size [2]. The scale of glaucoma in West Africa demands urgent recognition as both a clinical and systemic failure of health infrastructure. Population-based surveys consistently document Primary Open-Angle Glaucoma (POAG) prevalence rates far exceeding global norms, with recent pooled estimates suggesting 5.8% across African studies, while West African cohorts specifically demonstrate rates between 4.4% and 7.3% [2].

This burden manifests aggressively, characterized by rapid progression, presentation at younger ages (often under 50 years), and advanced visual field loss at diagnosis [11]. Unlike population-based data, clinic-based data reveals that most patients arrive in tertiary clinics with moderate-to-severe disease, rendering preservation of vision exceedingly challenging [12]. These epidemiological realities underscore a vicious cycle wherein late presentation intersects with aggressive pathophysiology, perpetuating high rates of irreversible visual impairment across communities already strained by poverty and competing health priorities.

Risk Factors and Phenotype of Glaucoma in West African Populations

Understanding the risk profile of West African glaucoma is essential for designing effective screening and prevention strategies. The major risk factors for POAG established through prospective cohort studies and clinical trials include elevated IOP, increased age, male sex, lower central corneal thickness (CCT), positive family history, African or African-Caribbean ancestry, and myopia [13,14].

The Nigeria National Blindness Survey provided granular insight into these variables within the local context. Univariable analyses demonstrated a linear relationship between intraocular pressure and disease risk, where each 1 mmHg increase in IOP was associated with a 1.26-fold increased risk of POAG [15]. Furthermore, central corneal thickness (CCT) emerged as a potent independent predictor. Individuals with a CCT of less than 555 micrometers faced a 4.2-fold increased risk compared to those with thicker corneas [15]. This finding is particularly salient given that the mean CCT in the surveyed population was approximately 540 micrometers, substantially thinner than the global average of 555 micrometers. This suggests that thin corneas, a known risk factor for faster disease progression, may be relatively common in Nigerian populations, rendering standard IOP adjustments critical [15,16].

Family history plays a pivotal but often neglected role in the risk profile [14]. A positive family history of glaucoma or blindness was elicited from approximately 6–8% of respondents in the Nigeria survey and was associated with a more than two-fold increased risk of prevalent POAG [17]. The implication is that in settings where awareness is low and screening is passive, familial cases go undetected and secondary cases in relatives accumulate without intervention [17,18].

Risk factors specific to African-descent populations merit further emphasis. A 2024 global systematic review and meta-analysis found that African and Caribbean populations had an annual cumulative incidence of POAG of approximately 23.46 per 10,000 person-years, substantially higher than European and Asian populations [14]. Moreover, the relationship between IOP and the risk of conversion from ocular hypertension to glaucoma was found to be steeper in African-descent individuals, suggesting a lower threshold for damage [19].

Disease Progression and Natural History

Understanding the trajectory of untreated glaucomatous disease clarifies the stakes of delayed diagnosis. In patients with untreated POAG, the rate of visual field deterioration averages approximately 0.8 decibels (dB) per year in global cohorts [20]. However, for West Africa, where treatment initiation is often delayed until advanced disease is present, progression rates in the time preceding diagnosis are likely substantial [21].

Modeling studies suggest that an individual with undetected POAG at age 50 with a mild visual field defect (mean deviation -5 to -10 dB) could progress to blindness (mean deviation < -20 dB) by age 65–70 if untreated [20,22]. This represents approximately 15–20 years of preventable visual loss [20]. The Ocular Hypertension Treatment Study (OHTS) and subsequent analyses demonstrate that early intervention is effective: topical IOP-lowering therapy reduced the 5-year incidence of conversion to glaucoma from approximately 9.5% in untreated groups to 4.4% in treated groups [23].

Diagnostic Capacity and Current Practice

Optometry and Diagnostic Infrastructure in Ghana and Nigeria

The 2023 cross-sectional study by Ocansey and colleagues serves as the most granular assessment of glaucoma diagnostic practice in West Africa 24. While the data in West Africa leans heavily on Ghana and Nigeria due to the sparseness of literature in the domain from the subregion,

Table 1: Diagnostic Capacity and Practice Patterns Among Optometrists in Ghana and Nigeria

Diagnostic Parameter	Ghana (n=238)	Nigeria (n=255)	Interpretation
Basic Assessment			
IOP (Tonometry)	98.0%	95.3%	High coverage of basic screening.
Optic Nerve Head Exam	98.7%	93.7%	High coverage of basic screening.
Slit-lamp Biomicroscopy	96.9%	74.9%	Significant gap in Nigeria for detailed anterior segment exam.
Advanced Diagnostics			
Visual Field Testing (Any)	~87%	~81%	Availability exists, but specific utilization often lags.
OCT Availability	87.6%	71.9%	Surprisingly high equipment presence, but likely underutilized.
Gonioscopy (Essential)	5.5%	11.2%	Critical Gap: The definitive test for angle closure is rarely done.
Level of Care Achieved*			
Level 3 (Optimal Care)	75.5%	29.7%	Ghana shows higher adherence to optimal protocols.
*Level 3 Care defined in study as: Basic assessment + Visual Fields + OCT + Gonioscopy [24].			

A significant disconnect exists between the availability of high-cost tools and the utilization of essential, low-cost diagnostics. While OCT is available in many urban centers, its utilization is limited by cost ($15,000–50,000 per unit) and training requirements. In contrast, gonioscopy lenses cost less than $1,000 yet are used by only 5–11% of practitioners. This skill deficit has critical clinical implications: without gonioscopy, distinguishing open-angle from angle-closure disease is impossible, leading to potential mismanagement and missed opportunities for prophylactic laser peripheral iridotomy. Consequently, adherence to global standards, such as those established by the American Academy of Ophthalmology (AAO) [26,27] and European Glaucoma Society (EGS) [28] regarding target IOP and disease staging, remains low, as the necessary diagnostic data is simply unavailable in routine practice. Therefore, rather than prioritizing expensive imaging, health systems should focus on a "minimum viable diagnostic package" for primary and secondary care, consisting of reliable tonometry and gonioscopy.

Practice Patterns and Workforce Variables

Beyond equipment, individual characteristics powerfully predict diagnostic practice. Optometrists with more than 10 years of experience were over seven times more likely to diagnose glaucoma compared to junior practitioners [24]. Furthermore, private practice optometrists were 3.33 times more likely to diagnose glaucoma than those in public facilities [24]. This suggests a system where experienced, motivated practitioners concentrate in urban, fee-for-service settings, systematically disadvantaging lower-income populations who rely on public, often rural, facilities staffed by less experienced personnel.

The Broader Eye Care Workforce

The shortage of ophthalmologists in sub-Saharan Africa remains a major constraint, with an average density of approximately 1.2 ophthalmologists per million population, far below the WHO recommendation of 8–15 per million [25,29]. In Nigeria, earlier assessments identified fewer than five glaucoma-trained sub-specialists in the entire country [30].

Primary health care (PHC) facilities, the foundation of the health system, are largely unprepared. An assessment of 233 PHCs in Nigeria found that while 95% had a room for eye exams, only 12% had a tonometer, and fewer than 5% of staff had received any formal training in eye disease screening [31]. Consequently, patients with early, asymptomatic glaucoma passing through PHCs may go undetected until visual loss prompts a referral.

The Care Pathway: Barriers from Awareness to Management

Conceptualizing glaucoma care as a pathway reveals systematic failures at every stage. Table 2 summarizes barriers to Glaucoma care along the patient pathway.

Physiological and Structural Aspects of Diagnosis

Intraocular Pressure and Optic Nerve Assessment

Standard Goldmann applanation tonometry is the gold standard but requires expensive slit lamps and expertise. While rebound tonometry (e.g., iCare) offers a portable alternative, the unit cost ($2,000–3,000) remains a barrier for PHCs. Furthermore, the reliance on Cup-to-Disc Ratio (CDR) assessment via direct ophthalmoscopy is prone to error. While OCT is available in many urban centers, its utilization is limited by cost ($15,000–50,000 per unit) and training requirements [34]. Recent data from Ghana suggests that local populations may have specific OCT normative values that differ from global databases, necessitating population-specific calibration [35].

Visual Field Testing and Gonioscopy

Automated perimetry is largely restricted to tertiary centers [36]. Frequency-doubling technology (FDT) has been used in screening but is not ubiquitous [36]. Emerging tablet-based perimetry (e.g., Eyecatcher) shows promise for low-resource settings but requires validation [36]. The most critical, low-cost technical gap remains gonioscopy [37]. Despite requiring equipment costing less than $1,000, it is performed by only 5–11% of optometrists, leading to significant underdiagnosis of angle-closure glaucoma and missed opportunities for prophylactic laser peripheral iridotomy [37].

Table 2: Barriers to Glaucoma Care Along the Patient Pathway

Pathway Stage	Key Barriers Identified	Impact
Awareness	Low Health Literacy: Only ~39% awareness in rural Ghana. Asymptomatic Nature: "No pain, no problem" mindset. Cultural Beliefs: Attribution of blindness to spiritual causes or aging.	Patients do not seek care until vision is irreversibly lost.
Access	Distance: 20–50 km travel often required. Cost: Transport costs often exceed consultation fees. Workforce: Shortage of specialists in rural areas.	High rates of non-attendance even after referral.
Diagnosis	Equipment: Lack of perimetry/OCT in secondary centers. Cost: Diagnostic tests require out-of-pocket payment. Skill: Low gonioscopy rates lead to misclassification.	Delayed diagnosis or misdiagnosis of glaucoma type.
Treatment Acceptance	Surgery Fear: Fear of iatrogenic blindness (30–40% refusal rates). Preference: Reliance on traditional healers/prayer. Cost: Surgery requires large upfront lump-sum payment.	Patients refuse the most effective long-term interventions.
Adherence	Cost: Monthly drops cost $10–35 USD (significant % of income). Side Effects: Redness/stinging without perceived benefit. Understanding: Lack of insight into chronic nature of disease.	Medication stops when money runs out or symptoms are absent.
Follow-up	Loss to Follow-up: 30–50% default within 1–3 years. System: Poor appointment systems and long wait times.	Disease progression goes monitoring; surgical failure undetected.

Source: [32,33]

Evidence-Based Interventions and Practical Solutions

Addressing the crisis requires a multi-pronged approach tailored to the West African context.

Community Outreach and Case-Finding

Community-based screening effectively reaches the undiagnosed. It has been demonstrated a 6.38% yield of glaucoma cases in Nigerian outreach programs, targeting older, poorer, and less educated populations than those found in clinics [33]. However, the study also revealed a critical drop-off: only 32.5% of referred patients actually attended the hospital for follow-up examination [33].

To bridge the gap between identification and clinical attendance, outreach must be multifaceted and must go beyond case-finding to trust-building. First, on-site health education is indispensable; it must explicitly address the asymptomatic nature of early glaucoma and dismantle the common misconception that lack of pain equates to lack of disease. Ideally, this education is delivered in local languages using culturally appropriate metaphors. Second, the logistical barriers of distance and cost must be actively dismantled through transportation support, such as vouchers or arranged minibus transport from community sites to referral clinics. Finally, the integration of community leadership, including religious organizations and town unions, is essential for trust. In Ilorin, Nigeria, such collaborative models were shown to improve both screening participation and referral adherence [38]. These leaders can help dismantle misconceptions about the "spiritual" nature of blindness and reinforce the medical necessity of treatment. Furthermore, logistical barriers must be addressed through transportation support (vouchers or arranged transport) to referral clinics.

Task-Shifting and Workforce Development

West Africa is uniquely positioned to leverage its Doctor of Optometry (OD) workforce. With structured Continuing Professional Development (CPD), optometrists can manage early glaucoma. To close the diagnostic gap, training programs should mandate clinical simulation labs specifically for gonioscopy, ensuring practitioners can identify angle-closure disease. This aligns with the "Balanced Eye Team" model, successful in Malawi,

utilizes ophthalmologists for surgery, optometrists for management, and nurses for screening [39]. Establishing clinical simulation labs for trabeculectomy training (as per the GLASS trial) can also rapidly upskill ophthalmology residents [40]. Crucially, this training must extend beyond the operating room to postoperative care. Task-shifting protocols must explicitly include nurse-led monitoring of trabeculectomy blebs for signs of scarring or infection, ensuring long-term surgical survival in rural settings where ophthalmologist follow-up is inconsistent.

Integration into Primary Health Care (PHC)

Glaucoma screening should be integrated into existing Non-Communicable Disease (NCD) clinics. Patients attending for hypertension or diabetes should receive simultaneous IOP and optic disc screening. This reduces patient travel burden and leverages existing chronic disease infrastructure.

Medication Access and Cost Containment

Cost remains the primary barrier to treatment initiation and adherence. Policy-level interventions are required to address this. While Selective Laser Trabeculoplasty (SLT) is increasingly advocated by the AAO and EGS [26-28] as a cost-effective first-line intervention, its widespread adoption in West Africa is currently constrained by high initial equipment costs and maintenance requirements similar to those of OCT. Therefore, optimizing medical therapy costs remains the most immediate priority. The optimization of Essential Medicines Lists (EML) and procurement policies constitutes a primary lever; ensuring that generic prostaglandin analogues and beta-blockers are not only listed but procured through regional pooled mechanisms can significantly reduce unit costs. Concurrently, the inclusion of glaucoma medications and surgical procedures within national health insurance schemes, such as those in Ghana and Nigeria, is vital to prevent catastrophic health expenditures for enrollees [41]. Furthermore, clinical workflows can be adapted to reduce patient burden; shifting to 3 or 6-month prescriptions for stable patients reduces the frequency of clinic visits and the associated indirect costs of transportation and lost wages.

Adherence Support and Telemedicine

Given the chronic nature of the disease, adherence support is as critical as the initial diagnosis. Technological solutions offer scalable methods to support adherence [9]. Automated reminder systems, particularly Short Message Service (SMS) interventions, have demonstrated efficacy in improving self-reported adherence in Nigerian pilot studies [42]. Beyond adherence, telemedicine models such as "store-and-forward" teleophthalmology offer a mechanism to democratize access. In these models, rural nurses or optometrists capture clinical data and images to transmit to urban specialists for review, effectively extending the reach of sub-specialty care to underserved areas [43].

Implementation Science and Health System Factors

Success depends on organizational readiness. Applying the Reach, Effectiveness, Adoption, Implementation, Maintenance (RE-AIM) framework clarifies the scalability of interventions [44]. Reach is extended to rural populations through task-shifting models, such as the Malawi Balanced Eye Team [45]. Effectiveness is evidenced by Nigerian pilot studies where SMS reminders increased medication compliance by approximately 20% [46]. Adoption is facilitated by integrating glaucoma screening into existing NCD clinics to leverage established patient flows. Implementation is operationalized through clinical simulation labs to build workforce capacity, while Maintenance is secured through sustainable financing mechanisms like national health insurance coverage. Programs must assess facility capacity (electricity, supply chains, staff motivation) prior to launch. Sustainability requires moving from donor funding to integration within government health budgets and insurance schemes. Robust monitoring and evaluation systems are non-negotiable to track referral uptake, treatment adherence, and visual outcomes.

Conclusion

Glaucoma in West Africa is a massive, largely preventable cause of blindness. Closing the gap requires systematic application of known interventions tailored to the region's specific socio-economic landscape. For national governments, the priority must be the formal declaration of glaucoma as a public health priority, accompanied by the mandate that national health insurance schemes provide comprehensive coverage for both medication and surgery. For health system leaders and hospital administrators, the focus must pivot toward equipping primary and secondary facilities with basic diagnostic tools and formalizing referral pathways, potentially through the implementation of "Balanced Eye Teams." The clinical workforce must commit to upskilling, particularly in gonioscopy, while simultaneously engaging in community education to improve health literacy. Finally, civil society and patient advocacy groups play a vital role in destigmatizing the disease and holding systems accountable. With sustained commitment across these sectors, West African health systems can significantly reduce glaucoma-related blindness, preserving the sight and quality of life of millions.

Disclosure statement

None to declare.

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REVIEW ARTICLE

Patient Perceptions of the Impact of the National Health Insurance Scheme on Healthcare Service Delivery in Ghana: A Systematic Review.

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Abstract

Background: Patients in Ghana have conflicting perceptions about the impact of the National Health Insurance Scheme (NHIS) on service delivery. While some acknowledge the beneficial effects of the scheme, others highlight its drawbacks. The objective of this study was to investigate how patients perceive the impact of the NHIS on service delivery in Ghana.

Methods: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied. Searches were conducted in five databases: ScienceDirect, Web of Science, PubMed, ProQuest, and Scopus. Included studies were written in English, published between 2015 and 2024, and peer-reviewed. Two reviewers independently assessed search results, extracted data, and assessed study quality.

Results: Out of 250 studies screened, 24 full-text articles were included. Patient perceptions of NHIS were largely positive, especially among rural and low-income groups who saw it as improving affordability and access. In contrast, urban and more educated patients were more critical, citing long wait times, drug shortages, and poor service quality. One study found no significant difference in service quality between insured and uninsured patients ($p = 0.47$). Common themes included financial access (70% of studies), service quality (50%), perceived fairness (50%), provider attitudes (40%), waiting times (30%), and drug availability (50%).

Conclusion: The study highlights the perceived benefits of NHIS in Ghana, including improved access to care, enhanced continuity of care, and alleviated financial burden. However, challenges such as negative attitudes of healthcare personnel, delays in accessing care, lack of coverage for critical services, drug unavailability, and concerns about service quality remain.

Keywords: impact, national health insurance scheme, patients, perception, service delivery

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Introduction

PGlobally, especially in sub-Saharan Africa, National Health Insurance (NHI) is considered the lifeblood of Universal Health Coverage [1]. Ghana has implemented several reforms in healthcare financing. Earlier reforms led to reduced utilization of services and increased inequities, prompting the implementation of a social health insurance scheme known as the National Health Insurance Scheme (NHIS). This initiative aimed to pool and share health risks across the population and reduce direct payment at the point of service delivery [2].

There are perceived benefits associated with the implementation of the NHIS in Ghana's healthcare system. A study by Christmals and Aidam[1] indicated that the NHIS has helped remove some socio-economic barriers to healthcare by making services accessible and affordable.

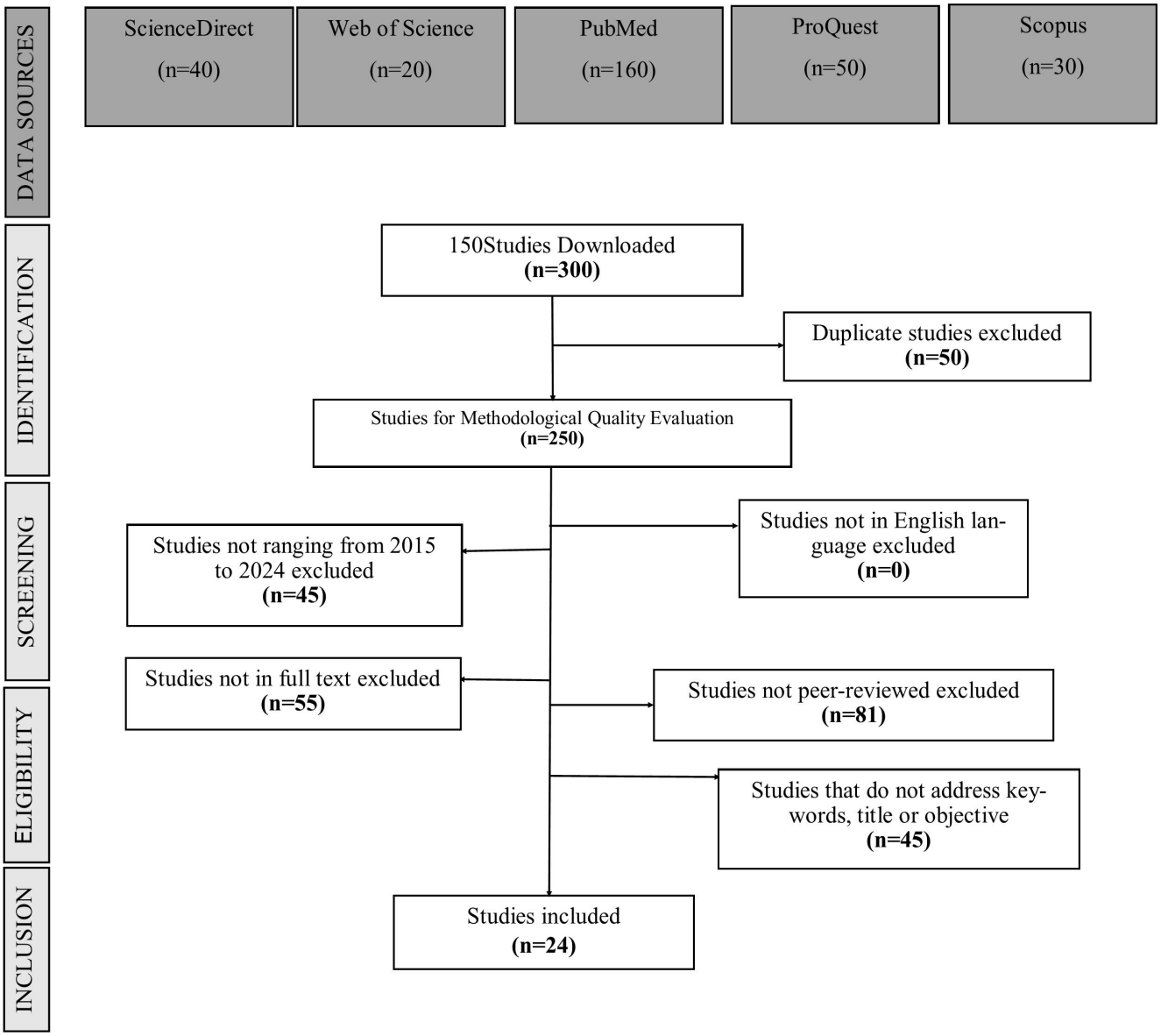

Figure 1. Flow Chart for Preferred Reporting Items for Systematic Reviews and Meta-Analyses

The scheme provides access and financial protection, which have contributed to improved health-seeking behaviors and reduced risky practices such as self-medication. Despite these perceived benefits, the implementation of the NHIS has also been associated with certain challenges in healthcare service delivery [1].

According to studies by Kipo-Sunyehzi et al.[3] and Zhang et al.[4], patients in Ghana have expressed conflicting perceptions regarding the impact of NHIS on service delivery. While some acknowledge its beneficial effects in increasing access to healthcare services, others highlight drawbacks such as long waiting times, inadequate drug supplies, and concerns about the quality of care. In addition, Akweongo et al.[5] reported that some insured clients refrained from renewing their NHIS membership due to perceived poor quality healthcare services received during visits to medical facilities. Others claimed that, despite having active membership cards, they were still required to pay for services [5].

Evaluating how well the NHIS meets the healthcare needs of the population depends largely on patients' perceptions of the system and its impact on service delivery [6]. Although previous studies have examined the impact of NHIS on service delivery in Ghana, less emphasis has been placed on comprehensively synthesizing patient perceptions. Therefore, this study seeks to fill this gap by synthesizing patient perceptions of the impact of the NHIS on healthcare service delivery in Ghana. The findings of this study will help to inform policy decisions and practice by enabling stakeholders to identify areas for healthcare delivery optimization and improvement under the scheme. Ultimately, this will enhance NHIS implementation to better meet the needs of patients [6].

Methods

This study aimed to synthesize patient perceptions of the impact of the National Health Insurance Scheme on

Table 1. Quality Assessment (Study Appraisal and Risk of Bias of Included Studies)

Author(s)	Study Design	Meets Inclusion Criteria (5/5)	Risk of Bias
Abredu et al., 2023	Mixed methods	✓	Low
Adisah et al., 2017	Qualitative	✓	Moderate
Nketiah-Amponsah et al., 2019	Quantitative	✓	Low
Baozhen et al., 2019	Quantitative	✓	Moderate
Otieno et al., 2022	Quantitative	✓	Moderate
Zhang et al., 2019	Quantitative	✓	Low
Umar et al., 2020	Quantitative	✓	Low
Dalinjong et al., 2019	Qualitative	✓	Moderate
Thapa et al., 2021	Qualitative	✓	Moderate
Ameyaw et al., 2021	Quantitative	✓	Low
Badu et al., 2019	Qualitative	✓	Moderate
Yaya et al., 2017	Quantitative	✓	Low
Bagnoli et al., 2019	Quantitative	✓	Low
Ayanore et al., 2018	Mixed methods	✓	Low
Duku et al., 2018	Quantitative	✓	Low
Debpuur et al., 2015	Qualitative	✓	Moderate
Alhassan et al., 2015	Qualitative	✓	Moderate
Okoroh et al., 2018	Quantitative	✓	Low
Tindyebwa et al., 2023	Mixed methods	✓	Low
Abuosi et al., 2016	Quantitative	✓	Low
Akweongo et al., 2021	Mixed methods	✓	Moderate
Kanmiki et al., 2019	Quantitative	✓	Low
Mkperedem et al., 2023	Qualitative	✓	Moderate
Andoh-Adjei et al., 2018	Mixed methods	✓	Low

healthcare service delivery in Ghana. The systematic review methodology was applied in this investigation. This method was deemed applicable because it allowed other researchers to evaluate the quality of the study and verify its findings. In addition, a systematic review was appropriate for this study because it is designed to minimize bias and error by using a structured and comprehensive approach to identify, select, and synthesize all available evidence related to the research objective. This investigation was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Inclusion Criteria

A study was considered for inclusion in this review if it was written in English, published between 2015 to 2024, available in full text, peer reviewed, and addressed the keywords, title or objectives of this study. The 2015 starting point was chosen to capture more recent and relevant evidence following key policy reforms and the evolving implementation of National Health Insurance Scheme in Ghana, which was initially launched in 2003.

Exclusion Criteria

Studies were excluded if they were conference abstracts, book chapters, editorials, commentaries, grey literature; if they were not written in English, published before 2015; not available in full text or not peer reviewed; or if they did not address the keywords, title or objectives of the study.

Identifying Relevant Studies (Information Sources)

Five international databases were used to gather English-language literature published between 2015 and 2024: ScienceDirect, Web of Science, PubMed, ProQuest and Scopus. These databases were purposefully selected for their extensive coverage and standardized indexing of relevant studies. Regional databases, such as African Journals Online (AJOL) were excluded to maintain consistency and accessibility.

To identify relevant studies that met the inclusion criteria, key search terms and phrases were combined using Boolean operators (AND, OR, NOT). The main keywords and phrases included: "National Health Insurance Scheme (NHIS)", "history", "implementation", "benefits", "challenges", "impact", "patients", "perceptions", "healthcare", "service delivery", and "Ghana". Search combinations were tailored for each database. For PubMed, ScienceDirect, ProQuest, and Web of Science, combinations included phrases such as: "Implementation of the National Health Insurance Scheme in Ghana", "History of the National Health Insurance Scheme in Ghana", "Benefits of the National Health Insurance Scheme in Ghana", "Challenges of the National Health Insurance Scheme in Ghana", and "Patients Perceptions of the Impact of the National Health Insurance Scheme on Service Delivery". In Scopus, the search focused on the later three combinations: benefits, challenges, and patient perceptions of NHIS impact on service delivery.

Study Selection

The selection of eligible studies adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. From the downloaded studies, 50 were eliminated due to duplication using Mendeley referencing software. The titles and abstracts were reviewed and agreement was reached on those requiring full screening. The full screening was conducted following the inclusion and exclusion criteria. Figure 1 shows the PRISMA flow diagram for the process of selecting eligible studies.

Study Appraisal

There were 250 remaining studies that qualified for evaluation under methodological quality. The methodological quality of all included studies was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Tools based on the study design. All included studies met the inclusion criteria and were appraised according. The results are summarized in Table 1. Five questions were posed to evaluate the 250 studies. The questions were:

1. Is the article written in English language?
2. Is the article published within the year range of 2015 to 2024?
3. Is the article available in full text?
4. Is the article peer-reviewed?
5. Does the article address the keywords, title, or objectives of this study?

For every question asked, a "Yes" or "No" response was required. This was a systematic way to assess the quality of the research. This evaluation included studies that answered "Yes" to all five questions. Following the evaluation, 24 studies were included in this review.

Risk of Bias Assessment

In addition to meeting the predefined inclusion criteria, each included study was assessed for risk of bias using simplified criteria adapted from the ROBIS tool. This evaluation considered the clarity of the research objectives, the appropriateness of the study design, transparency in methodology, data quality control, and completeness of reporting. Studies were categorized as having a low, moderate, or high risk of bias. The results of this appraisal are presented in Table 1.

Data Extraction (Charting data)

Two independent reviewers extracted data from the included studies using a standardized and piloted charting form that captured publication details (year, authors, study design, and title) and key findings related to patient perceptions of the NHIS and its impact on service delivery. Although inter-rater reliability was not quantified, consistency was ensured through adherence to the study protocol, and discrepancies were resolved by consensus. The charting form was piloted on ten studies that were not included in the final review to enhance clarity and reliability.

Data Synthesis

The findings from the included studies concerning patient perceptions of the impact of the NHIS on service delivery were synthesized to identify patterns and themes. To achieve this, the studies were carefully reviewed to identify key subject areas and recurring themes. The identified subject areas and themes were re-examined to gain an in-depth understanding of the results. These subject areas and themes were then classified based on their associations and relatedness.

Results

This section of the systematic review presents the findings.

Search Results

Table 2 shows the distribution of downloaded studies across the five selected databases and the included studies with their percentages. A total of 300 studies were downloaded following a search of the five databases. Of these, 20 were from Web of Science, 160 from PubMed, 50 from ProQuest, 40 from ScienceDirect, and 30 from Scopus.

Fifty (50) studies were excluded for being duplicates, 81 studies were excluded for not being peer-reviewed journal articles, 55 studies were excluded for not being available in full text articles, 45 studies were excluded for not being published between 2015 and 2024, and 45 studies were excluded for not addressing keywords, title and objectives of the study were. No study was excluded for not being written in English. Twenty-four (24) studies were included in this review.

Table 2. Distribution of Downloaded, Included Studies and Percentages of Included Studies

Databases	Number of downloaded studies	Number of Included Studies	% of Included Studies
Science Direct	40	5	21
Web of Science	20	4	17
PubMed	160	8	33
ProQuest	50	5	21
Scopus	30	2	8

Themes Generated from the Data

This review identified six main themes in patient perceptions of the impact of NHIS on healthcare delivery in Ghana: financial access, fairness of care, quality of care, attitudes of healthcare professionals, waiting times, and availability of drugs.

About 70% of the studies reported improved financial access through reduced costs and better service continuity, although 40% noted persistent out-of-pocket payments for drugs and tests, especially among vulnerable groups [6,7]. Perceptions of fairness of care varied, with poorer rural patients generally expressing more positive views than wealthier urban patients (reported in 50% of studies) [6].

Views on quality of care were mixed: approximately 50% of the studies reported patient satisfaction, while the remainder highlighted issues such as discrimination, claim

Table 3. The perception of patients about the impact of NHIS on service delivery

Year of publication	Authors	Study Design	Study Title	Key findings
2023	Abredu et al.	Mixed Methods	Factors influencing the free maternal health care policy under the national health insurance scheme provision for skilled delivery services in Ghana: a narrative literature review	Challenges like out-of-pocket payments for services excluded from NHIS coverage, as well as the absence of coverage for critical services like ambulance usage during emergencies, contribute to the mixed views of patients on the impact of the scheme on service delivery.
2017	Adisah- Atta.	Qualitative	Financing Health Care in Ghana: Are Ghanaians Willing to Pay Higher Taxes for Better Health Care? Findings from Afrobarometer	Ghanaians have voiced differing opinions regarding the effects of the NHIS on service delivery. Overall patient satisfaction with the NHIS is influenced by their experiences with drug availability, waiting times, and service quality.
2019	Nketiah-Amponsah et al.	Quantitative	The perception subscribers of quality of services provided by the National Health Insurance Scheme in Ghana - what are the correlates?	Although many people are benefiting from the NHIS, there are worries about how this may affect the quality of care. Over time, patients have complained about increased waiting times, higher costs, and decreased satisfaction with medical care. An assessment of the NHIS by both insured and uninsured clients revealed poor quality of services provided to insured clients by the NHIS-accredited facilities in the areas of stock-out of essential drugs and bad attitude of staff as some of the marked drawbacks of the scheme. Insured clients indicated that essential drugs are often not available at the health facility.
2019	Baozhen et al.	Quantitative	Assessing Factors Affecting the Patronage of Health Insurance Schemes: An Evidence of Ghana	There are differences in views of patients on how the NHIS affects service delivery, and some are not happy with the quality of care they received through the program. Individuals who pay cash for care instead of utilizing their insurance cards have reportedly received better care.
2022	Oteieno et al.	Quantitative	Health services availability and readiness moderate cash transfer impacts on health insurance enrolment: evidence from the LEAP 1000 cash transfer program in Ghana	The perception patients of the NHIS in Ghana are influenced by factors such as service availability, quality of care, and administrative processes. Some individuals may perceive the NHIS positively if they experience improved access to healthcare service without financial burden. However, challenges in service delivery, including long waiting times, limited facility resources and perceived service quality issues, can lead to negative perceptions of the NHIS.
2019	Zhang et al.	Quantitative	Trends and projections of universal health coverage indicators in Ghana, 1995-2030: A national and subnational study	Patients in Ghana have conflicting opinions about the National Health Insurance Scheme (NHIS). While some acknowledge the schemes beneficial effects on increasing access to healthcare services, others draw attention to drawbacks such as lengthy waiting times, shortage in drug supply, and problems with the quality of care.
2020	Umar et al	Quantitative	The shared experiences of insured members and the uninsured in health care access and utilization under national health insurance scheme in Ghana: Evidence from the Hohoe Municipality	Patients have voiced issues over the attitude of healthcare personnel, specifically pointing out that public health institutions tend to be ruder and more disrespectful than private ones.
2017	Dalinjong et al	Qualitative	The association between health insurance status and utilization of health services in rural Northern Ghana: evidence from the introduction of the National Health Insurance Scheme	The perception of patients of the National Health Insurance Program (NHIS) differ, some are happy that the program may offer reasonably priced medical services, while others complain about issues like long waiting times, difficulty in accessing specialized care, and drug shortages.

Table 3. The perception of patients about the impact of NHIS on service delivery

Year of publication	Authors	Study Design	Study Title	Key findings
2021	Thapa et al	Qualitative	Perception Towards National Health Insurance Scheme among Enrollees of Central Terai: A qualitative Study	Respondents were dissatisfied with the services they received. Their concerns were about the quality of care they received, regarding drug availability, interaction between patients and providers when seeking medical care, and long waiting times.
2021	Ameyaw et al	Quantitative	Is the National Health Insurance Scheme helping pregnant women in accessing health services? Analysis of the 2014 Ghana demographic and Health survey	Regarding NHIS subscription, we discovered that women who had enrolled in the program were more likely to emphasize how it helps expectant mothers get access to healthcare.
2019	Badu et al	Qualitative	Perceived satisfaction with health services under National Health Insurance Scheme: the perspectives of clients	Eighty-one percent of insured clients felt that the NHIS do not cover all medical expenses.
2017	Yaya et al	Quantitative	Urban-rural difference in satisfaction with primary healthcare services in Ghana	The were shortages of drug supply with the NHIS which led to insured clients being dissatisfied with the services they received and were discouraged from enrolling on the scheme.
2019	Bagnoli et al	Quantitative	Does health insurance improve health for all? Heterogeneous effects on children in Ghana	Respondents believed that it removes a financial burden for lower-income households, which helps in the improvement of health among insured children.
2018	Ayanore et al	Mixed Methods	Predictors of Health Care Service Quality among Women Insured Under National Health Insurance Scheme in Ghana	The view of patients of low quality for meeting their health care demands will definitely equate to patients making Out-of-Pocket Payment (OOP).
2015	Debpuur et al	Qualitative	An exploration of moral hazard behaviors under the national health insurance scheme in Northern Ghana: a qualitative study	Patients who were insured were discouraged from going to seek medical care at the health facilities because they have to join long queues as a result of the large number of patients seeking medical care.
2015	Alhassan et al	Qualitative	Comparison of perceived and technical Healthcare quality in primary health Facilities: Implications For	Clients were not satisfied with the quality of services they received.
2018	Okoroh et al	Quantitative	Evaluating the impact of the national health insurance scheme of Ghana on out-of-pocket expenditures: a systematic review	All insured members made Out-Of-Pocket payment for medicines, although they paid less as compared to the uninsured clients.
2023	Tindyebwa et al	Mixed Methods	Expectations of clients, insurers, and providers: a qualitative responsiveness assessment among private health insurance sector in Kampala-Uganda	Clients experienced low service care responsiveness which was characterized by long waiting times.
2016	Abuosi et al	Quantitative	Health insurance and quality of care: Comparing perceptions of quality between insured and uninsured patients in hospitals in Ghana	Both the insured and the uninsured had equal positive perception regarding the quality of services they received. However, the insured paid less to access healthcare as compared to the uninsured.
2021	Akweongo et al	Mixed Methods	Out-of-pocket payments by insured clients for health care under the national health insurance scheme in Ghana	About 47% of the respondents who were insured reported out-of-pocket payments of Out-Patient Department services.
2019	Kanmiki et al	Quantitative	Out-of-pocket payment for primary healthcare in the era of national health insurance: Evidence from northern Ghana	The NHIS has reduced the financial burden of accessing healthcare by reducing the out-of-pocket payments made by patients.
2023	Mkperedem et al	Qualitative	Perception among NHIS–HMO Enrollees of the Attitudes of Medical Personnel during Outpatient Care in Lagos Hospitals	Some clients had a positive perception of the overall attitude of healthcare professionals during out-patient care. They said the attitudes of the medical personnel towards them were humane and respectful.
2018	Duku et al	Quantitative	Perceptions of healthcare quality in Ghana: Does health insurance status matter?	People sign up for health insurance in order to protect themselves financially from having to pay a disproportionate amount of money out of pocket for high-quality medical care. Clients who were insured showed dissatisfaction with the services they received because of long waiting time, inadequate provision of information, partial queuing system, bad attitude of staff, and poor quality of drugs available at accredited facilities.
2018	Andoh-Adjei et al	Mixed Methods	Does a provider payment method affect membership retention in a health insurance scheme? a mixed method study of capitation payment for primary care in Ghana.	The respondents stated that the speed at which the NHIS processes claims from healthcare providers affects the quality of care.

delays, and poor services in public facilities [8,9,7]. Attitudes of healthcare professionals also varied, with about 40% of studies reporting respectful behavior, while others documented rudeness, particularly in public facilities [10,11].

Waiting times and drug shortages were common concerns, reported in approximately 30% and 50% of studies, respectively, including long queues and limited drug availability [12,13]. Additional barriers, such as incomplete service coverage and dissatisfaction leading some insured patients to seek private care, were noted in 35% of studies [14,15].

Discussion

The findings from this study revealed that patients have different views regarding the impact of the NHIS on healthcare service delivery in Ghana. These differences in perception are influenced by access to healthcare services (service availability), quality of care, financial access, service coverage, waiting times, and the attitudes of healthcare personnel. Other influencing factors include demographic characteristics such as socio-economic status, wealth status, education level and gender [6,13,16].

Some patients had a positive perception of the NHIS because they perceived it as enhancing the continuity of healthcare by reducing the financial burden on patients. Andoh-Adjei et al.[6] and Bagnoli et al.[17] also noted that poorer, less-educated rural patients tended to view NHIS services more favorably, likely because they lack other healthcare alternatives for comparison. In contrast, their urban counterparts, who tend to be more educated and wealthier, were more critical of NHIS service quality.

Patients generally had a positive perception of the NHIS, as it is recognized to enabling insured clients to benefit from reduced healthcare costs and improved access to healthcare services [7,18,19]. This is consistent with a study by Christmals and Aidam[1] which indicated that NHIS helps improve the accessibility and affordability of healthcare services. In addition, a study by Dalinjong and Laar[20] noted that some patients were please that the scheme offered reasonably priced medical services. However, insured clients still made out-of-pocket payments, although these were lower compared to those uninsured clients [8,21]. It was also observed that pregnant women had greater access to NHIS healthcare services [19].

In contrast, despite the free maternal healthcare policy integrated into the NHIS, studies by Abredu et al.[14], Andoh-Adjei et al.[6], and Adisah-Atta[22] reported that the costs for some services such as drugs, laboratory tests, ultrasounds, and emergency transportation, are not fully covered by the scheme. Women who cannot afford these out-of-pocket payments are deprived of accessing essential maternal health services, which contradicts the intended "free care" policy under the NHIS, thus, potentially undermining its goal of financial risk protection.

Patients had a positive perception of the attitudes of healthcare professionals. They reported that medical personnel treated them were humanely and respectfully[10], which contrasts with the findings of Duku et al.[11], Nketiah-Amponsah et al.[13], and Umar et al.[7], who indicated that insured clients were dissatisfied with services due to poor attitude of the healthcare staff. Patients also noted that healthcare personnel in public health institutions tend to be ruder and less respectful than those in private institutions. Measures such as in-service training and corrective (sanction) policies should be implemented to ensure that the attitudes and behavior of healthcare professionals align with the standards, procedures, and principles governing their work.

Both the insured and the uninsured patients had equally positive perceptions regarding the quality of services they received [8]. However, others expressed dissatisfaction with the quality of services [9,17,23]. Challenges reported included experiences of discrimination by healthcare workers against insured clients, lack of coverage for critical services such as ambulance use during emergencies, and perceived poor quality of care at government healthcare facilities [6,7,11,14,24,25]. Some patients noted that the perceived poor quality of care could be influenced by the speed at which the NHIS processes claims from healthcare providers, stating that the services they receive are affected by delays in claims processing [6].

Furthermore, some patients were reluctant to renew their NHIS membership due to delays in accessing care with NHIS cards, concerns about the overall quality of healthcare under the scheme, and decreased satisfaction with medical services. Some insured patients even opted to pay higher fees at private facilities for what they perceived as better-quality care [7,13,26]. Regarding subscribers who opted to pay for healthcare services, a study by Baozhen et al.[15] noted that individuals who made out-of-pocket payments instead of utilizing their insurance cards reportedly received better care.

Insured patients were discouraged from seeking medical care at health facilities because they had to join long queues due to the large number of people seeking medical care[12]. It was also found that insured clients expressed dissatisfaction because essential drugs were often unavailable at the facilities, and those that were available were frequently of poor quality [7,11,13,27].

The findings of this study indicate that patient perceptions of the impact of the NHIS on service delivery vary. Some patients reported positive perceptions of the scheme, while others highlighted challenges such as additional charges, long waiting times, discrimination against insured clients, lack of coverage for critical services, and concerns about the quality of care. These findings suggest that, although the implementation of the NHIS in Ghana has had some perceived positive impact on healthcare service delivery, patient concerns regarding the scheme still require attention to enhance its effectiveness.

Implications for Policy, Practice, and Future Research

This study explored patient perceptions of the impact of the NHIS on service delivery in Ghana. The findings reveal that patient perceptions of NHIS vary. These results have several policy implications. Policymakers and healthcare administrators should address the identified challenges to improve the NHIS's effectiveness in delivering affordable,

high-quality healthcare to all Ghanaians. Measures must be implemented to ensure that healthcare professionals' attitudes and behaviors align with established standards, procedures, and principles. For example, incentive programs could motivate providers to maintain professionalism and deliver respectful care. Regular in-service training and monitoring of staff conduct can also promote more empathetic patient interactions.

To reduce long waiting times, additional healthcare providers should be recruited to improve the provider-to-patient ratio. Additionally, streamlining administrative processes and expediting claims reimbursement can minimize delays in service delivery. Policy interventions should also consider the specific challenges faced by rural populations, such as limited facilities, longer travel times, and reduced access to skilled providers. Rural patients often reported more favourable views of the NHIS, likely due to fewer alternatives, whereas urban patients, with more options, tended to be more critical. Policy efforts should address rural barriers while also enhancing service quality in urban areas.

Furthermore, mechanisms should be established to monitor and evaluate the scheme's operations and to collect feedback from subscribers to identify areas needing improvement and guide reforms. Future research should explore strategies necessary for the NHIS to achieve its intended goals.

Limitations of the Study

The study acknowledges certain limitations, such as the exclusion of non-English studies, as the linguistic diversity in Ghana may influence perceptions. In addition, the exclusion of studies published before 2015 may limit the scope of the review. The study also excluded grey literature, which may have introduced publication bias and limited the scope of the findings by omitting relevant but non-peer-reviewed data. However, the findings from this study remain relevant to the National Health Insurance Scheme, the Ghana Health Service, and the Ministry of Health for supporting the effective delivery of healthcare services under the National Health Insurance Scheme in Ghana.

Conclusion

This study provides a comprehensive overview of the diverse perceptions patients regarding the NHIS and its impact of on service delivery. The study highlights perceived benefits such as improved access to care, enhanced continuity of care, and reduced financial burden on patients. However, it also identifies perceived challenges including the negative attitudes of healthcare personnel, delays in accessing care, concerns about service quality and coverage scope, long waiting times, and the unavailability of drugs.

Based on the findings, it is recommended that policymakers and healthcare administrators prioritize addressing the identified challenges to optimize the effectiveness of the NHIS in providing affordable and quality healthcare services to all Ghanaians. Measures should be taken to curb the negative provider attitudes, streamline access to care, improve service quality, and expand the scope of coverage to meet the diverse healthcare needs of the population.

Furthermore, implementing mechanisms for ongoing monitoring and evaluation, as well as soliciting feedback from NHIS subscribers, can help identify areas for improvement and ensure that the scheme remains responsive to the evolving needs and expectations of beneficiaries. By addressing these recommendations, policymakers can enhance the ability of the NHIS to achieve its objectives of improving healthcare accessibility, affordability, and quality for all Ghanaians. Implementing these measures will help the NHIS to remain relevant and responsive to subscribers' needs and contribute to the overall improvement of healthcare services in Ghana.

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Authors' contributions

Conceptualization, NKM and EO; formal analysis, investigation, and data synthesis, EO; formal data analysis and results interpretation, EO; writing, original draft preparation, EO; writing, review and editing, EO and NKM. All authors have read and agreed to the published version of the manuscript.

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Competing interests

The authors declare that they have no competing interests

Availability of data and materials

Pieces of Literature analyzed during the current study are available online and can also be made available through the corresponding author upon request.

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Abbreviations

NHI National Health Insurance

NHIS National Health Insurance Scheme

PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses

UHC Universal Health Coverage

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RESEARCH ARTICLE

Biosafety of *Beauveria bassiana* as a biopesticide: no effects on sex hormones in experimental rats

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Abstract

Background: Although biological control is regarded as a safer and more sustainable alternative to chemical pesticides for insect pest management, the effect of biocontrol agents on the endocrine system of non-target organisms remain inadequately characterized. This study investigates the hormonal responses of Wistar rats as a non-target mammalian model following exposure to the entomopathogenic fungus Beauveria bassiana.

Materials and methods: The fungus was isolated from diseased variegated grasshopper. Veen's media was used to isolate the entomopathogenic fungus from the insect cadavers. Spore suspensions of the fungus were prepared used to infect another batch of the insect to confirm entomopathogenicity. The inoculum was standardized and injected into experimental rats intraperitoneally. Rats were observed for a period of 7days before being sacrificed. Blood was collected into heparin tube through ocular puncture and analysed for selected reproductive hormones. The rats were examined to ascertain they are in uniform reproductive cycle to minimize the spiking or reduction in hormonal concentration.

Results: Results showed a slight change in some hormonal levels in challenged rats compared to rats within the control group. Progesterone levels were 6.67±1.16a ng/mL and 6.33±0.58a ng/mL in rats within the test and control groups respectively. Testosterone levels were 3.40±0.20a ng/mL and 3.50±0.17a ng/mL respectively in the test and control groups. Follicle Stimulating Hormone level were 2.00±0.10a mIU/mL and 1.93±0.12a mIU/mL while Lieutenizing Hormone were 16.40±0.10a and 16.43±0.06a in rats within the test and control groups respectively. Despite the reduction, the values were still within the acceptable range with the exception of testosterone.

Conclusion: The study showed that usage of B. bassiana as an entomopathogenic fungus in the formulation of biopesticides may pose less risk to the handler and the environment upon its deliberate release or accidental exposure

Keywords: entomopathogens, biopesticides, FSH, LH, progesterone, testosterone

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Introduction

Microbial toxicology is a scientific discipline involving the study of structure and mechanism related to the toxic effects of microbial agents and the toxins they produce [1, 2]. It is a specialized branch of toxicology that focuses on the interactions between microorganisms and toxins. It involves the study of how microorganisms produce toxins, how these toxins affect human health and the environment, and how microbial activity can influence the toxicity of various substances. This discipline encompasses technology advances in research related to microbiological aspects of toxicology. Microbial toxins are toxins produced by microorganisms, including bacteria, algae, viruses and fungi. They can be endotoxins which are produced as components of the outer membrane which are released when bacteria die and their cell walls break apart. They can also be exotoxins secreted by bacteria during microbial growth and metabolism [3]. Microbial toxins are important virulence determinants responsible for microbial pathogenicity and/or evasion of the host immune response. These toxins can exert their adverse effects by disrupting cellular membranes, inhibiting protein synthesis, interfering with signal transduction

pathways and triggering immune responses. Microbial toxins can equally have significant effects on the endocrine system, disrupting hormonal balance and function. Some microbial toxins can interfere with the synthesis or secretion of hormones. Toxins can also bind to hormone receptors, either mimicking the hormone's action (agonist effect) or blocking the hormone's action (antagonist effect). Some toxins interfere with intracellular signalling pathways that are activated by hormones, leading to inappropriate cellular responses [4].

Before any drug, substance or agent can be certified for usage, there must be toxicological analysis of its effects upon its use so as not to exert adverse effects on the human population and its environment [5]. While a lot of work has been carried out on the toxicological potentials of medically important microbial strains, not a lot has been done on environmental strains due to the assumption that environmental strains usually use in biological control are largely benign [6-10]. Once an organism is released into the environment, its behaviour can no longer be controlled. Thus, a previously benign organism can become pathogenic after release. Even the few toxicological work carried out largely involve the evaluation of the histopathological and haematological parameters. There is a less common work been done on hormonal evaluation. Biocontrol agents like *B. bassiana* are usually considered as benign. However, certain strains of the fungus is known to produce a variety of toxic secondary metabolites, including beauvericin, bassianolide, and tenellin, which have cytotoxic and ionophoric properties. Beauvericin in particular can disrupt calcium homeostasis in mammalian cells, leading to apoptosis and cellular dysfunction. Such disruptions can affect gonadal cells thus impairing the synthesis of sex steroids like testosterone, estrogen and progesterone [11]. In order to prevent a potential plague especially in the case of *B. bassiana*, the toxicological effects have to be confirmed before its usage can be encouraged.

Methods

Collection of *Z. variegatus*

Z. variegatus were sourced from cassava Farm using sweep nets [12]. They were transferred to the laboratory, provided with fresh water leaves and sterile water allowed to acclimatize and watched for the onset of disease symptoms. Individuals exhibiting symptoms such as lethargy, colour change, abnormal outgrowths and reduced feeding rate were separated from the population for maceration and subsequent isolation of microorganisms [13-16].

Isolation of *B. bassiana* from insect cadavers.

Cadavers of *Z. variegatus* were removed from the cages and surfaced sterilized by generously rinsing in 5 percent sodium hypochlorite, followed by 75 percent ethanol. Cadavers were then further rinsed in plenty of sterile distilled water. The cadavers were then left to dry out naturally for 48 hours [13], transferred to desiccators for humid incubation at room temperature as described by Luz and Farques[17]. Sporulating cadavers as shown in Figure 1 were regarded as being positive for the growth of *B. bassiana*. The sporulating fungi on cadavers were inoculated onto Veen's medium for the isolation of *B. bassiana* and incubated at 25 °C for 72 hours [18]. Veen's medium was prepared by dissolving 5 g of Peptone, 10 g of glucose and 6 g of agar into 500 ml of distilled water. The pH was adjusted to 6.3 with 1M of HCL and then autoclaved for 20 minutes at 120 °C. The medium was allowed to cool to 60°C after which 0.5ml of streptomycin, 0.5ml of tetracycline, 0.5 ml of dodine and 2.5 ml of cyclohexamide were added [19].

Figure 1. The growth of *B. bassiana* on *Z. variegatus* cadaver.

Further identification of sporulating fungi

Two drops of cotton blue lactophenol were placed on a clean grease-free microscopic slide and a small piece of mycelium from the Veen's media was removed with sterile inoculating needle, transferred onto the stain on the slide and covered with clean slip as described by Fawole and Oso [20]. The identification of molds were done by comparisons of the observed morphological characteristics beneath the microscope in accordance with standard methods.

Confirmation of entomopathogenicity of *B. bassiana*

B. bassiana was inoculated onto fresh Potato Dextrose Agar (PDA) plates and incubated at 27 °C for 14 days for sporulation to take place. Spores and conidia were harvested from these plates with a 0.1 percent Tween 80 solution. Conidia stock suspensions were stored at 4 °C until being used. The spores suspensions obtained were dispensed into plastic aspirators. New batch of apparently healthy grasshoppers *Z. variegatus* were infected with the suspension. Control experiment was set up by spraying separate populations of insects with sterile saline [21-23].

Collection and rearing of wistar rat for hormonal analysis

Male rats were used for testosterone analysis while female rats were used for progesterone, follicle stimulating hormone as well as luteinizing hormones analysis. The parent rats were purchased from the animal house of the Department of Physiology, University of Ibadan, Nigeria. The rats were housed in suitable cages which allow the free flow of air and also contain wood shavings as beddings.

Each sample group contains seven rats each. The rats were bred in the animal house of the University and kept under standard conditions in a well-ventilated room at temperature of 26.0 ± 2.0°C. Rats experienced conditions of 12 hours light/dark cycle for 5weeks and fed with a standard rodent pellet and water. Pellets were purchased from certified Top feed mills outlet in Ondo City. After the acclimatization period the rats were used for the experimental work. All experiments were carried out in accordance with guidelines of Experimental Animal Ethic Committee, refrigerated to preserve the integrity of cells and prevent enzyme denaturation [24].

Preparation of spore suspensions for the infection of wistar rats

Each of the fungi was inoculated onto fresh PDA plates and incubated at 27 °C for 14 days for sporulation to take place. Spores and conidia were harvested from these plates with 0.1 percent Tween 80 solution and sterile glass rods. The number of active spore and conidia was determined and adjusted for subsequent inoculation into the experimental animals [25].

Inoculation of *B. bassiana* into albino rat

Laboratory animals were injected with 1ml of the prepared microbial suspension after being adjusted to 106 sfu/ml using a spectrophotometer. The pure cells and conidia of fungi were injected into each rat intraperitoneally using the 1ml syringe [26]. Rats were watched for seven days before being sacrificed and their blood harvested for hormonal analysis

Collection of blood from infected wistar rat

The albino rats were made to fast overnight and sacrificed after seven days. The rats were put into air-tight jar containing diethyl ether and were slightly anaesthetized. Blood samples were collected from the overnight fasted rats under anaesthesia via tail and ocular vein puncture using heparin bottle immersed in ice-cold water. The clot was removed by centrifuging at 4000 rpm for 15 minutes; the resulting supernatant which was the serum was collected into plain tubes and labelled accordingly for hormonal test [27].

Hormonal assay on infected animals

Testosterone

The serum testosterone concentration was quantitatively determined using the direct human testosterone enzyme immunoassay (EIA) kit as described by the manufacturer's protocol which adopted the principle of Tietz[28] with modifications from Nnamah et al. [29]. The testosterone EIA is based on the principle of competitive binding between testosterone HRP conjugate for a constant amount of rabbit anti-testosterone.

The number of desired coated wells in the holder was secured. 10 µl of standards, specimen and controls were dispensed into appropriate wells. 100 µl of testosterone-HRP conjugate reagent was dispensed to each well and mixed thoroughly for 30 seconds and incubated at 37°C for 90 minutes for the standards, specimen and control. The microwells were rinsed and flicked for 5 minutes with washing buffer. 100 µl of 3,3',5,5'-Tetramethylbenzidine (TMB) reagent was dispensed into each well, mixed gently for 5 seconds and incubated at room temperature for 20 minutes inside a dark chamber. The stop solution is added to terminate enzymatic reactions and stabilize color development allowing for accurate measurement. The blue colour completely turned to yellow and the absorbance was read at wavelength of 450 nm with a micro titre well reader within 15 minutes of the preparation.

Follicle stimulating hormone

The serum FSH was quantitatively determined using the direct human serum follicle stimulating enzyme immunoassay (EIA) kit as described in the manufacturer's protocol, which adopted the principle of Tietz[28].

Microplate wells for each serum reference, control and samples to be assayed were in duplicate. 25 µl of each calibrators, control serum and samples were pipetted into appropriate wells. 100 µl conjugate was pipetted into each well for control and sample, except blank and incubated on a thermoshaker for 30 minutes at 37°C. The wells were washed 5 times with 300 µl of working washing solution per well and tapped firmly against absorbance paper to ensure that it is dry. 100 µl of TMB substrate was pipetted into each well at timed interval and incubated for 30 minutes at room temperature in a dark place. 150 µl of stopping reagent was pipetted into each well and mixed gently for 5-10 seconds. The plate was read on microplate reader at 450 nm within 20 minutes after.

Luteinizing hormone

The serum LH was quantitatively determined using the direct human serum luteinizing enzyme immunoassay (EIA) kit as described in the manufacturer's protocol, which adopted the principle of Tietz [28].

Microplate wells for each serum reference, control and samples to be assayed were in duplicate. 25 µl of each calibrators, control serum and samples were pipetted into appropriate wells. 100 µl conjugate was pipetted into each well for control and sample serum, except blank and incubated on a thermoshaker for 30 minutes at 37°C. The wells were washed 5 times with 300 µl of working washing solution per well and tapped firmly against absorbance paper to ensure that it is dry. 100 µl TMB substrate was pipetted into each well at timed interval and incubated for 30 minutes at room temperature in a dark place. 150 µl of stopping reagent was pipette into each well and mixed gently for 5-10 seconds, the plate was read on microplate reader at 450 nm within 20 minutes after addition of the stopping reagent.

Progesterone

The serum progesterone was quantitatively determined using microplate immunoenzymometric assay kit as described in the manufacturer's protocol, which adopted the principle of Tietz [28]. To 0.025 ml of each calibrator, control and serum samples were pipetted into microplate wells. 0.10 ml of conjugate was pipetted into each well and the micro-plate was swirled gently for 20-30 seconds to mix and incubated for 60 minutes at room temperature, the content of the micro-plate was decanted and 0.30 ml

washing solution was added repeatedly four times. 0.10 ml of TMB Substrate was added and incubated for 25 minutes at room temperature in a dark place. 0.15 ml of stopping reagent was pipetted into each well. The plate was read on microplate reader at 450 nm within 20 minutes after.

Statistical Analysis

The data obtained from the hormonal assays were subjected to statistical analysis to compare the means of groups as well as the standard deviation. T-Test was applied to compare the test and control groups. $P < 0.05$ was considered to be significant

Results

Isolation of *B. bassiana* from *Z. variegatus*

B. bassiana was isolated from the cadavers of the insects using the Veen's media. The fungus has a whitish muscadine growth when re-cultured on SDA. It was kept on slants for further analysis while a new subculture was prepared to incubate for 14 days for sporulation and subsequent harvest to take place. The microscopic feature of the fungus is described in Table 1.

Hormonal assay on infected animals

Rats injected with *B. bassiana* showed physical and stress-related symptoms including slightly hunched posture, raised fur and reduction in the rate of feeding. The Hormonal assay as represented in Table 2, generally showed a slight reduction in the level of the hormones assayed for in infected rats group compared to the rats in the control group with the exception of the follicle stimulating hormone which was hardly affected in rats infected with *B. bassiana*. The highest difference was noticed in the progesterone level which reduced in the rats within the test group compared to those in the control group.

Discussion

The isolation of *B. bassiana* from *Z. variegatus* as demonstrated in this study further affirmed that Beauveria bassiana is a well-known entomopathogenic fungus that infects a wide range of insect hosts. The grasshopper species used in this study is a significant agricultural pest in Africa, causing extensive damage to crops [30]. The use of *B. bassiana* as a biological control agent has been studied due to its ability to naturally infect and kill *Z. variegatus* thus decimating the numbers of insect pest that may destroy crops and cause significant damage to food crops.

The infection of *Z. variegatus* by spraying the insects with the spore suspensions of Beauveria bassiana also supports the submissions by Balogun and Fagade[31] that the entomopathogenic fungus infects the insect through the contact of the chitinous cuticle with the conidia (spores) of the fungus. The spores adhere to the cuticle of the grasshopper, germinate, and penetrate the insect's body, eventually leading to its death. The fungus proliferates within the host and exhibits its presence by producing a whitish muscardine growth as noticed on the grasshopper cadavers (plate 1) used in this study thus producing toxins and disrupting physiological processes (Goettel and Inglis[21]).

Table 2. Effect of entomopathogens on hormonal levels in experimental rats

Hormone	*B. bassiana*	Control
Progesterone (ng/mL)	6.67±1.16a	6.33±0.58a
Testosterone (ng/mL)	3.40±0.20a	3.50±0.17a
Luteinizing (ng/mL)	16.40±0.10a	16.43±0.06a
Follicle Stimulating (mIU/mL)	2.00±0.10a	1.93±0.12a
Active Straight Leg Raise	1.96±0.73	2.39±0.59*

The virulence of *B. bassiana* on *Z. variegatus* can be attributed to its proven ability to produce proteases, chitinases, and lipases. The insect cuticle contains a waxy layer composed mainly of lipids, which acts as a hydrophobic barrier preventing water loss and pathogen entry. All of these are enzymes that can work in synergy to degrade insect cuticle thereby providing an entry point for the fungal hyphae which eventually lead to their sicknesses and subsequent death [32]. Aside this, *B. bassiana* has also been shown to degrade the chitin covering of insects facilitated by a combination of mechanical force and enzymic degradation [33].

Behavioural signs exhibited by rats inoculated with B. bassiana including the hunched posture, piloerection and reduction in food intake reflects the progression of infection as well as the immune response of the host [34].

Although the Hormonal assay which showed a difference in progesterone levels in rats challenged with *B. bassiana* and unchallenged rats. However, the change in progesterone level is still within the acceptable range for female rats in the estrus phase. A slight drop was only noticed in the values of Luteinizing hormone in both test and control rats. The decrease in the values of luteinizing hormone is also still within the normal range of female rats within the estrus phase while the slight change in follicle stimulating hormones is well within the normal range for female rats within the puberty age range. A reduction in the testosterone level of rats challenged with *B. bassiana* is only slightly below the baseline level for male rats that are not

Table 1. Examination of B. bassiana under the microscope

Isolate	Cultural characteristics	Microscopic examination	Suspected Organism
VM1	Powdery mycelia which is whitish to pale yellow.	Conidia are hyaline, short and globose or ovoid in shape Conidiogenous cells are flask-shaped, rachiform, proliferating and aggregating into sporodochia.	*Beauveria bassiana*

engaged in sexual activities.

Since progesterone is a steroid hormone (hormone of pregnancy) [35] which plays an important role in the preparation for and maintenance of pregnancy [36], the likely hood of the occurrence of gestational anomaly in the administration and usage of *B. bassiana* may be unlikely since the values obtained in the test and control rats group are still within the acceptable rate.

Hormonal assay showed that testosterone level was slightly reduced in rats challenged with B. bassiana below the baseline values. Since testosterone is involved in health and general well-being and the prevention of osteoporosis [37], insufficient levels of testosterone may arise from the repeated accidental exposure to these microorganisms when used as biopesticides. However, testosterone reduction can be supplemented in such cases through the usage of testosterone boosting therapeutics.

Levels of follicle stimulating hormone was relatively higher to what was obtained in the control rat group but both values are still within the acceptable range. This enzyme is needed to regulate follicular growth and maturation of ovaries, trigger ovulation in females [38]. There is a reduced likelihood that subsequent use and exposure of females to the entomopathogenic strain may affect or prevent the maturation of the ovaries and even affect subsequent implantation of a foetus after conception

LH is a crucial gonadotropin produced by the anterior pituitary gland which plays a significant role in the regulation of reproductive functions. Impaired LH production may lead to a decrease in testosterone synthesis thus resulting to reduced libido, delayed ovulation, erectile dysfunction, and infertility. Insufficient LH during adolescence can also delay the development of secondary sexual characteristics [38]. Although the LH levels within rat in the test group is slightly lower compared to those within the control group, it is unlikely that the reduction will result to a major impairment of the reproductive functions because the reduction falls within acceptable LH levels within the rats.

Conclusion

This study shows that the exposure of wistar rats to B. *bassiana* within a 7-day period has no major effects on their reproductive hormones thus highlighting and further emphasizing the safety of these agents when employed as biocontrol agents. These results is a good indication that it might be safe to integrate these agents in pest management practices without adversely affecting non-target mammalian species, including humans. However, further research especially on long-term studies, varied environments and extended exposure are necessary to better understand the broader impacts of these agents on reproductive health so as to further affirm their safety across different environmental and biological contexts.

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RESEARCH ARTICLE

Exploration of Histone H2B and Some Biochemical Markers in Sudanese Women with Breast Cancer

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Abstract

Background: H2B protein is associated with breast cancer and is thought to be an effective biomarker. This paper investigates the potential role of the H2B protein as a tumor marker and its association with some biochemical molecules in breast cancer patients.

Methods: This study was a retrospective hospital-based case-control study. A total of 121 Sudanese breast cancer patients with different stages of the disease and 31 healthy individuals as the control group were included in this study. A pretested structured questionnaire was used to collect participants' data, such as age, sex, district, type of therapy, and cancer stage. Blood samples were collected for laboratory investigation. H2B protein was measured by Enzyme-Linked Immunosorbent Assay (ELISA), and biochemical tests were measured by a spectrophotometer. The study data were analyzed by the GraphPad Prism software version 5.00.

Results: Most cases of breast cancer were prevalent in Khartoum, Darfur, Kordofan, and Gezira, respectively. They were commonly treated with surgery, chemotherapy, and radiation therapy. The diagnostic role of the H2B protein was investigated, and the mean value was found to be significantly different ($p < 0.05$) from the control group. Regarding the disease stages, the mean values in the late stages (stages II and III) showed significant differences ($p < 0.01$ and < 0.001, respectively) compared to the control group. However, there is no significant difference ($p > 0.05$) between the mean value in the early stage (stage I) and the control group. This result showed that the H2B protein can be used as a biomarker for breast cancer. Moreover, the H2B mean values were positively associated with cholesterol and lactate dehydrogenase (LDH), and negatively correlated with blood glucose and protein levels.

Conclusion: The results demonstrated a significant link between epigenetic (histone H2B levels) and metabolic changes (glucose, LDH, and cholesterol) in breast cancer patients, especially at the late stages.

Keywords: Breast cancer, Tumor marker, H2B protein, Blood glucose, Lactate dehydrogenase; Cholesterol

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Introduction

Breast cancer (BC) is a heterogeneous disease, comprised of various subtypes, which have different risk factors for incidence, therapeutic response, disease progression, and preferential organ sites of metastases [1]. BC is the primary causes of deaths among females and expected to reach around 3.2 million new cases worldwide by 2050 [2,3]. In Sudan, breast cancer was the most predominant malignancy among Sudanese female cancer patients [4]. Most cases were young-aged women; about 40% were below 45 years (mean age of 50) with late stages of the disease [4]. Therefore, the diagnosis and prediction of breast cancer are essential for the identification and control of the disease [5,6]. Although different breast cancer biomarkers are approved by the FDA as a diagnostic biomarkers, such as cancer antigen 15-3 (CA15-3) and carcinoembryonic antigen (CEA), they become less sensitive in the diagnosis of breast cancer progression and metastasis [7,8]. Additionally, other biomarkers like estrogen receptors (ERs), progesterone receptors (PRs), and human epidermal growth factor receptor 2 (HER2) are routinely used in breast cancer diagnosis [9]. However, their sensitivity and selectivity remain a challenge due to the presence of complex event that involves the mutation or deregulation of multiple genes [10].

It is well established that histone variants are associated

with the molecular and biological features of tumor cells [11]. Moreover, histone variants are conventional histones, comprising non-allelic isoforms (H2A, H2B, H3, and H4), that are encoded by independent genes and serve as diverse epigenetic marks by regulating the transcriptional outcomes [12]. For example, the H2B protein undergoes post-translational modifications (PTMs) and regulates fundamental processes in cancer development, including DNA damage response and expression of tumor-associated genes [13]. The incorporation of H2B into chromatin results in compact nucleosome configurations that change chromatin accessibility and trigger oncogenic and pro-inflammatory pathways [14]. In breast cancer, there are several deregulated H2B histone variations. In aggressive subtypes of breast cancer, such as triple-negative and HER2-enriched tumors, H2B overexpression is more common and is linked to a lower overall survival rate. Higher expression of the variation is seen in Asian, African American/Black populations, and in young female patients [15]. The available information comes solely from in vitro mechanistic investigations and tissue-based expression research [15], indicating a considerable gap in the translational potential of H2B as a blood biomarker. Moreover, blood-based H2B inquiry should be prioritized above traditional tissue analysis because of its non-invasive nature, demonstrated clinical effectiveness across a variety of disorders, capacity to capture systemic heterogeneity, and biological stability [16]. This strategy has revolutionary potential for real-time monitoring, early diagnosis, and tailored therapeutic interventions. However, to our knowledge, there is limited information on H2B blood levels as a diagnostic biomarker for breast cancer. Therefore, this study aims to investigate the diagnostic value of the H2B protein in the circulating blood of breast cancer patients and its possibility to be a biomarker. Furthermore, some biochemical parameters such as blood glucose, total protein, total cholesterol and LDH were measured to study their relation to H2B proteins and tumor stage progression.

Materials and Methods

Study population

The population consists of breast cancer patients and controls, healthy subjects matched to the case in terms of age. Breast cancer patients of any age who are taking different types of cancer treatment were included, based on their willingness. Breast cancer patients with diagnostic necrosis were excluded. In the context of the control group, healthy people of age relevant to cancer patients were included in this study. However, people taking medications before sample collection were excluded from being included in the control group.

The sample size was calculated using the following formula: n = N/1+ N (d)2; where n: sample size, N: Population, and d: degree of significance (0.05). The estimated study population (N) is 175 patients. Therefore, n= 175/(1+(175×0.0025)) = 122. The sample size of this study was about 122 patients.

Data collection and analysis

Secondary Data

A pretested structured data sheet containing closed questions, including age, gender, and district, has been used.

Assessment of H2B protein levels

Venous blood samples from 122 breast cancer patients and 31 healthy individuals were collected in test tubes containing EDTA as an anticoagulant, mixed for 10 min, and centrifuged at 1000 g (relative centrifugal force, RCF) for 5 min. Then, the H2B protein levels were measured by an ELISA Kit as described by Lodish et al. [17]. Briefly, the Kit components were equilibrated for 20 minutes at room temperature. Standards (9 ng/ml, 6 ng/ml, 3 ng/ml, 1.5 ng/ml, and 0.75 ng/ml) were added to designated wells, and a control diluent was added to the control well. Sample wells received 40µl of diluent buffer followed by 10µl of the sample. The plate was shaken, sealed, and incubated at 37°C for 30 minutes, then washed five times. HRP-conjugated anti-H2B antibody was added to wells (except control), followed by another incubation. TMB substrates A and B were added, mixed, and incubated in the dark at 37°C for 15 minutes, resulting in blue shades. After adding a stop solution, the color changed to yellow, and absorbance was read at 450 nm within 15 minutes.

Biochemical investigations

Venous blood samples from previously described individuals were taken in plain test tubes and centrifuged at 1000g (RCF) for 5 min. Then, the levels of blood glucose, total cholesterol, total protein, and lactate dehydrogenase (LDH) were investigated according to the corresponding manufacturer's instructions, using Biosystem commercial kits (Barcelona, Spain).

Data analysis

The study data were analyzed by the GraphPad Prism software version 5.00. Data are presented as the mean ± SEM. Student's t-test (two-tailed; Mann-Whitney) was used to compare the two independent groups (e.g., patients vs. control group). ANOVA with Tukey's post-hoc test was used to evaluate the pair-wise comparisons across groups. The two-sided probability level $p < 0.05$ was considered statistically significant. Differences with $p < 0.05$ are described as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

Ethical consideration

The ethical committee of the Ministry of Health, Khartoum State, approved this study in 2022. Consent was obtained from each patient, and permission from the Ministry of Health and from the general manager of the hospital was obtained.

Results

Demographic characteristics of breast cancer patients

A total of 121 breast cancer patients, selected randomly, were found to be females with a mean age of 47.5 ± 1.74 years. The most cases were prevalent in Khartoum state (29. 75%), followed by Darfur (15.70%), Kordofan (13.22%), Gezira (10.74%), Northern State (9.09 %), White Nile (8.26%), Sennar (4.96 %), the Nile River (4.15 %), the Blue Nile (2.48 %), and Gadarif (1.65 %), respectively.

Clinical characteristics of breast cancer patients.

The majority of patients in this study were in early disease stages, and they were treated with different therapeutic types. Regarding disease stages, out of 121 subjects who were diagnosed as breast cancer patients, 55 (45%) were at stage I, 37(31%) were at stage II, and 29 (24%) were at stage III, as shown in Table 1. In the context of treatment, 41 patients (22.3%) undergone surgery, 111 patients (60.3%) were treated with chemotherapy either alone or in combination with other therapy, 28 patients (15.2%) were treated with radiotherapy either alone or in combination with other therapy, and only 4 patients (2.2%) were treated with hormone therapy (Table 1).

Table 1. Clinical characteristics of breast cancer patients based on their disease stages and treatments

Patient Characteristics	Number of Patients	Percentage (%)
Cancer stage		
Stage I	55	45
Stage II	37	31
Stage III	29	24
Treatment*		
Surgery	41	33.88
Chemotherapy	111	91.74
Radiation therapy	28	23.14
Hormone therapy	4	3.31
*The percentages of this group are based on the patients who received each treatment.		

Blood biomarkers

H2B protein

H2B protein levels were investigated in breast cancer patients and the control group, and the results were presented in Table 2. The H2B protein levels were found to be higher in breast cancer patients compared with the control group. The mean value of H2B protein was significantly increased (P<0.05) in breast cancer patients, Figure 1.

The mean values of H2B protein were compared with the control group among the breast cancer stages (Table 3 and Figure 2). Patients in stage III showed the highest mean value, followed by stage II, while the patients in stage I and the control group showed the lowest mean value. Based on these results, it can be concluded that stages III and II tend to have high levels of H2B. Statistical tests confirmed that there are significant changes in the late stages (stages II and III, respectively, whereas there is no significant change (p> 0.62) in stage I compared to the control group.

Table 2. The mean value of H2B protein among breast cancer patients and the control group.

Parameters	Number of subjects	H2B protein levels (Mean ± SEM)
Patients	121	2.93 ± 0.07243
Control	30	2.51 ± 0.09194
p-value	-	< 0.05 (0.0007)

Table 3. The mean values of H2B protein among the stages of breast cancer patients and the control group.

Parameters	Control	Stages of breast cancer patients		
		T	N	M
H2B protein level (Mean ±SEM)	2.51± 0.092	2.57± 0.055	3.23± 0.152	3.79± 0.206
p-value	-	> 0.05*	< 0.01	< 0.001

Table 4. The mean levels of blood glucose, total protein, cholesterol, and LDH among breast cancer patients and the control group

Blood Biomarker	Control (No. 30)	Patients (No. 121)	p-value*
Blood glucose	91.87 ± 2.105	59.31 ± 2.990	<0.001
Total protein	7.18 ± 0.244	6.94 ± 0.086	>0.05
Total cholesterol	185 ± 6.364	244 ± 5.470	<0.001
LDH	347 ± 9.684	563 ± 18.93	<0.001
*Data presented as the mean ± SEM. p value computed by using two-tailed (Mann-Whitney)			

Table 5. Correlation of biochemical markers with H2B blood levels in breast cancer patients.

Biochemical Marker	Correlation Coefficient (Pearson's r)	p-value (two-tailed)
Cholesterol	0.1756	0.0310*
LDH	0.0834	0.3325
Glucose	-0.1291	0.1271
T. Protein	0.2455	0.0025**
*p < 0.05, **p < 0.01		

Considering the breast cancer stages, the mean values of biochemical markers were also compared with the control group (Figure 3). The patients in stage II showed the lowest glucose mean levels followed by stages III and I, respectively. Statistical analysis demonstrated that there is a significant reduction in the blood glucose levels (Figure 3A) in all tumor stages (I, II and III) when compared to the control group. However, there are no significant differences in total protein levels among all stages (Figure 3B).

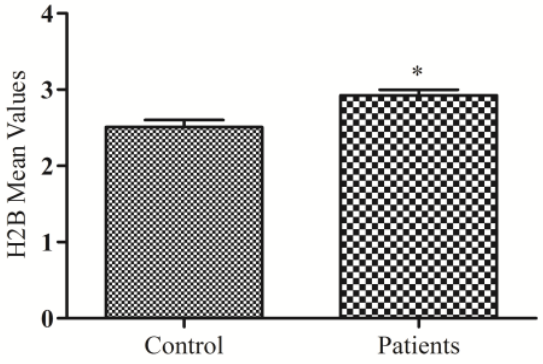

Figure 1. Comparing the H2B mean levels in control and breast cancer patients. Graph bars represent the mean ± SEM. *, P < 0.05; Two-tailed (Mann-Whitney) test.

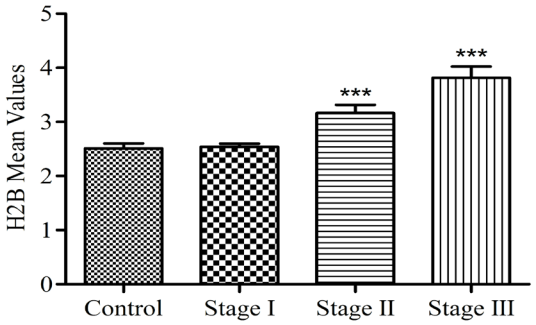

Figure 2. The H2B mean levels in control and breast cancer stages. Graph bars represent the mean ± SEM. **, P< 0.01; ***, P < 0.001, One way ANOVA (Tukey's Multiple Comparison Test).

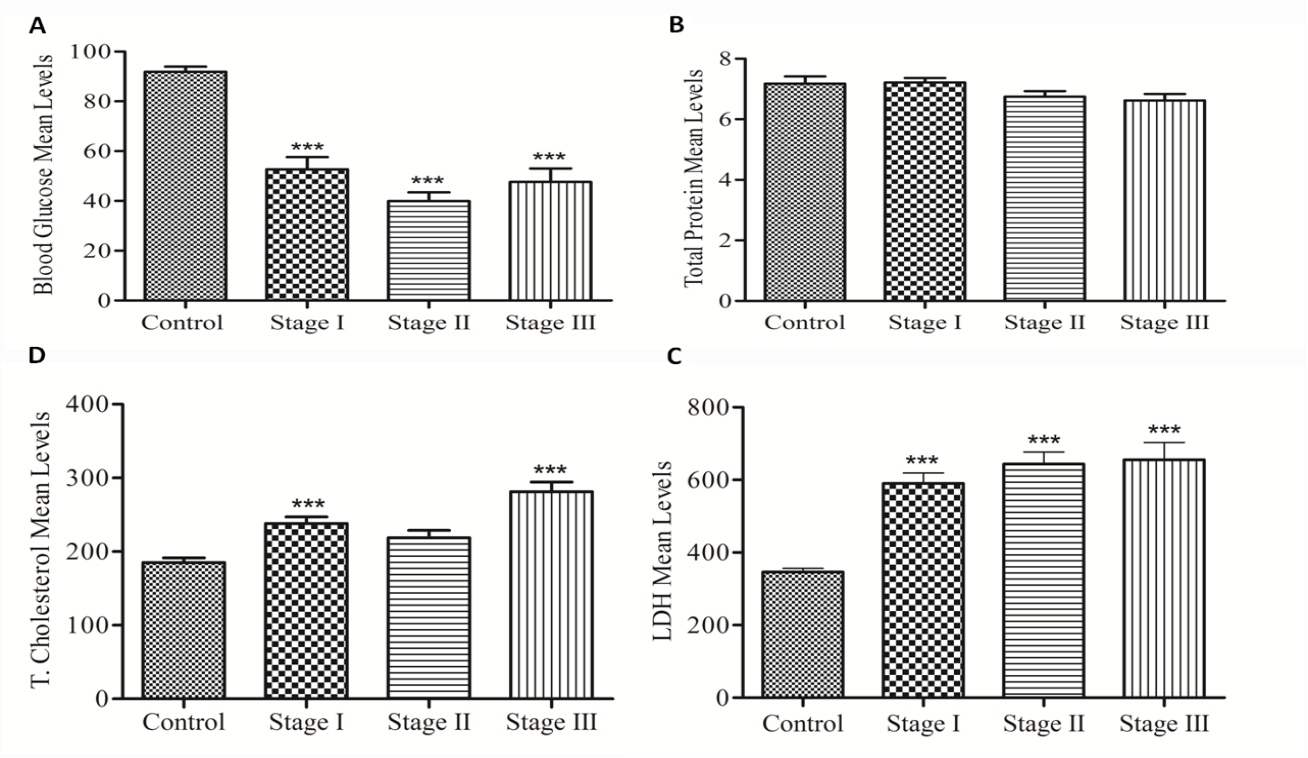

Figure 3: The biochemical marker mean levels in control and breast cancer stages. Graph bars represent the mean ± SEM. One-way ANOVA (Tukey's Multiple Comparison Test) is used to compute the significant changes (p-value) between the control and all tumor stages I, II, and III.

Then, the mean values of total cholesterol and LDH, in all cancer stages, were compared with the control group (Figure 3C and 3D). The mean values of total cholesterol and LDH in breast cancer stages (stages I, II and III) were found to be higher than the healthy control group. Figure 3D confirmed that there are significant differences in cholesterol levels between cancer stages I and III. However, there are no significant changes between patients in stage II and the control group. Similarly, the mean values of LDH in breast cancer stages (stages I, II and III) were found to be significantly higher than the control group (Figure 3C).

Discussion

Factors associated with breast cancer patients

This study was a case-control-based hospital study; it was conducted in the Radio Isotope Center in Khartoum (RICK), which is a reference hospital for Sudanese cancer patients. A total of 121 patients with breast cancer were included and selected randomly in the study. All patients were found to be females. Breast cancer in Sudanese males is considered rare; it constitutes 3.5-4%, as reported by Amani et al. [18].

In this study, the median age in Sudanese patients with breast cancer was found to be 48 years; this result is considered to be in line with previous results reported by Elhoweris [19]. Most cases in this study were prevalent in Khartoum state, Darfur, Kordofan, Gezira, the Northern, White Nile, Sennar, Nile River, Blue Nile, and Gadarif, respectively. This result is in agreement with those previously reported by Ali and Saeed [20,21]. These differences in prevalence may be due to several factors, such as the different rates of screening and diagnosis, poverty, a lack of primary health care in some states, and traditional mineralization, as well as genetic and environmental factors.

The majority of patients in this study were in early disease stages; this may support the successful screening and early detection programs or rapid death without diagnosis. Or due to relatively indolent growth rates in breast cancer patients, which can be detected by early symptoms or screening, allowing a long pre-clinical window [22].

According to this study, the most common treatments for patients with breast cancer in Sudan are chemotherapy, surgery, radiation therapy, or combined therapy, depending on the patient's status and the stages of the disease. There is also hormone therapy, but until now it's been rare.

H2B protein as a predictive biomarker for breast cancer

An elevation of histone H2B most directly associates with increased cellular proliferation and turnover in the tumors. However, its release into blood circulations is a complex biomarker that can be influenced by greater tumor burden, necrotic cell death and active secretion [16]. Therefore, it is a complicated mechanism of dynamic tumor biology [16]. The results showed that the concentration of H2B protein increases when the disease progresses to the late stages (stages II and III). This result is in line with the result of a study conducted by Kang and his colleagues [23]. They measured H2B gene expression in normal and tumor tissues; their study showed that H2B genes were differentially expressed in cholangiocarcinoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, and cutaneous melanoma. Although their study applied to other tumors on a genetic level, the result supports our result based on the fact that the proteins are the products of expressed genes. Moreover, the data from the HPA database showed that the protein expression of H2B genes was more highly expressed in glioma than in normal tissues; this result is also in line with our result regardless of the type of cancer. Besides that, a high frequency of H2B gene alteration was shown in mature B-cell tumors and ovarian epithelial cancer; this H2B gene alteration may lead to mutations that are subsequently associated with different types of cancer, including breast cancer. An explanation for the expression of the H2B protein in breast cancer may be the epigenetic role of histone variants in cancer development. Jia and colleagues [24] reported the importance of these variants in cancer progression and development. Together, these results may support our finding that there is an increase in H2B levels, particularly in the late stages. Therefore, high expression of the H2B protein level can be used as a diagnostic biomarker for breast cancer.

Other associated biochemical markers for breast cancer

Cancer cells consume more glucose than normal cells to fulfill their energy needs for rapid growth and proliferation. The finding indicates that tumor cells in breast cancer patients can reprogram the glucose metabolism by increasing the rate of glycolysis, the Warburg effect, which can lead to hypoglycemia [25]. Based on these facts, our results showed that the mean values of the blood glucose in all tumor stages (stages I, II, and III) were less than the mean value of the control group. However, in the context of total protein levels, our results showed that there is no significant difference between the control group and patients in all stages of the tumors. This result disagrees with the finding that the mean values of the protein concentration in serum of breast cancer patients were significantly higher than those in healthy individuals with a statistical significance ($P<0.05$) [26]. This controversial maybe due to the genetic and metabolic conditions that differ from one population to another.

Furthermore, nutritional behavior is another factor that may affect the ratio of protein levels. It is known that the concentration of the plasma total protein depends on the balance between the rate of synthesis and the rate of loss, and this balance is controlled by the metabolic genes [27]. However, metabolic reprogramming is a feature of cancer cells, and it includes reprogramming of protein metabolism as well as amino acids. Glutamine is an important amino acid for cancer metabolism, and a source of this amino acid is protein catabolism. Theoretically, this means that cancer cells may degrade protein more than in normal conditions, and this study did not prove this assumption.

In addition to protein, the cholesterol level is sought to be higher in breast cancer [28]. As well, the current results showed that the mean values of cholesterol were found to be higher in breast cancer patients than in the control group. These results are similar to a finding reported by Ehmsen et al. [29], who concluded that the increased de novo synthesis of cholesterol is a feature of breast cancer cells and inhibiting the cholesterol synthesis decreases the growth of cancer stem cells. In addition, a study conducted by Wang et al. [30] concluded that many cancer cells overexpress low-density lipoprotein receptor (LDLR) compared to normal cells; the enhancement of cholesterol uptake by cancer cells leads to their rapid proliferation and progression. This finding also falls in line with our results. Although we did not find significant increases in cancer stage II, we can conclude that breast cancer cells may share the same feature of de novo synthesis of cholesterol.

To this end, LDH is one of the most important enzymes, which converts pyruvate into lactate in the hypoxic tumor microenvironment and leads to its acidification. The result of this study showed that there are significant increases in LDH levels among the cancer stages I, II, and III. Of note, this change is noted when the disease goes into the late stages, indicating the potential role of LDH in breast cancer. Moreover, the high levels of LDH enzyme may effectively convert glucose into lactate, explaining the decreased levels of glucose in breast cancer patients. These findings are in line with those of Ratnikov et al. [31], who reported that tumor cells convert 60% to 80% of glucose to lactate; the rate may rise up to 90% in a hypoxic tumor microenvironment. Previous findings [32] also reported

the high levels of LDH and its potential role in different cancer types, including breast cancer. Overall, excessive accumulation of lactate in breast cancer plays a significant role in tumor drug resistance [33].

Conclusion and Perspective

Among all types of cancers in Sudan, breast cancer is the most prevalent. The results demonstrated that the H2B protein is increased in the late stages of breast cancer, confirming its value as a tumor marker. Moreover, the hypoglycemia is considered a normal result in breast cancer patients because tumor cells consume more glucose. The study did not find significant changes in the total protein. However, the elevated level of cholesterol in this study was found to play a role in breast cancer development and proliferation. Moreover, the increased levels of LDH as a result of metabolic reprogramming can be used as a significant tumor marker in breast cancers. Collectively, there was a positive association between H2B, T. cholesterol, and LDH mean levels in breast cancer patients and tumor stages. In contrast, the blood glucose levels were negatively associated with the H2B levels and tumor stages. Moreover, there were no changes in the total protein levels among the tested groups.

Despite these promising results, a large sample size is recommended to confirm the potential role of H2B protein in breast cancer patients. In addition, more clinical and molecular investigations are required to know the mechanisms and the risks behind breast cancer and other cancer types in Sudan. Although some biochemical tests, such as glucose and total protein, are investigated, their exact relation with H2B in cancer is not clear.

Author contributions

Dr. Ahmed M. E. Abdalla and Ahmed I. M. Ahmed designed the primary concept, idea, and wrote the first draft. Dr. Yasir A. Taha contributed to the data analysis, draft revision, and interpretation of the topic. Dr. Ahmed M. E. Abdalla and Dr. Yasir A. Taha approved the final version and decided for all aspects of the work integrity.

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Data Availability Statement

No data were used to support this study.

Conflict of interest statement

All the authors declare no conflicts of interest.

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RESEARCH ARTICLE

The Association of Polysomnographic Variables with BMI, Gender, and Age in Sudanese Adults with Obstructive Sleep Apnea

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Abstract

Background: Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder characterized by repeated upper airway collapse during sleep. Despite its global prevalence, data on OSA in Sudanese populations remain limited. This study aimed to explore the relationship between polysomnographic variables and body mass index (BMI), gender, and age in Sudanese adults with OSA.

Methods: This retrospective study analyzed polysomnography data from 251 Sudanese adults. Given the non-normal distribution of sleep data, non-parametric tests were used. OSA severity was classified using the apnea-hypopnea index (AHI). Key parameters—AHI, arousal index, desaturation index, sleep efficiency, and snoring—were analyzed against BMI, age, and gender using Mann-Whitney U tests, Kruskal-Wallis tests, and Spearman's rank correlation. A negative binomial generalized linear model (GLM) was used to assess predictors of AHI.

Results: BMI showed a moderate positive correlation with AHI (Spearman's rho = 0.307, $p < 0.001$) and desaturation index (rho = 0.342, $p < 0.001$). AHI increased significantly across BMI categories ($p < 0.001$), with obese class III patients showing the highest median AHI. Age was negatively correlated with sleep efficiency (rho = -0.230, $p < 0.001$). Males had significantly higher arousal index ($p < 0.001$) and snoring ($p = 0.016$) than females, but AHI did not differ significantly between genders. The GLM regression confirmed BMI as a significant independent predictor of higher AHI (Incidence Rate Ratio = 1.024, $p = 0.002$).

Conclusion: In this Sudanese cohort, BMI was the most significant factor associated with OSA severity. Increasing age was linked to poorer sleep efficiency. While males exhibited more sleep disruption, OSA severity (AHI) was comparable between genders, highlighting the importance of considering OSA in females. These findings underscore the need for weight management strategies in managing OSA in this population.

Keywords: Obstructive Sleep Apnea, Polysomnography, Body Mass Index, Apnea-Hypopnea Index, Sudanese Population.

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Introduction

Recurrent episodes of upper airway narrowing or obstruction during sleep, which result in a significant decrease or halt of airflow, oxygen desaturation, and frequent arousals, are the hallmarks of obstructive sleep apnea (OSA) [1,2]. In the United States, it is a prevalent illness that is more common in men and becomes more prevalent as people age, with an estimated 26% prevalence among patients between the ages of 30 and 70.3 The apnea-hypopnea index (AHI), or the average number of apneas and hypopneas per hour of sleep, is used to gauge the

severity of the disorder. When an AHI of ≥5 is combined with daytime sleepiness, it is referred to be OSA, and when it is ≥5, it is called OSA syndrome. Daytime sleepiness was classified as mild, moderate, or severe by the American Association of Sleep Medicine based on how it affected social interactions during the day [3]. Patients with obstructive sleep apnea are being treated differently in each nation based on their individual symptoms. Although significant efforts are being made in settings with adequate resources to detect and treat obstructive sleep apnea, research indicates that even in industrialized nations, the majority of instances of obstructive sleep apnea go misdiagnosed and untreated. Obstructive sleep apnea is often not well known in underdeveloped nations, and available diagnostic and treatment solutions are frequently unavailable or have not been modified for environments with limited resources [4]. Obstructive sleep apnea is linked to a significant economic and societal burden because to its complex and social effects. The estimated cost of diagnosing and treating obstructive sleep apnea in the United States was $12.4 billion in 2015 [4]. Research indicates that obstructive sleep apnea plays a significant role in adverse health outcomes and that in general, treating this illness helps to improve sleep-related quality of life and reduce related negative clinical consequences [5]. A group of cardiovascular risk factors known as metabolic syndrome (MS) include high blood pressure, overweight or obesity, hypertriglyceridemia, low HDL-C (high-density lipoprotein cholesterol), and glucose intolerance. When three or more of the five distinct components are present in the same person, MS is diagnosed in adults and, more recently, in children and adolescents. MS is a significant risk factor for chronic illnesses and is becoming more common due to a sedentary lifestyle and increased childhood obesity [6]. When there is no other neuromuscular, mechanical, or metabolic explanation for hypoventilation, obesity (body mass index (BMI) ≤30 $kg \cdot m^{-2}$), sleep disturbance, and daytime hypercapnia (arterial carbon dioxide tension ($PaCO_2$) ≥45 mmHg at sea level) during wakefulness are combined to form obesity hypoventilation syndrome (OHS). An apnea/hypopnea index (AHI) of ≤5 occurrences h^{-1} indicates obstructive sleep apnea (OSA), which affects about 90% of individuals with OHS. Concomitant severe OSA is seen in about 70% of patients (AHI ≤30 events$\cdot h^{-1}$) [7]. The prevalence of OSA has not been directly examined between racial groups in many research. Furthermore, comparisons of OSA prevalence among research are limited by the absence of uniform criteria for defining OSA. However, the data that is currently available shows that the prevalence of OSA is higher among African Americans, Hispanics, and Native Americans than among US whites, whereas the prevalence among Asians seems to be on level with whites [8]. Sudanese adults represent a distinct population for OSA research due to their unique genetic predispositions, environmental exposures, and cultural practices, which may influence the condition's prevalence, severity, and clinical presentation [9]. Variations in dietary habits, body composition, and access to healthcare may further modulate the relationship between key risk factors, such as BMI, and OSA severity [10]. While obesity is a recognized major risk factor for OSA, non-obese individuals may also develop OSA due to structural abnormalities of the upper airway, underscoring the complexity of the condition. Despite the growing prevalence of obesity in Sudan and its known association with OSA, limited studies have investigated these associations in Sudanese adults, highlighting the need for focused research in this population [11,12]. Existing evidence suggests that OSA is more prevalent in males and increases with age, with obesity playing a significant role in its development and progression [13,14]. However, gender and age differences in OSA severity and clinical manifestations remain poorly understood, particularly in African populations. For instance, females with OSA often report symptoms such as fatigue, insomnia, and mood disturbances, while males more commonly present with snoring and observed apneas. Hormonal fluctuations, particularly in women, add another layer of complexity to understanding how gender impacts OSA severity [15]. Additionally, older adults often experience reduced sleep efficiency and worsening OSA severity due to age-related changes in upper airway anatomy and neuromuscular control. This study aims to investigate the association of polysomnographic variables with BMI, age, and gender in Sudanese adults diagnosed with OSA. This research meets the need for region-specific data by focusing on an understudied population. The findings are expected to provide valuable insights into the multifactorial nature of OSA and inform tailored management strategies for a diverse population.

This study hypothesizes that in Sudanese adults with OSA, higher BMI and male gender will be associated with greater OSA severity, as indicated by a higher AHI, while increasing age will be associated with reduced sleep efficiency.

Methods

This study retrospectively analyzed 251 adult patients diagnosed with obstructive sleep apnea (OSA) through polysomnography (PSG) conducted between January 2020 and January 2021. Participants were recruited from the Sleep Study Center at the Military Hospital in Khartoum, Sudan. Patients were identified based on clinical indications for OSA, including symptoms like excessive daytime sleepiness, loud snoring, witnessed apneas, and unrefreshing sleep. Recruitment was conducted through referrals from primary care physicians and specialists and direct consultations at the sleep center. Ethical approval for the study was obtained from the Faculty of Medicine's ethics committee at the International Africa University (IRB Approval Number: IAU-EC-2020-015).

Inclusion and Exclusion Criteria

Inclusion criteria required participants to be adults aged 18 years or older with a confirmed diagnosis of OSA based on PSG results. Exclusion criteria included the presence of other sleep disorders (e.g., central sleep apnea, restless leg syndrome), significant comorbidities that could influence sleep parameters (e.g., advanced heart failure, chronic obstructive pulmonary disease), or prior treatment for

OSA (e.g., continuous positive airway pressure therapy). Patients with incomplete or poor-quality PSG data were also excluded from the analysis. The sample size was determined by the number of eligible patients who underwent PSG at the center during the study period.

Polysomnography and Classification

Each participant underwent a single night of laboratory-based PSG using the Grass-Comet Plus system. The apnea-hypopnea index (AHI) was calculated to classify OSA severity according to the World Health Organization's criteria: no/minimal OSA (AHI < 5), mild OSA (AHI = 5–15), moderate OSA (AHI = 16–30), and severe OSA (AHI > 30). Body mass index (BMI) was calculated as weight divided by the square of height (kg/m²). Participants were categorized into four age groups: 18–30, 31–49, 50–64, and 65 years and above.

Statistical Analysis

Data analysis was performed using Python (version 3.11) with the scipy, statsmodels, and pyreadstat libraries. The normality of continuous variables was assessed using the Shapiro-Wilk test. All key sleep parameters (sleep efficiency, arousal index, AHI, desaturation index, snoring) demonstrated significant deviation from a normal distribution (all $p < 0.001$), confirming the appropriateness of non-parametric methods.

Descriptive statistics are presented as median and interquartile range (IQR). The Mann-Whitney U test (an equivalent of the independent samples t-test) was used to compare medians between two independent groups (gender). The Kruskal-Wallis test was employed to analyze differences across more than two groups (BMI and age categories), as it is robust to unequal group sizes and non-normal distributions. Spearman's rank correlation coefficients were calculated to evaluate the monotonic relationship between continuous variables (BMI, age) and sleep parameters.

To control for the increased risk of Type I errors from multiple comparisons, a Bonferroni correction was applied to the significance level ($\alpha = 0.05$), resulting in a corrected threshold of $p < 0.002$ for the 25 primary tests conducted.

Given the skewed, overdispersed count nature of the AHI, a negative binomial generalized linear model (GLM) was employed to assess the independent effects of BMI, age, and gender on AHI. This approach does not assume normality of the outcome variable and is more appropriate than standard linear regression. Results from the GLM are reported as Incidence Rate Ratios (IRR) with 95% confidence intervals (CI). For all tests, a p-value ≤ 0.05 was considered statistically significant, with the Bonferroni-corrected threshold applied for multiple comparisons.

Results

A total of 251 patients were diagnosed with obstructive sleep apnea (OSA), comprising 134 males (53%) and 117 females (47%). The demographic characteristics of the study participants, including age, gender, and body mass index (BMI) categories, are summarized in Table 1. The majority of participants were aged 50–64 years (42.6%), followed by those aged 65 years and above (29.5%). Regarding BMI, 34.3% of participants were classified as having obesity class III (BMI ≥ 40 kg/m²), highlighting the strong association between obesity and OSA in this cohort. Only 2.4% of participants had a normal BMI, while the majority fell into overweight or obese categories. This distribution underscores the importance of BMI as a key factor in the prevalence and severity of OSA.

Table 1: Demographic Distribution of Patients (N=251)

Characteristic	Category	N (%)	Min-Max
Age			19-90
	18-30	11 (4.4%)	
	31-49	59 (23.5%)	
	50-64	107 (42.6%)	
	Over 65	74 (29.5%)	
Gender			
	Males	134 (53.4%)	
	Females	117 (46.6%)	
BMI			18.9-84.3
	Normal (18.5-24.9)	6 (2.4%)	
	Overweight (25-29.9)	30 (12.0%)	
	Obesity Class I (30-34.9)	73 (29.1%)	
	Obesity Class II (35-39.9)	56 (22.3%)	
	Obesity Class III (≥40)	86 (34.3%)	

Polysomnographic parameters across BMI categories are presented in Table 2. While sleep efficiency did not differ significantly across BMI groups, the apnea-hypopnea index (AHI), desaturation index, and snoring all increased significantly with higher BMI categories (all $p < 0.05$). A clear dose-response relationship was observed, with participants in the obesity class III group exhibiting the highest median AHI of 51.0 (IQR: 20.9–77.5), compared to a median of 4.3 (IQR: 2.7–7.5) in the normal weight group.

The correlation between continuous BMI, age, and sleep study parameters is shown in Table 3. BMI was moderately and positively correlated with AHI (rho = 0.307, $p < 0.001$) and desaturation index (rho = 0.342, $p < 0.001$). Age was weakly but significantly negatively correlated with sleep efficiency (rho = -0.230, $p < 0.001$), indicating that older individuals tend to have reduced sleep efficiency.

Age-related differences in sleep study parameters are summarized in Table 4. Sleep efficiency showed a significant decline with increasing age ($p = 0.005$), with the oldest group (≥65 years) exhibiting the lowest median sleep efficiency. AHI values also varied significantly across age groups ($p = 0.027$), with the highest values observed in the 31–49 age group.

Gender differences in sleep study parameters are presented in Table 5. Males had significantly higher arousal index and snoring intensity compared to females. However, AHI and desaturation index values did not differ significantly between genders.

Table 2: Sleep Study Parameters by Body Mass Index Groups (Median (IQR))

Parameter	Normal (n=6)	Overweight (n=30)	Obesity I (n=73)	Obesity II (n=56)	Obesity III (n=86)	p-value
Sleep Efficiency	88.7 (85.5-92.0)	85.9 (75.1-91.3)	84.6 (77.0-92.0)	79.0 (67.0-90.3)	79.6 (67.7-92.0)	0.218
Arousal Index	10.7 (9.2-15.6)	11.7 (6.5-15.9)	17.0 (7.9-27.4)	17.3 (7.2-33.0)	17.3 (10.5-30.3)	0.128
AHI	4.3 (2.7-7.5)	10.4 (5.1-43.2)	35.0 (13.5-57.3)	45.5 (26.5-69.6)	51.0 (20.9-77.5)	<0.001*
Desaturation Index	2.7 (2.0-5.4)	18.0 (8.5-56.6)	40.0 (17.4-69.3)	64.9 (31.8-81.8)	63.0 (26.1-84.0)	<0.001*
Snoring	23.1 (5.7-30.4)	12.6 (7.6-29.2)	23.0 (14.0-34.6)	28.3 (15.3-36.2)	28.5 (20.5-40.6)	0.024*
*Significant at p < 0.05. Results for AHI and Desaturation Index also survive Bonferroni correction (p < 0.002).						

Table 3: Spearman Correlation of BMI and Age with Sleep Study Parameters

Parameter	vs. BMI (rho)	p-value	vs. Age (rho)	p-value
Sleep Efficiency	-0.156	0.014*	-0.230	<0.001*
Arousal Index	0.181	0.005*	-0.025	0.703
AHI	0.307	<0.001*	0.038	0.545
Desaturation Index	0.342	<0.001*	0.101	0.114
Snoring	0.191	0.002*	0.058	0.360
*Significant at p < 0.05. Results for AHI and Desaturation Index also survive Bonferroni correction (p < 0.002).				

Table 4: Sleep Study Parameters by Age Groups (Median (IQR))

Parameter	18-30 (n=11)	31-49 (n=59)	50-64 (n=107)	≥65 (n=74)	p-value
Sleep Efficiency	85.0 (81.0-89.5)	88.6 (79.1-94.0)	81.4 (68.6-91.5)	78.9 (66.0-89.0)	0.005*
Arousal Index	16.9 (9.7-19.8)	22.0 (10.5-31.2)	15.0 (6.9-26.1)	15.2 (7.4-24.4)	0.374
AHI	4.0 (2.3-34.6)	49.2 (17.9-73.7)	41.5 (11.7-68.5)	38.5 (15.9-59.3)	0.027*
Desaturation Index	5.0 (2.5-39.5)	43.0 (19.7-84.0)	47.9 (18.8-77.3)	56.8 (25.7-74.6)	0.083
Snoring	14.8 (1.4-23.6)	28.7 (10.6-42.0)	26.2 (15.2-35.7)	27.5 (17.6-36.1)	0.050
*Significant at p < 0.05. Only Sleep Efficiency vs Age survives Bonferroni correction (p < 0.002).					

Age-related differences in sleep study parameters are summarized in Table 4. Sleep efficiency showed a significant decline with increasing age (p = 0.005), with the oldest group (≥65 years) exhibiting the lowest median sleep efficiency. AHI values also varied significantly across age groups (p = 0.027), with the highest values observed in the 31–49 age group.

Gender differences in sleep study parameters are presented in Table 5. Males had significantly higher arousal index and snoring intensity compared to females. However, AHI and desaturation index values did not differ significantly between genders.

Finally, the negative binomial GLM regression analysis (Table 6) identified BMI as the only significant independent predictor of AHI. For each 1-unit increase in BMI, the AHI was expected to increase by a factor of 1.024 (p = 0.002), holding other variables constant. Age and gender were not significant predictors in this model.

Discussion

This study presents a comprehensive analysis of the associations between polysomnographic variables and key demographic and anthropometric factors in a cohort of 251 Sudanese adults diagnosed with obstructive sleep apnea (OSA). Using robust non-parametric statistical methods including the Mann-Whitney U test, Kruskal-Wallis test, and Spearman correlation, we ensured valid inference despite the skewed distribution of AHI and related parameters. The negative binomial generalized linear model was particularly suitable, outperforming standard linear regression by addressing violations of normality and homoscedasticity.

Our primary finding is that body mass index (BMI) is the most significant and consistent predictor of OSA severity in this population. This conclusion is supported by both the Kruskal-Wallis test, which revealed a clear dose-response relationship, and the regression model, which identified BMI as the sole significant independent predictor of AHI. The strong association between BMI and AHI (Spearman's rho = 0.307) aligns with extensive international literature that identifies obesity as the primary modifiable risk factor for OSA [16,17]. Notably, each 1-unit increase in BMI was associated with a 2.4% increase in AHI, underscoring the importance of weight management in clinical strategies for OSA in Sudan. The high prevalence of Class III obesity (34.3%) in our cohort further reinforces this point.

Historical and global studies have consistently linked Obstructive sleep apnea-hypopnea syndrome (OSAHS) to excess weight, particularly among middle-aged obese individuals. One study found that a 10% weight gain increased the risk of OSA sixfold [13]. Another reported that moderate to severe OSA (AHI ≥15) was present in 11% of normal-weight men, 21% of overweight men, and 63% of obese men [14]. Similarly, in women, prevalence rates were 3%, 9%, and 22%, respectively [18]. A study by Yalım found

Table 5: Gender Differences in Sleep Study Parameters (Median (IQR))

Parameter	Males (n=134)	Females (n=117)	p-value
Sleep Efficiency	86.0 (77.3-93.1)	78.8 (67.2-89.0)	0.002*
Arousal Index	19.2 (10.5-31.0)	13.3 (6.0-21.7)	<0.001*
AHI	43.9 (22.2-66.9)	33.0 (8.4-65.5)	0.065
Desaturation Index	49.5 (23.0-72.8)	45.4 (13.6-81.4)	0.770
Snoring	28.3 (16.4-40.5)	24.3 (10.8-34.7)	0.016*
*Significant at $p < 0.05$. Arousal Index and Sleep Efficiency differences survive Bonferroni correction ($p < 0.002$).			

Table 6: Negative Binomial GLM Results for Predictors of AHI

Predictor	Incidence Rate Ratio (IRR)	95% Confidence Interval	p-value
BMI	1.024	1.009 – 1.039	0.002*
Age	0.999	0.989 – 1.009	0.816
Gender (Male vs. Female)	1.122	0.859 – 1.467	0.399
*Significant at $p < 0.05$.			

that 42.6% of OSA patients were overweight and 26% had first-degree obesity, with each unit increase in BMI leading to a 1.538-unit rise in AHI [19]. Mechanistically, obesity contributes to pharyngeal airway collapsibility by increasing adipose tissue around the airway and reducing lung volumes due to visceral fat, thereby increasing mechanical loads on the respiratory system [20].

Our analysis also revealed a significant negative correlation between age and sleep efficiency, consistent with known age-related changes in sleep architecture. However, age was not an independent predictor of AHI in our regression model, suggesting that while older individuals may experience poorer sleep quality, OSA severity is more strongly influenced by BMI. Supporting this, Fietze et al.[12] reported that OSA prevalence increases with age in both sexes, though women tend to be diagnosed later. Their study also identified associations between OSA and factors such as gender, age, BMI, waist-to-hip ratio, snoring, alcohol consumption (in women), and cardiovascular diseases, while daytime sleepiness showed no significant link [13].

Gender differences in sleep parameters were also observed. Males exhibited higher arousal indices and snoring intensity, indicating more disrupted sleep [21]. However, AHI values did not differ significantly between genders [22,23]. This challenges the stereotype of OSA as a predominantly male disorder and suggests that females may present with different symptoms—such as fatigue and insomnia—despite having comparable disease severity. This highlights the need for heightened clinical awareness and diagnostic vigilance in women, who may be underdiagnosed [21].

Further, our findings showed significant variations in sleep efficiency, arousal index, and snoring intensity between genders, but no differences in desaturation index or AHI. A meta-analysis exploring sex differences in insomnia risk emphasized the need to understand gender-specific sleep patterns [15]. Women frequently report more sleep disturbances, including insomnia and nightmares, which are twice as common compared to men [23,24]. These discrepancies are often linked to hormonal fluctuations throughout the menstrual cycle [25,26]. Additionally, modern lifestyle factors—such as increased work demands, psychosocial stress, and additional responsibilities like childcare—may exacerbate sleep deprivation in women. While the adverse effects of sleep restriction are well-documented in men, they remain underexplored in women [27].

Finally, our study demonstrated a positive association between severe OSA and comorbid conditions such as diabetes, hypertension, and metabolic syndrome. Notably, clinical parameters in women showed stronger associations with OSA compared to men. Hongyo et al. also found that OSA severity increases with age, identifying male gender, BMI, and age as independent risk factors for severe OSA in elderly patients [17].

Conclusion

In this cohort of Sudanese adults with OSA, increasing BMI was the most powerful and consistent predictor of OSA severity. Age was primarily associated with a decline in sleep efficiency rather than an increase in AHI. While males exhibited greater sleep fragmentation, OSA severity was comparable between genders, underscoring the importance of diagnosing OSA in females. These findings highlight the critical role of weight management in mitigating OSA severity in the Sudanese population and emphasize the need for nuanced, gender-aware clinical assessments.

Study Limitations

This study has several limitations. Its retrospective, cross-sectional design prevents the establishment of causality. the study did not include other important anthropometric measurements like neck circumference or data on comorbidities such as hypertension and diabetes, which are known confounders. Future research should aim to address these limitations through prospective, community-

based studies that include a wider range of clinical and demographic data.

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Conflict of interest

The authors declare that there is no conflict of interest in this work

Ethical consideration

The data of this work has been taken from the sleep study center, and verbal consent has been taken from all patients who participated in this work. The authors took permission from, the ethical committee in the faculty of medicine at the National University of Africa.

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RESEARCH ARTICLE

Edible Insect Phobia and Associated Factors Among Women of Reproductive Age in the Central Region of Ghana

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Abstract

Background: Food insecurity and micronutrient deficiencies remain significant public health concerns among women of reproductive age in Ghana. Edible insects offer a nutrient-dense, sustainable protein source. However, edible insect phobia may limit the acceptance and consumption of edible insects. This study explored edible insect phobia and its associated factors among women of reproductive age in the Central Region of Ghana.

Methods: A cross-sectional study was conducted between March and June 2024 at a tertiary hospital in the central region of Ghana. Three hundred forty women of reproductive age (15-49 years) were recruited. Data were collected using two validated psychometric scales including the Food Neophobia Scale and Insect Phobia Scale, along with sociodemographic characteristics and insect consumption history. Associations were examined using chi-square tests, Fisher's exact tests, and Spearman's rank correlation in R version 4.5.1.

Results: Edible insect phobia was statistically significantly associated with level of education ($p = 0.016$), ethnic group ($p < 0.001$), pregnancy status ($\chi^2(2) = 6.46$, $p = 0.040$, Cramer's V = 0.151, 95% Confidence Interval [CI] = 0.064–0.252), awareness of insects as food ($\chi^2(2) = 17.45$, $p < 0.001$, Cramer's V = 0.234, 95% CI = 0.138–0.330), and prior insect consumption ($\chi^2(2) = 16.54$, $p < 0.001$, Cramer's V = 0.228, 95% CI = 0.123–0.334). Edible insect phobia was negatively correlated with age ($r = -0.223$, 95% CI = -0.325 to -0.116, $p < 0.001$) and number of pregnancies ($r = -0.163$, 95% CI = -0.270 to -0.053, $p = 0.003$), but not with food neophobia score ($r = 0.097$, 95% CI = -0.020 to 0.209, $p = 0.074$).

Conclusions: In this sample, predominantly composed of pregnant women attending antenatal care services, higher phobia of edible insects among women of reproductive age was associated with younger age, fewer pregnancies, lower educational level, certain ethnicities, pregnancy status, unawareness, and no previous history of consuming edible insects. Interventions targeting these factors may help reduce edible insect phobia and increase acceptance of edible insects as dietary proteins among similar populations.

Keywords: Entomophagy; Alternative protein; Edible insect phobia; Women of reproductive age; Ghana

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Introduction

Food insecurity and deficiencies in protein, iron, zinc, and other essential nutrients remain a significant public health concern in Ghana and worldwide. Among women of reproductive age, nutrient deficiencies contribute to a heightened risk of anemia, compromised immune function, poor pregnancy outcomes, and intergenerational health consequences [1,2]. As efforts to combat malnutrition evolve, attention has increasingly turned to underutilized, nutrient-dense food sources, particularly edible insects. Edible insects offer a compelling nutritional profile, rich in high-quality protein, unsaturated fatty acids, and micronutrients such as iron, zinc, and vitamin B12 [3,4].

According to van Huis et al. [5], over 2,000 insect species are consumed globally, with beetle larvae, caterpillars, crickets, and termites among the most common. Their nutrient density and lower environmental footprint have led the Food and Agriculture Organization (FAO) to endorse the practice of eating insects as a sustainable food solution in the face of rising food insecurity and climate change [3].

Despite global promotion of edible insects as food, actual consumption patterns vary. A systematic review by Florença et al. [6] identified key determinants of edible insect consumption, including sex, age, food neophobia, disgust sensitivity, cultural norms, nutritional beliefs, safety concerns, and the mode of presentation. Psychological aversion, sometimes referred to as "insect phobia," also emerges as a significant impediment to the widespread practice of consuming insects [3]. These attitudes are particularly concerning among women of reproductive age, who play a central role in household food provisioning and dietary decision-making. However, the bulk of empirical studies have been conducted in Europe, with relatively fewer from Africa, mainly Nigeria, Kenya, and South Africa [6].

In Ghana, the practice of eating insects is not a new phenomenon. Indigenous communities across the country have traditionally consumed species such as termites, grasshoppers, caterpillars, and the African palm weevil larvae [7]. A study by Anankware et al. [4] cataloged the commonly consumed insects in Ghana, including species such as the black soldier fly, shea tree caterpillar, and African termite. These insects are often harvested seasonally and prepared by frying, roasting, or grinding into powder for incorporation into meals. Yet, the 2022 Ghana Demographic and Health Survey reported that only 0.2% of women of reproductive age had consumed any form of edible insect in a certain 24-hour reference period 8. This underutilization contrasts with the nutritional potential of insects and may be partly explained by psychosocial and environmental barriers. In a mixed-methods study in northern Ghana, Kubuga et al. [2] found that while reasons for insect consumption included affordability, cultural familiarity, and perceived health benefits, deterrents were disgust, negative sensory attributes, limited availability, and concerns about hygiene. While most existing Ghanaian research on edible insects focuses on children, the factors influencing edible insect phobia among women of reproductive age remain understudied. This study, therefore, investigated edible insect phobia and its associated factors among women of reproductive age in Ghana. The findings may inform culturally sensitive nutrition education, product development, and policy interventions to harness the nutritional benefits of edible insects, improve dietary diversity, and contribute to sustainable solutions for malnutrition in Ghana.

Materials and Methods

Study Design

TThis was a cross-sectional study conducted at the Cape Coast Teaching Hospital, a tertiary hospital located in Cape Coast, Central Region of Ghana. The Cape Coast Teaching Hospital was chosen as the study site because it serves as a regional referral facility for the Central Region of Ghana. The underlying assumption was that selecting the Cape Coast Teaching Hospital would enable a diverse sample to be captured in the study, given the impracticality of obtaining a sampling frame for our target population.

Sample and Sampling Procedure

The target population for this study was women of reproductive age in the Central Region of Ghana. Eligibility criteria included women aged 15 to 49 years, willingness to participate in the study, and ability to provide informed consent. Individuals who identified as vegetarians were excluded. It was not feasible to use a random sampling method due to the impracticality of obtaining a sampling frame of our target population; hence, the study participants were recruited using convenience sampling. The initial target sample size was 384, determined using Cochran's formula based on an assumed 50% prevalence of edible insect phobia, a 5% margin of error, and a 95% confidence interval. Constraints, including limited participant flow and the end of the data collection period, resulted in a final sample of 340 participants, 88.5% of the target.

Measures

Data were collected in person between March and June 2024 by five final-year nutrition students under the supervision of a nutrition officer and registered dietitian. The data collection questionnaire included sociodemographic characteristics, awareness, and history of insect consumption, as well as psychological constructs from two validated psychometric scales 3. Although the psychometric scales were originally developed and tested in Italy, we assessed and confirmed their internal consistency for use among women of reproductive age in our setting. We conducted reliability checks on the two psychometric scales prior to full data collection using a sample of 20 women of reproductive age.

Food Neophobia Scale

The food neophobia scale was used to assess general reluctance to try unfamiliar foods 3. It consists of 10 items, with responses captured on a five-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Five items (Q1, Q4, Q6, Q9, and Q10) were reverse-coded to ensure that higher scores across all items consistently reflected greater food neophobia. All item scores were summed up to generate a total food neophobia score. Internal consistency was assessed during a pilot reliability check conducted with 20 women of reproductive age, yielding acceptable reliability (Cronbach's $\alpha = 0.71$, 95% CI: 0.47–0.87, Feldt test).

Insect Phobia Scale

The Insect Phobia Scale was used to assess emotional and cognitive discomfort related to edible insect consumption [3]. It included six negatively worded items addressing disgust,

perceived hygiene, concerns about taste and texture, and social unacceptability. Responses were rated on the same five-point Likert scale (1 = strongly disagree to 5 = strongly agree), and all items were summed to generate a total insect phobia score. No reverse coding was required, as all items were phrased to align directly with the construct being measured. Internal consistency was evaluated in the same pilot assessment, demonstrating good reliability (Cronbach's α = 0.81, 95% CI: 0.65–0.92, Feldt test).

Likelihood of Consuming Insect-Based Products

Participants were asked to indicate their likelihood of consuming eight different insect-based food products using a five-point Likert scale (1 = Very Unlikely to 5 = Very Likely). The products included insect nuggets, insect burgers, spreads, crunchy larvae, insect soup, protein shakes, powdered insect spice mixes, and insect flour for baking. The items varied in terms of their visibility and recognizability, capturing a range of psychological responses toward insect-based foods. Responses with higher scores indicate greater willingness to consume the respective product.

Data Analysis

All statistical analyses were conducted using R version 4.5.1 9. Prior to selecting statistical tests, the normality of continuous variables was assessed using the Shapiro–Wilk test, histograms, and boxplots. Where distributions deviated from normality, log transformations were applied, and normality was reassessed using the same methods. Despite attempts at transformation, the continuous variables remained non-normally distributed. Consequently, non-parametric tests were employed throughout the analysis. Because no formal cutoffs exist for categorization, the insect phobia scale and food neophobia were divided into tertiles (33% increments) to represent low, moderate, and high phobia groups. Associations between total insect phobia scores and continuous variables (e.g., age, number of pregnancies, and food neophobia scores) were assessed using Spearman's rank correlation. Relationships between insect phobia categories and sociodemographic variables were examined using Chi-square or Fisher's exact tests, with Monte Carlo approximations applied when cell counts were sparse. Effect sizes for categorical comparisons were reported using Cramer's V. Descriptive responses on the likelihood of consuming various insect-based foods were visually summarized using a diverging stacked bar chart to display the distribution of Likert-scale responses across food types.

Results

Sociodemographic Characteristics and Insect Consumption History of Study Participants.

The median age of the women was 31 years (interquartile range [IQR], 27–35), and they had a median of 2 pregnancies (IQR, 2–3). Most participants were married (69.1%), employed (89.4%), and had completed either senior high school (27.9%) or tertiary education (41.2%). The majority identified as Akan (89.7%) and Christian (92.6%). Most participants were currently pregnant (85.3%). While 72.1% were aware that insects can be eaten as food, only 23.5% reported ever consuming them. Among those who had eaten insects, taste was the most commonly stated reason (13.2%), followed by curiosity (7.4%). Among those who had not eaten insects, the most frequently cited reasons were unfamiliarity (33.8%), disgust (29.4%), fear of illness (14.7%), and a preference for traditional meat (10.3%) (Table 1).

Table 1. Self-Reported Sociodemographic Characteristics of a Sample of Women of Reproductive Age at a Tertiary Hospital in Ghana Between March 2024 and June 2024.

Variables	N = 340
Age	31 (27 - 35)
Number of pregnancies	2 (2 - 3)
Marital status	
Not married	105 (30.9%)
Married	235 (69.1%)
Level of education	
None	10 (2.9%)
Primary	5 (1.5%)
JHS	90 (26.5%)
SHS	95 (27.9%)
Tertiary	140 (41.2%)
Employment status	
Unemployed	36 (10.6%)
Employed	304 (89.4%)
Ethnic group	
Akan	305 (89.7%)
Ga	10 (2.9%)
Ewe	10 (2.9%)
Mole-Dagbani	5 (1.5%)
Hausa	5 (1.5%)
Guan	5 (1.5%)
Religious affiliation	
Christianity	315 (92.6%)
Islam	25 (7.4%)
Current pregnant	290 (85.3%)
Aware of insects as food	245 (72.1%)
History of insect consumption	80 (23.5%)
Reason for eating insects	
Because it tastes good	45 (13.2%)
By curiousity	25 (7.4%)
Reason for not eating insects	
Fear of illness	50 (14.7%)
It disgusts me	100 (29.4%)
Has not seen it before	115 (33.8%)
I prefer real meat	35 (10.3%)

Note: Age and number of pregnancies are presented as medians with interquartile ranges (Q1 – Q3). For several items related to insect consumption (e.g., reasons for eating or not eating insects), participants could select more than one applicable response; therefore, the combined percentages in these categories exceed 100%. JHS = Junior High School; SHS = Senior High School. History of insect consumption refers to any prior experience of eating insects, encompassing both regular dietary practice and occasional or one-time exposure.

Table 2. Associations Between Sociodemographic Variables and Insect Phobia Category of a Sample of 340 Women of Reproductive Age at a Tertiary Hospital in Ghana Between March 2024 and June 2024.

Variable	Test	Statistic	p-value	Cramer's V effect size	95% Confidence Interval
Marital Status	Chi-Square	χ^2 (2) = 3.54	0.170	0.117	0.03, 0.224
Level of education	Fisher's Exact (Monte Carlo)	N/A	0.016	N/A	N/A
Employment status	Chi-Square	χ^2 (2) = 2.72	0.256	0.106	0.022 , 0.216
Ethnic group	Fisher's Exact (Monte Carlo)	N/A	<0.001	N/A	N/A
Religious affiliation	Chi-Square	χ^2 (2) = 1.93	0.381	0.095	0.021 , 0.178
Pregnancy status	Chi-Square	χ^2 (2) = 6.46	0.040	0.151	0.064 , 0.252
Awareness of insects as food	Chi-Square	χ^2 (2) = 17.45	<0.001	0.234	0.138 , 0.330
History of insect consumption	Chi-Square	χ^2 (2) = 16.54	<0.001	0.228	0.123 , 0.334

Note: Statistical tests examine associations between each sociodemographic variable and insect phobia categories (low, moderate, and high). N/A indicates that test statistics and effect sizes were not computed due to the use of Fisher's Exact Test with Monte Carlo simulation for variables with sparse or large contingency tables.

Table 3. Spearman's Correlation Between Insect Phobia and Other Variables of a Sample of 340 Women of Reproductive Age at a Tertiary Hospital in Ghana Between March 2024 and June 2024.

Variable	Test	Statistic	95% Confidence Interval	p-value
Age	Spearman's Correlation	r = -0.223	-0.325 , -0.116	<0.001
Number of Pregnancies	Spearman's Correlation	r = -0.163	-0.27 , -0.053	0.003
Total Food Neophobia Score	Spearman's Correlation	r = 0.097	-0.02 , 0.209	0.074
Note: Spearman's correlation was used to assess the association between the total insect phobia score and the listed variables				

Associations Between Sociodemographic Characteristics, Insect Consumption History, and Insect Phobia

Associations between sociodemographic characteristics, insect consumption history, and insect phobia among the study participants are summarized in Tables 2 and 3. The total insect phobia score had a median of 22 (IQR = 8). Among the study participants, 35.3% (n = 120) had low insect phobia, while 32.4% (n = 110) had moderate and 32.4% (n = 110) had high levels of insect phobia. There was sufficient evidence to suggest associations with insect phobia for age, number of pregnancies, participants' level of education, ethnic group, pregnancy status, awareness of insects as a food source, and history of insect consumption. There was insufficient evidence to support associations for marital status, employment status, religious affiliation, or food neophobia score.

Likelihood of Consuming Different Types of Edible Insect-Based Foods

Figure 1 illustrates the likelihood of consuming various edible insect-based foods among respondents. Figure 2 shows images of insect food products that were shown to the study participants. Overall, there was a higher likelihood of consuming insects in the form of spice mix and flour. In contrast, crunchy larvae and insect-based burgers were least favored, with a majority indicating they were "very unlikely" to consume these products.

Discussion

The findings of this study indicate that although most women of reproductive age are aware of edible insects as a food source, only a small fraction have consumed them, with unfamiliarity, disgust and fear of illness cited as reasons for not doing so. Insect phobia was associated with factors such as education, ethnicity, pregnancy status, and prior exposure to edible insects. Awareness and previous consumption of insects were associated with lower levels of insect phobia, while younger age and fewer pregnancies were linked to higher levels of fear. Furthermore, insect-based foods in processed forms, such as spice mixes and flour, had a higher likelihood of acceptance.

Several studies have investigated edible insect consumption, with a primary focus on nutritional composition and consumer acceptance in various populations. However, a paucity of research has examined insect phobia specifically among women of reproductive age. For instance, Anankware et al. [4] examined the nutritional profiles of five important edible insect species in West Africa. Ayensu et al. 10 assessed the impact of palm weevil larvae-fortified biscuits on the nutritional status of rats. Similarly, Chamoun et al. [1] and Laar et al. [11] investigated consumer acceptance of insect-fortified foods and the potential of palm weevil larvae as a source of nutrition; however, neither study focused on insect-related fears and phobias. Studies, such as that by Coley et al. [12], have examined the feasibility of insect consumption among pregnant women

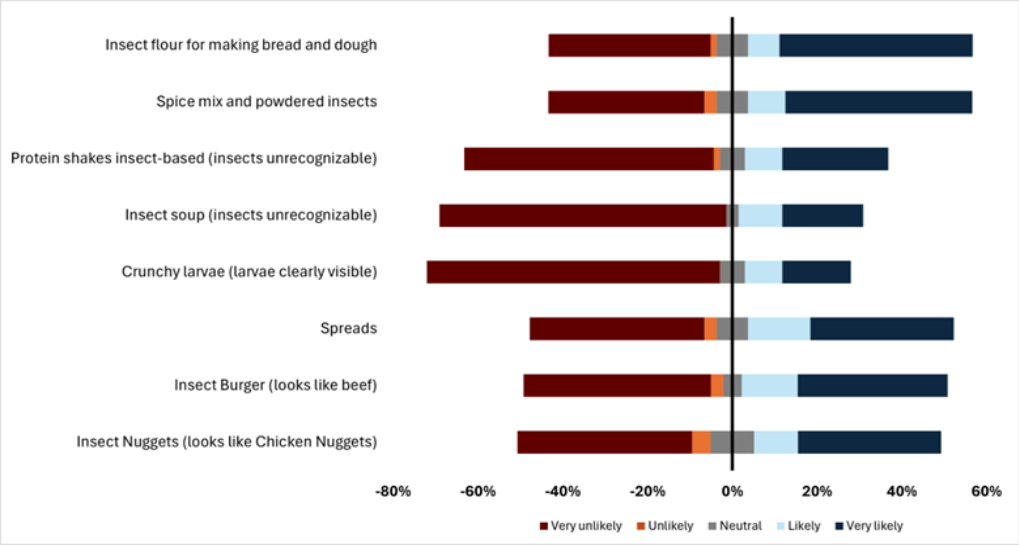

Figure 1. Likelihood of consuming various insect-based food products among 340 women of reproductive age in Cape Coast, Ghana, March–June 2024. The diverging stacked bar chart illustrates the distribution of intent, with the neutral category centered on the zero-axis to distinguish between negative and positive sentiment. Numerical values (Very Unlikely; Unlikely; Neutral; Likely; Very Likely) for each category are as follows: Insect flour for bread/dough (38.2%; 1.5%; 7.4%; 7.4%; 45.6%); Spice mix and powdered insects (36.8%; 2.9%; 7.4%; 8.8%; 44.1%); Protein shakes (58.8%; 1.5%; 5.9%; 8.8%; 25.0%); Insect soup (67.6%; 0.0%; 2.9%; 10.3%; 19.1%); Crunchy larvae (69.1%; 0.0%; 5.9%; 8.8%; 16.2%); Spreads (41.2%; 2.9%; 7.4%; 14.7%; 33.8%); Insect Burger (44.1%; 2.9%; 4.4%; 13.2%; 35.3%); and Insect Nuggets (41.2%; 4.4%; 10.3%; 10.3%; 33.8%).

Figure 2. Insect Food Products Shown to a Sample of 340 Women of Reproductive Age at a Tertiary Hospital in the Central Region of Ghana Between March 2024 and June 2024.

in Liberia. Parker et al. [7] and Kubuga et al. [2] focused on the nutrient contribution of edible insects in children's diets. This study is novel in its specific focus on psychosocial and demographic constructs among women of reproductive age.

Our findings are largely consistent with existing literature. Similar to previous studies, we found that psychological factors such as unfamiliarity, disgust, and fear of illness were identified to influence the willingness to consume insects [6]. Additionally, our results agree with the results from Coley et al. [12] and Chamoun et al. [1] that consumer acceptance increases when insects are presented in less recognizable forms. The statistically significant associations we identified between insect phobia and age, pregnancies, and educational attainment suggest that experiential

exposure and education may mitigate psychological barriers to edible insect consumption. Younger participants likely had fewer opportunities for exposure and thus higher discomfort, which aligns with Kubuga et al. [2]'s assertion that familiarity reduces neophobia. The inverse relationship between educational attainment and insect phobia suggests that knowledge may alleviate fear.

Furthermore, the observed ethnic variations reflect culturally embedded perceptions and practices regarding food, highlighting the need for culturally tailored intervention approaches. The association between pregnancy status and insect phobia may be explained by hormonal and cognitive changes that heighten sensory sensitivity and risk perception during pregnancy. Hormonal fluctuations can trigger neurophysiological and cognitive shifts that amplify disgust responses, making pregnant women more averse to unfamiliar foods such as insects [13]. Additionally, increased concern for food safety during pregnancy may contribute to heightened risk perception, as reflected in participants' reported fear of illness. Furthermore, women with fewer pregnancies may have had less exposure to edible insects, which could contribute to higher phobia levels. The preference for processed forms of edible insects, such as spice mixes and flour, rather than whole insects, strongly indicates that presentation forms and sensory perceptions significantly impact food acceptance. This suggests that strategically designed insect-based products could be essential in normalizing and encouraging edible insect consumption.

This study has limitations that warrant careful consideration. First, the cross-sectional design limits the ability to draw causal inferences regarding the observed relationships. Second, the use of convenience sampling restricts the generalizability of the findings. Importantly, participant recruitment was heavily concentrated among pregnant women, who constituted over 85% of the sample and were primarily accessed through antenatal care services. Consequently, the findings should be interpreted within the context of pregnant women receiving antenatal care. Additionally, reliance on self-reported data introduces the possibility of recall bias and social desirability bias, which may have influenced participants' reporting of insect consumption practices and related psychological responses. The use of specific food vehicles in the visual aids, such as burgers and nuggets, may also have affected participants' willingness to consume insects independently of the insect content itself, as these Western-style food presentations may not align with participants' usual dietary contexts.

Despite these limitations, the study has notable strengths that help mitigate their potential impact. Although the final sample size of 340 participants fell slightly short of the initial target of 384, it is considered adequate to detect meaningful associations given the observed effect sizes (e.g., Cramer's V values ranging from 0.151 to 0.234 and Spearman's correlations of r = -0.223 and r = -0.163). Therefore, the study retains sufficient statistical power to support the robustness of the main associations examined. Data collection was conducted by trained nutrition students under the supervision of a qualified nutrition officer and a registered dietitian, which likely enhanced data quality and minimized measurement error. Furthermore, the use of validated scales to assess insect phobia strengthens the reliability and rigor of the psychological measurements employed in this study.

Implications for Research and Practice

Future research exploring similar phenomena in diverse settings could provide insights into the cultural influences on insect consumption practices. Additionally, qualitative methods might provide deeper insights into the underlying reasons for insect phobia and attitudes toward entomophagy. The findings of this study demonstrate the importance of considering psychological and sociodemographic characteristics when designing interventions to improve edible insect consumption among women of reproductive age. The preference for insect-based products in processed forms, such as spice mixes and flours, suggests that visibility and texture may play a role in food acceptance. To enhance the acceptability of edible insects, product development efforts should prioritize familiar, less visually identifiable formats that align with local culinary practices. For example, incorporating insect flour into commonly consumed staples (e.g., porridges, pastries, or stews) may reduce aversion and normalize consumption. Public health education should also emphasize the safety, nutritional benefits, and environmental sustainability of edible insects, using culturally relevant messaging to dispel fears and misconceptions. Furthermore, interventions should be tailored to subgroups identified as more likely to exhibit insect phobia, particularly younger women, those with lower education, and women with fewer pregnancies, by integrating experiential learning opportunities such as tasting events, cooking demonstrations, or peer-led campaigns. Engaging ethnic and community leaders in advocacy may also help address culturally rooted perceptions and foster greater social acceptance of insect-based foods.

Conflict of Interest

The authors state no conflict of interest.

Author contribution.

Dr Opoku-Antwi conducted a literature search, read every reference, evaluated every article and exported the data. The paper was initially written by JAK, and then it was edited by both authors. The final manuscript was reviewed and approved by all authors.

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RESEARCH ARTICLE

Mental Health Literacy and Stigma among Pharmacists toward Patients with Mental Illness: A Cross-Sectional Survey in Sudan

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Abstract

Background: Pharmacists, as the most accessible healthcare providers, play a significant role in disease management, yet their involvement in mental health services is limited by stigma, inadequate training, and negative perceptions. The ongoing conflict in Sudan has exacerbated mental health challenges, thus making it essential to assess pharmacists' readiness to address these issues. This study aimed to evaluate the mental health literacy, stigma, and comfort levels of Sudanese pharmacists in providing care to patients with mental health disorders.

Methods: A descriptive cross-sectional study was conducted using an online questionnaire that was distributed to Sudanese pharmacists via social media platforms. The questionnaire, adapted from a previously validated tool, measured knowledge, attitudes, stigma, and comfort in managing mental health conditions versus cardiovascular diseases. SPSS version 29 was used to analyze the data with a significance level of $P < 0.05$.

Results: A total of 413 pharmacists participated (73% female; most aged 25–30 years). Pharmacists demonstrated a high recognition of depressive disorders 94%, but moderate awareness of anxiety 62% and obsessive-compulsive disorder 68%. While pharmacists showed good knowledge of common psychiatric medications, with amitriptyline 76% and haloperidol 65% being the most recognized drugs, 37.8% of them viewed pharmacist consultation negatively. Pharmacists predominantly associated mental health patients with negative outcomes, such as increased likelihood of suicide 76.8%, violence 75.8%, and illegal drug use 70.0%. Comfort levels in discussing psychiatric symptoms and providing medication counseling (21.8%) were significantly lower compared to those for cardiovascular conditions (40%).

Conclusion: This study identified significant gaps in mental health care capacity. While baseline disorder recognition was high, pharmacists demonstrated limited knowledge of psychiatric pharmacotherapy, low professional confidence, and prevalent stigma, commonly associating mental illness with negative outcomes like violence and suicide. Comfort in managing mental health conditions was substantially lower than for cardiovascular diseases. These findings underscore an urgent need for systemic interventions and continuous pharmacist education combining advanced clinical knowledge, counseling skills, and stigma mitigation. Training should employ active learning, including case studies and role playing, while fostering collaboration with mental health professionals. Additionally, future initiatives must account for variability among displaced pharmacists, such as employment background and conflict exposure, which can affect their competency and comfort in mental health care.

Keywords: Mental health literacy, Stigma, Mental illness, Pharmacists, Sudan

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Introduction

Mental health is a state of mental well-being that enables people to cope with the stresses of life, realize their abilities, learn and work well, and contribute to their community [1]. In Sudan, however, mental health has historically received limited attention in terms of policy development, service provision, and research. The development of comprehensive mental health services has been significantly hindered by long-standing economic

constraints and political instability. As of 2020, a total of 878 professionals nationwide were involved in mental health service delivery, equating to approximately 2.05 mental health professionals per 100,000 population in a country of nearly 45 million people [2]. Additionally, inequitable allocation of mental health services, poor health education, and fear of stigma were the other identified barriers to treatment [3].

The continuous conflict between Sudan's military and paramilitary forces adds to the country's history of violence and its negative impact on mental health. Evidence from Khartoum State indicates that approximately 13% of secondary school students experience depression and anxiety, while 23% of pregnant women have been diagnosed with prenatal mental disorders [2]. Among internally displaced populations, the mental health burden is markedly higher, with 53% affected by mental disorders, including major depressive disorder (24.3%), and generalized anxiety disorder (23.6%) [2].

Pharmacists are essential in the management of mental health conditions by optimizing drug therapy, educating patients on their medications, and ensuring safe and effective use [4]. Research has shown that clinical pharmacists can reduce drug-related problems and healthcare costs, improve a patient's quality of life, and lower mortality rates [5]. The expanding role of pharmacists in mental healthcare isn't just for specialists; it also includes community pharmacists as part of multidisciplinary teams, who are among the most accessible healthcare professionals [6,7]. Community pharmacists are well-positioned to identify individuals at risk of mental health crises, screen for mental illnesses, and provide education to patients and caregivers. There is an opportunity and a need to better utilize pharmacists internationally in the provision of mental health care [6].

Pharmacists face several barriers in providing effective care for mental health patients, which can hinder their crucial role in supporting comprehensive treatment. These barriers include pharmacists' lack of knowledge or confidence, their attitudes toward people with mental health disorders, and the belief that such patients would be unwilling or incapable of understanding medication information [8]. Additionally, privacy in community pharmacies, limited accessibility of community pharmacists to patients' medical and/or medication records, and the perception that providing medication information is the physician's role further contribute to these challenges [9,10]. Other frequently cited barriers were the inability to monitor outcomes, as patients may never return to the pharmacy, and the lack of easy follow-up [11].

When it comes to the word "stigma", it is used to refer to a mark of shame or disgrace, or to some related ideas such as stereotyping or rejection [12]. Pharmacists can shape how patients perceive their mental illness. However, when pharmacists themselves show negative attitudes, such as discomfort or hesitation during interactions with patients, this can lead to inappropriate counselling or a failure to provide professional medical services. Unfortunately, many pharmacists hold negative stereotypes or stigmas about mental health disorders [11,13]. The term 'mental health literacy' (MHL) refers to knowledge and beliefs about mental disorders that aid their recognition, management, or prevention [14].

Pharmacists' knowledge, attitude, and stigma were studied in different communities. These studies proved that the baseline knowledge of pharmacists was adequate, but not their advanced knowledge, especially in schizophrenia and bipolar disorder, which are related to a higher level of stigma [15,16]. In contrast, some studies showed a low level of stigma from pharmacists toward mental health patients [17].

In Sudan, stigma towards mental disorders remains high, influenced by cultural, religious, and societal factors as well as augmented by the limited mental health services available. The present socio-political conflict in Sudan has caused a rise in symptoms of mental illness, but most patients come to the facilities only when their condition is confirmed and advanced [18-20]. To date, no research has evaluated mental health literacy and stigma among pharmacists in Sudan, a significant gap given their influential role. Addressing this gap is vital for understanding and improving pharmacists' attitudes, knowledge, and practices. Such insights can directly inform strategies to enhance mental health training, elevate the quality of care, promote treatment adherence, and reduce stigma-related barriers to accessing treatment

Materials and Methods

Study Design and Setting

This observational cross-sectional study was conducted to assess pharmacists' literacy and stigma toward patients with mental health disorders in Sudan between September 2023 and March 2024. Data was collected through an online questionnaire for Sudanese pharmacists across different states in Sudan.

Study population

The target population consisted of licensed pharmacists eligible to practice in Sudan. The specific inclusion and exclusion criteria were as follows:

Inclusion Criteria:

- Licensed pharmacists currently practicing in Sudan, and pharmacists displaced by the ongoing conflict but who were previously licensed and practicing within Sudan.
- Provision of voluntary informed consent to participate.

Exclusion Criteria:

- Pharmacy students or interns.
- Pharmacists practicing exclusively outside of Sudan without prior professional experience within the country.

- Individuals unwilling or unable to provide informed consent.
- Submission of incomplete survey responses.

Sample size and technique

As an official, updated registry of pharmacists in Sudan was unavailable, the minimum sample size was calculated using Cochran's formula for an infinite population: $n=Z2\times p\times q/e^2$. Where Z = 1.96 (for a 95% confidence level), p = 0.5 (the assumed population proportion for maximum variability (50%)), q = 1 - p, and e = 0.05 (the margin of error). This calculation yielded a minimum required sample size of 385 participants. To account for potential non-response and incomplete submissions, data were collected from 413 pharmacists. A convenient sampling method was applied for Sudanese pharmacists currently working in Sudan. The survey link was distributed to targeted groups across social media platforms (Whatsapp, Facebook and Telegram) for Sudanese pharmacists across different regions in Sudan

Data collection tools and methods

Data was collected through a self-administered, previously validated questionnaire adapted from a previous study with the authors' consent [15]. Face validity was assessed by a psychiatrist and two PhD holders (Pharmacy Practice and Clinical Pharmacy), and adjustments were made upon their recommendations. Content validity was assured by an extensive review of the literature [15]. The survey instrument was piloted among a small group of pharmacists (n=30), and revised for length, flow, and clarity. The questionnaire had a Cronbach's alpha of 0.82. The questionnaire was administered in English, which is the language of instruction at Sudanese pharmacy schools.

The tool contained 74 items: multiple-choice, Likert scale, and checkbox questions, and was divided into four sections:

- First section (socio-demographics): Age, gender, pharmacy setting (community, hospital), and pharmacy practice experience
- Second section (MHL (knowledge, attitude and practice)): Knowledge was assessed in terms of symptom recognition (cases of depression, anxiety, and obsessive-compulsive disorder (OCD)), medication classification, and the helpfulness of a range of interventions. Cases for generalized anxiety disorder (GAD) clinical presentation (physical, psychological, and cognitive symptoms), and the diagnostic criteria of OCD were developed based on the Diagnostic and Statistical Manual of Mental Disorders – fifth edition (DSM-V). Attitude and practice were assessed through rating the benefits of seeking help and perceived opinions about medications' safety and effectiveness. The guidelines used for medication indications were the Clinical Practice Guidelines by American Psychiatric Association (APA).
- Third section (Mental health stigma (MHS)): MHS was measured with reference to the Opening Minds Scale for Health Care Providers (OMS-HC) that included different examined dimensions of stigma, social distance, social responsibility, dangerousness and recovery. These dimensions have been established among healthcare providers; 'Social distance' refers to the desire to maintain distance from people with MHD, and 'social responsibility' represents one's emotional reactions towards patients with MHD.
- Fourth section (Comfort providing pharmaceutical care to patients with MHD): The willingness and comfort of pharmacists to provide services to patients with MHD (depression and schizophrenia) compared with those suffering from cardiovascular diseases were assessed by rating their comfort on a scale of 1–5.
- For the scoring system, pharmacist knowledge was assessed using 23 items covering: symptom recognition (3 items), medication classification (5 items), pharmacologic (7 items), and nonpharmacological interventions (8 items). Each correct response was scored as 1, and incorrect or uncertain as 0. Participants were classified as poor (0-7), moderate (8-15), and good knowledge (16-23). Stigma was assessed using 24 items comprising three domains: perceived dangerousness and recovery (6 items), comfort differentials (6 items), and interaction willingness (12 items). Each item contributed one point; pharmacists were classified as having mild stigma (0-8), moderate stigma (9-16), and severe stigma (17-24).
- Variables
- Independent variables: Demographic characteristics; Age, gender, pharmacy setting (community, hospital), and pharmacy practice experience (Grade, academic degree, year of graduation).
- Dependent variables: pharmacists' knowledge, attitude, and stigma.

Statistical Analysis

Data was entered into Microsoft Excel and cleaned for consistency and completeness. All statistical analyses were conducted using the Statistical Package for Social Sciences, version 29 (Armonk, NY: IBM Corp). Descriptive statistics were used to summarize pharmacists' demographics, MHL, and Mental Health Stigma (MHS). Inferential statistics using the chi-square test were applied to examine differences in associations between demographic characteristics or knowledge level with the MHS. A p-value of less than 0.05 was considered statistically significant.

Ethical consideration

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Ethical approval (protocol number: FPEC-51-2024) was granted by the Research Ethics Committee of the Faculty of Pharmacy, University of Khartoum, prior to the commencement of the study. All participants were provided with a comprehensive description of the study's aims. Written informed consent was obtained from each participant before data collection commenced. Participants retained the right to withdraw from the study at any time, without providing a reason and without penalty. Confidentiality and anonymity of all participant data were strictly maintained throughout the research process.

Table 1. Sociodemographic characteristics of the study participants (n= 413)

Variables		Frequency (%)
Gender	Female	300 (73)
	Male	113 (27)
Age	25 – 30	318 (77)
	>30	61 (15)
	<25	34 (8.2)
State	Khartoum	163 (39.5)
	Red Sea	59 (14.2)
	River Nile	36 (8.7)
	Gezira	30 (7.3)
	Kassala	26 (6.3)
	Northern	24 (5.8)
	Qadarif	11 (2.7)
	White Nile	10 (2.4)
	Others	54 (13)
Type of Pharmacy Degree	Pharmacy	394 (95)
	PharmD (clinical)	19 (4.6)
Highest academic degree	BSc	354 (86)
	MSc	49 (12)
	PhD	10 (2.4)
Occupation	Community pharmacists	296 (72)
	Hospital pharmacists	49 (12)
	Clinical pharmacists	23 (5.6)
	Others	45 (10.4)
Year of graduation		2021 (IQR: 2018–2022)

Results

Demographic Characteristics

A total of 413 pharmacists agreed to participate in the study. As shown in Table 1, the majority, 73%, were females, and 77% were aged 25-30 years. Participants were from various states, with the highest representation from Khartoum (39.5%). About 72% were community pharmacists, and 86% of them held a bachelor's degree in pharmacy.

Assessment of pharmacists' knowledge of mental health literacy (MHD)

In terms of accurate symptom recognition, 94% of pharmacists correctly identified the symptoms of depression, 68% identified those of obsessive-compulsive disorder (OCD), and 62% recognized the symptoms of anxiety. Regarding the participants' knowledge of commonly used psychiatric medications, Amitriptyline, as an antidepressant, was the most recognized drug and was identified by 76% of pharmacists (Figure 1).

Figure 2 summarizes pharmacists' knowledge of both pharmacological and non-pharmacological interventions for different mental health conditions. Regarding pharmacological treatments, 97.5% of pharmacists associated antidepressants with depression, while antipsychotics were mainly linked with schizophrenia (87%). Mood stabilizers were correctly associated with bipolar disorder by 69% and with schizophrenia by 45% of respondents. In this study, 24% of respondents identified herbal remedies as being related to the management of depression and anxiety. Analgesics and antibiotics were mentioned as not useful in any mental condition by 46% and 78%, respectively.

In terms of non-pharmacological approaches, 57% of pharmacists identified cognitive behavioral therapy (CBT) as beneficial for depression. Relaxation techniques and hypnosis were also linked to depression by 57% and 19%, respectively. Physical activity was associated with both depression 79% and anxiety 57%. Confinement in a psychiatric ward was considered necessary for the management of schizophrenia 74% and bipolar disorder 52%. Moreover, 47% of pharmacists correctly recognized the role of electroconvulsive therapy (ECT) in the treatment of schizophrenia (Figure 2).

Assessment of pharmacists' attitude and practice towards MHD

The perceived helpfulness of various support options for mental health patients among pharmacists showed that seeking help from a psychiatrist was rated as most helpful by 91% of the pharmacists, while consulting a pharmacist was rated as helpful by only 37.8% (Table 2). As shown in Table 3, only 15.7% of the pharmacists agreed that they had received sufficient training in mental health conditions,

Table 2. Pharmacists' attitude towards the helpfulness of different interventions for the management of mental

Characteristic	Frequency (%)		
	Harmful	Neutral	Helpful
Get spiritual help	113 (27.4)	12 (2.9)	288 (69.7)
Seek the help of a pharmacist	217 (52.5)	40 (9.7)	156 (37.8)
Seek the help of a psychiatrist	30 (7.3)	7 (1.7)	376 (91.0)
Seek the help of a social worker	171 (41.4)	79 (19.1)	163 (39.5)
Talk to a close family member	160 (38.7)	37 (8.9)	216 (52.4)
Talk to a friend	156 (37.8)	35 (8.5)	222 (53.7)

Table 3. Assessment of agreement among pharmacists on training and understanding of mental health conditions, in addition to psychiatric medications

Characteristic	Frequency (%)				
	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Sufficient training in mental health conditions	67 (16.2)	139 (33.7)	130 (31.5)	65 (15.7)	12 (2.9)
Sufficient training on psychiatric medications	53 (12.8)	99 (24.0)	128 (31.0)	107 (25.9)	26 (6.3)
Adequate understanding of mental health conditions	36 (8.7)	59 (14.3)	122 (29.5)	158 (38.3)	38 (9.2)
Adequate understanding of psychiatric medications	41 (9.9)	39 (9.4)	90 (21.8)	176 (42.6)	67 (16.2)

Table 4. Assessment of perceived dangerousness and recovery characteristics among patients with MHD given by pharmacists

Characteristic	Frequency (%)		
	No difference	Less likely	More likely
Attempt suicide	25 (6.1)	71 (17.2)	317 (76.8)
Be a productive worker	69 (16.7)	311 (75.3)	33 (8.0)
Be violent	64 (15.5)	36 (8.7)	313 (75.8)
Develop social relationships	52 (12.6)	308 (74.6)	53 (12.8)
Have a healthy marriage	56 (13.6)	326 (78.9)	31 (7.5)
Take illegal drugs	77 (18.6)	47 (11.4)	289 (70.0)

with 42.6% of them believing that they had a good understanding of psychiatric medications.

Assessment of the pharmacists' stigma towards MHD

Table 4 assesses the perceived dangerousness and recovery of patients with MHD. The majority (76.8%) of respondents perceived those patients with MHD were most likely to attempt suicide. In contrast, having a healthy marriage was perceived as the least likely outcome, as reported by 78.9% of respondents. As shown in Figure 3, providing medication counseling for psychiatric medications was identified as the most uncomfortable task by 21.8% of pharmacists, whereas discussing cardiovascular conditions was considered the most comfortable by 40% of respondents.

Figure 4 illustrates pharmacists' willingness to interact with individuals with depression and schizophrenia. Socializing

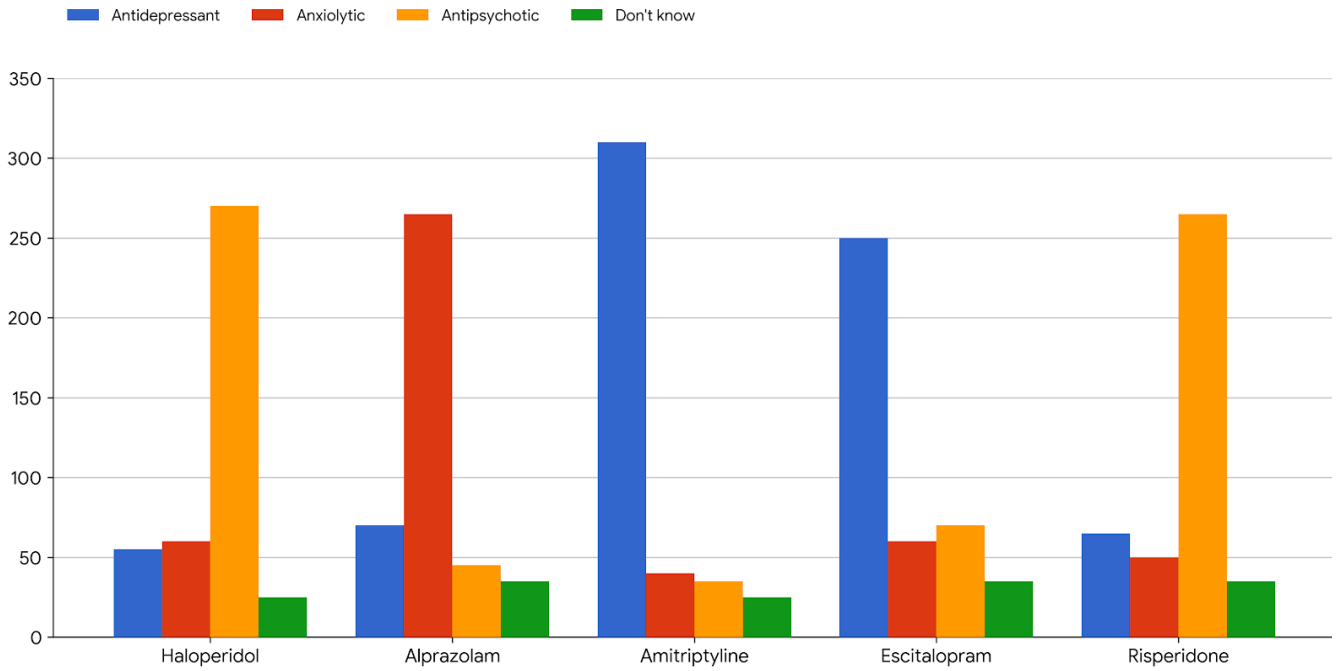

Figure 1. Knowledge of pharmacists regarding the classification of psychiatric medications.

Table 5. Association between pharmacists' stigma level towards MHD and demographic characteristics (gender, age, and academic degree), and Knowledge levels.

Characteristic	Frequency (%)			p-value
	Mild Stigma (N = 87)	Moderate Stigma (N = 161)	Sever Stigma (N = 165)	
Gender				
Female	67 (77)	126 (78)	107 (65)	
Male	20 (23)	35 (22)	58 (35)	0.09
Age				
< 25	11 (13)	11 (6.8)	12 (7.3)	
25 - 30	57 (66)	127 (79)	134 (81)	0.13
> 30	19 (22)	23 (14)	19 (12)	
Academic Degree				
BSc	74 (85)	140 (87)	140 (85)	
MSc	11 (13)	18 (11)	20 (12)	
PhD	2 (2.3)	3 (1.9)	5 (3.0)	0.4
Knowledge Levels				
Poor Knowledge	40 (46%)	83 (52%)	76 (46%)	
Moderate Knowledge	41 (47%)	63 (39%)	68 (41%)	
Good Knowledge	6 (6.9%)	15 (9.3%)	21 (13%)	0.07

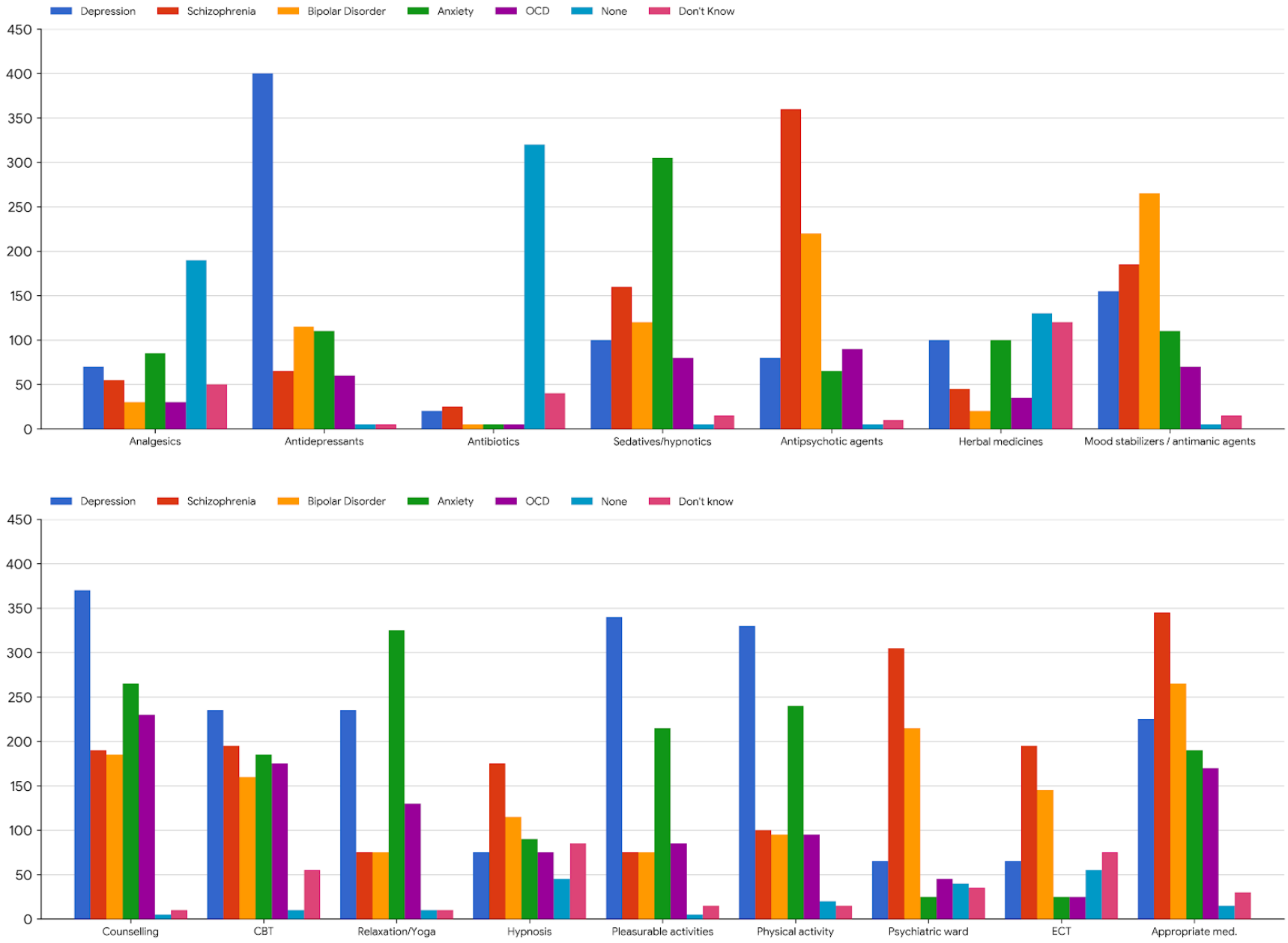

Figure 2. Knowledge of pharmacists regarding their ability to recognize pharmacologic and non-pharmacologic interventions for.

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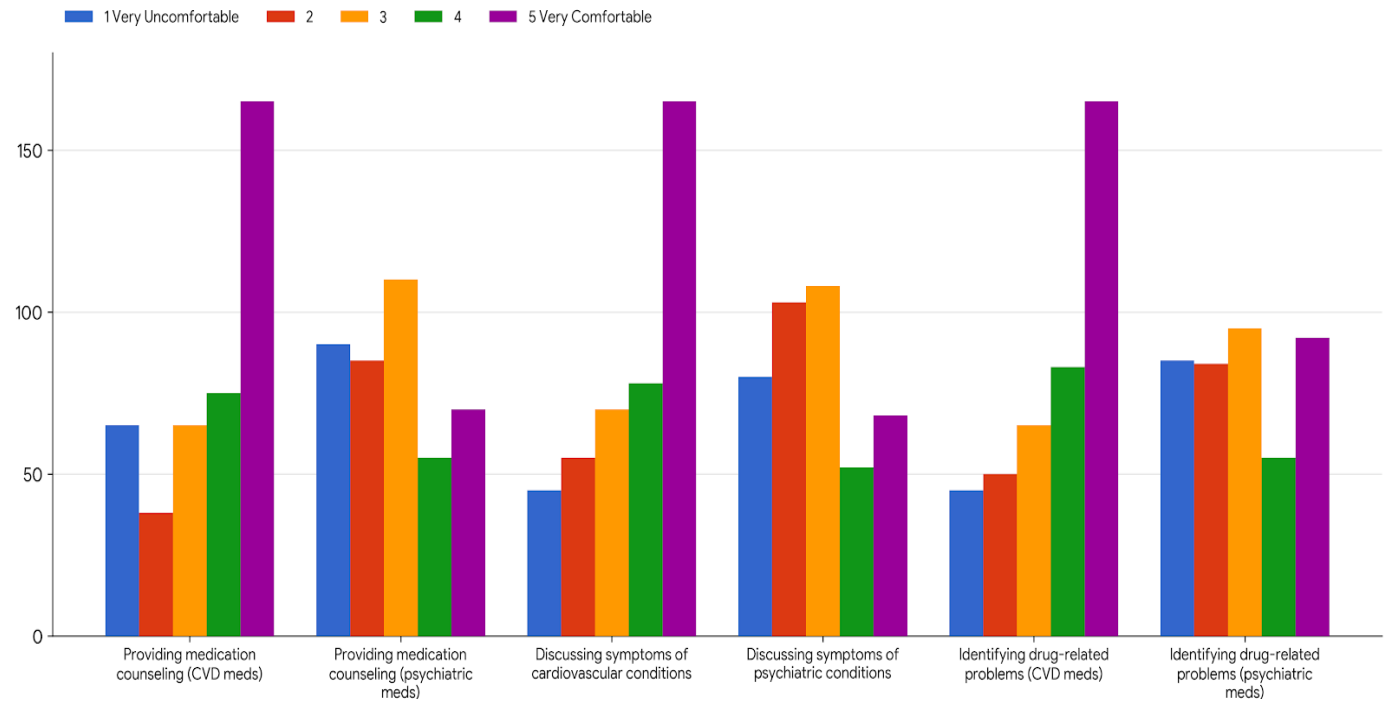

Figure 3. Knowledge of pharmacists regarding the classification of psychiatric medications.

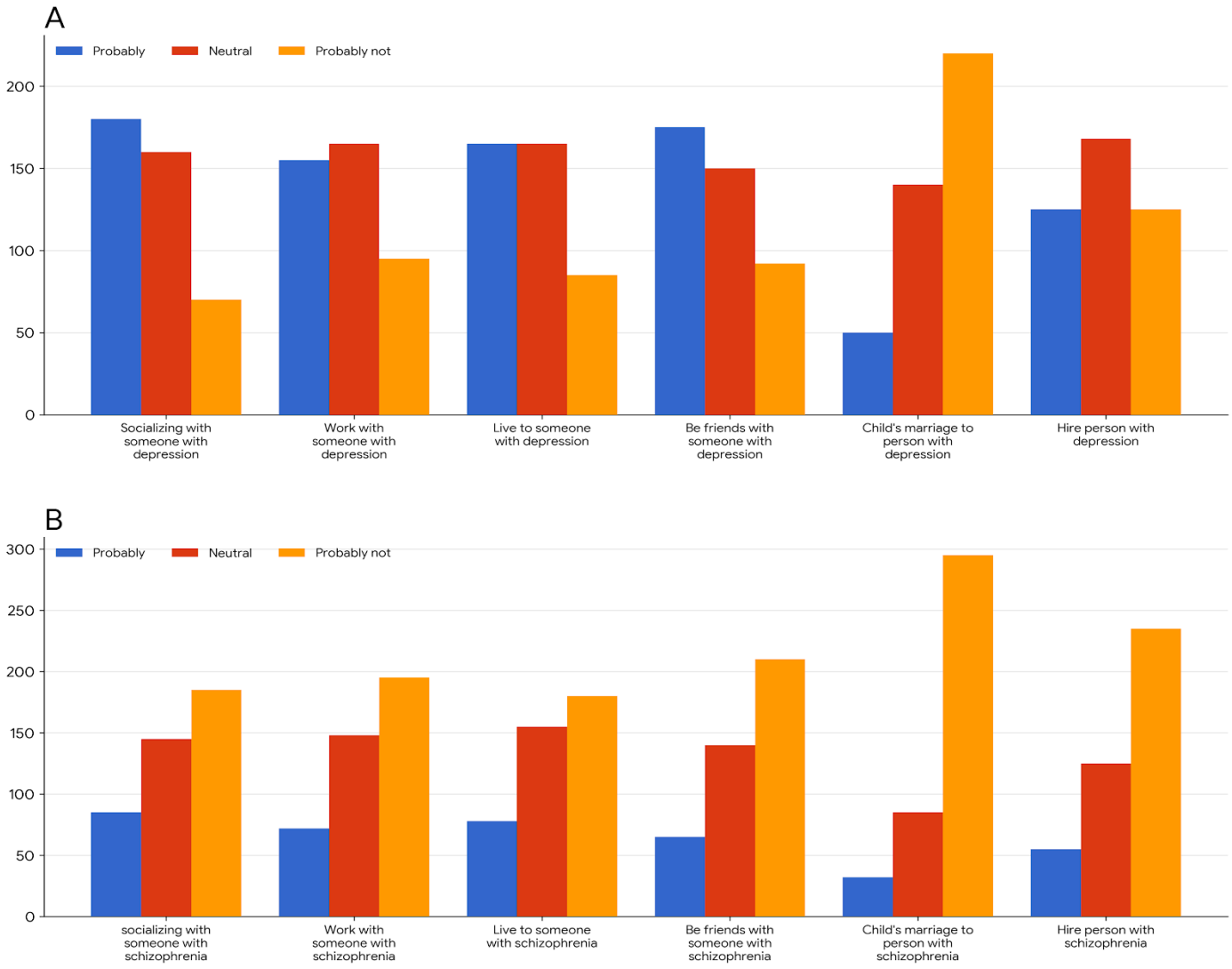

Figure 4. Assessment of pharmacists' willingness to interact with patients suffering from depression (A) and schizophrenia (B).

with individuals with depression (42.1%) or schizophrenia (15.5%) was the most accepted form of interaction. In contrast, having a child marry an individual with either condition was the least accepted scenario, reported by 12.6% of respondents for depression and 8.0% for schizophrenia.

Association between pharmacists' stigma level and demographic characteristics or knowledge levels

The distribution of pharmacists according to their level of stigma towards patients with mental health disorders (MHD) across key demographic and knowledge variables is detailed in Table 5. No statistically significant associations were found between stigma levels and gender ($p = 0.09$), age group ($p = 0.13$), highest academic degree ($p = 0.4$), or self-reported knowledge levels ($p = 0.07$).

Discussion

Despite the significant impact of mental illness diseases, to the best of our knowledge, we believe that this is the first study that has tried to evaluate the knowledge, attitude, practice, stigma, and behavioral responses towards people with mental illness among pharmacists in Sudan. Due to the ongoing socio-political instability and conflict, research showed an increase in symptoms of mental illnesses [18]. It is the need of the hour to call for urgent intervention and further research aimed at reducing stigmatizing beliefs and providing optimal care for those who suffer from mental health conditions. The study surveyed a total of 413 pharmacists, the majority of whom were under thirty years female, community pharmacists, and holding a bachelor's degree in pharmacy. Similar demographic characteristics were reported in many studies carried out in Sudan [21-23]. The large representation of the female gender could be justified by the real situation of the students in universities, with a gender ratio of at least 2:1 in most colleges [24,25].

The assessment of knowledge regarding MHD among participants showed that when it came to classifying major categories of medications, such as antidepressants, anxiolytics, and antipsychotics, pharmacists demonstrated an accuracy rate of 66%, about two-thirds of the cases across all three groups. The findings are consistent with a study of pharmacists in the MENA region, which reported that baseline knowledge of mental health disorders was sufficient for recognizing depression (93.3%), anxiety (63.7%), and obsessive-compulsive disorder (68%) [15]. In contrast, another study highlighted that although there is overall willingness and interest among community pharmacists to engage in services for patients with mental illness, there is a significant need for interventions to improve knowledge and attitudes related to mental illness [26].

To further evaluate their understanding of MHD pharmacotherapy, pharmacists were asked not only to sort medications by their correct primary indications but also to identify their potential for multiple indications. This evaluation revealed a gap in knowledge regarding the complexity and varied applications of psychiatric medications across different MHD-related situations. Such as while 87% of pharmacists correctly identified antipsychotics as primarily indicated for schizophrenia, only 53% recognized their use in the management of bipolar disorder. Our findings were at odds with the findings of research on medication therapy management (MTM), which underscores the importance of pharmacists' knowledge in identifying both primary and secondary indications of drugs [27].

The study revealed that a substantial proportion of pharmacists considered confinement in a psychiatric ward necessary for the management of schizophrenia (74%) and bipolar disorder (52%), reflecting ongoing stigma and outdated treatment approaches. Similar findings have been reported in previous studies showing that stigmatizing beliefs among health care professionals continue to favour institutional care [28].

Regarding the assessment of pharmacists' knowledge of non-pharmacological interventions, most participants recognized cognitive-behavioral therapy (CBT) as an effective treatment for depression. This is supported by a systematic review demonstrating that CBT significantly reduces depressive symptoms, especially when interventions are tailored to individual needs [29].

Additionally, in terms of correct symptom recognition, the study participants demonstrated a high level of recognition for depressive symptoms but lower awareness of anxiety-related and compulsive behavior. This could be justified by research on text-based estimation of depression that suggests that linguistic patterns associated with depressive symptoms are easier to identify, which might contribute to higher recognition rates [30]. Similar findings were reported in a study that examined the MHL of the British community [16].

The low perceived helpfulness of pharmacist consultation (37.8%) observed in this study represents a critical finding that reflects cyclical barriers to the expansion of the pharmacist's professional roles. The reported low comfort and training gaps reinforce stigma and limiting pharmacist's professional practice in mental health care. As stated in the literature, Pharmacists viewed their role as integral to providing mental health services; however, progress is impeded by challenges such as stigma, fragmented care, and training gaps [31]. The study findings align with a study showing that unfavorable attitudes toward mental illness are prevalent among pharmacists [32]. This differs from other analyses of literature, where health professionals view their field as the most beneficial [15]. This could be explained by what was reported on literature, which revealed that pharmacists face several barriers that may hinder them from performing their role, including a lack of confidence, time, payment, privacy in the current community pharmacies, lack of coordination with other health care providers, communication challenges, and stigma [33.34].

Lack of training is an important barrier in most professions. Our findings highlighted a considerable gap in training, as a low percentage of the pharmacists (15.7%) agree with the statement "I have had sufficient training in mental health

conditions". Due to that, it is important to emphasize the importance of continuous mental health training and new medical education methods for pharmacists to address gaps in their knowledge, eliminate stigma, and improve their confidence in delivering mental health services [35-39]. Nevertheless, researchers have demonstrated that this mindset can shift by incorporating more patient-focused approaches in curriculum development for topics related to MHD. A systematic review evaluating mental health training programs for pharmacists, pharmacy students, or staff showed that changes in participants' attitudes, skills, stigma, knowledge, and confidence were the most common outcomes evaluated in the studies, with overall significant improvement on those outcomes after the training [40]. In addition, individuals experiencing mental health disorders in Sudan often face significant societal stigma, leading them to seek spiritual and religious healing methods before considering professional medical assistance 41. This trend is further exacerbated by the negative attitudes and practices of healthcare providers toward mental health conditions [42,43].

Stigma was assessed based on different spectrums. The first was the degree of dangerousness and recovery. Three-quarters of the pharmacists found that patients with MHD are less likely to develop a positive outcome, like being reproductive or having a healthy marriage. Nevertheless, they believed that individuals with MHD are at a higher risk of developing harmful behaviors, such as experiencing suicidal thoughts, engaging in illegal drug use, or being violent [44]. These findings are like a study which also reported that the pharmacists stated that the presence of mental health facilities in the area poses a risk to the population in that area [17].

Secondly, pharmacists exhibited less comfort and willingness when providing essential services, such as discussing medication-related issues and offering counseling, to patients with mental health disorders compared to providing the same services to cardiovascular patients. This discomfort may stem from the stigma associated with psychotropic medications, which, in turn, could contribute to reducing follow-up monitoring for drug-related problems among mental health patients and subsequently poor medication adherence among these patients [32]. Which could be justified by the fact that pharmacists generally felt more at ease and confident when discussing symptoms related to cardiovascular conditions than when addressing symptoms of psychiatric conditions [13]. The observed differences in comfort levels likely reflect greater familiarity with cardiovascular medication, clearer clinical guidelines, and more structural support rather than stigma alone.

Regarding the pharmacists' ability to build different relationships with patients with depression and schizophrenia, the study revealed that while some pharmacists are willing to engage with individuals suffering from depression, many remain reluctant, particularly when it involves personal relationships and family matters. This reluctance aligns with findings from another study that have reported stigmatizing attitudes toward patients with depression [45]. Similarly, concerns have been raised about the inadequate attitudes and practices among Sudanese physicians in treating depression [46], and these are further challenges faced by individuals seeking mental health care in Sudan. In addition, building a relationship with a patient with schizophrenia presented greater challenges, but this was not the case in a study conducted in Australia, where participants showed the same level of stigma for both schizophrenia and severe depression [47]. In contrast, other researchers highlighted a low level of mental health stigma but high levels of schizophrenia literacy [48]. Regarding our findings, we didn't find any significant associations between age, gender, and overall stigma [49]. Unlike other observations where a more positive attitude was associated with older age and males, as reported by a Nigerian study [50].

Several limitations should be acknowledged in our study. The first limitation is self-report bias, as the questionnaire dealt with a sensitive issue like mental health disorders, participants might have been reluctant to provide honest answers out of concern for being stigmatized or judged. This could have affected the reliability of the data. Also, the use of convenience online sampling may have introduced bias, as pharmacists with reliable internet access and greater familiarity with online surveys were more likely to participate. This may have disproportionately represented younger, urban pharmacists while underrepresenting older or rural practitioners. Second, the use of cardiovascular medication users as the control group may limit generalizability, as this population may not represent users of other medications. Differences in comfort levels may reflect not only stigma but also greater familiarity with cardiovascular treatments, clearer clinical guidelines, and more established structural support for cardiovascular care in pharmacy practice.

Conclusion

This study identified significant gaps in mental health care capacity. While baseline disorder recognition was high, pharmacists demonstrated limited knowledge of psychiatric pharmacotherapy, low professional confidence, and prevalent stigma, commonly associating mental illness with negative outcomes like violence and suicide. Comfort in managing mental health conditions was substantially lower than for cardiovascular diseases. These findings underscore an urgent need for systemic interventions, including integrating mental health education, which should entail continuous education programs and workshops that focus on advanced pharmacotherapy knowledge, patient counseling skills, and stigma-reduction strategies. Training should also incorporate case-based learning, role-playing exercises to build confidence in patient interactions, and interprofessional collaboration with physicians and mental health specialists. Future studies should consider heterogeneity among displaced pharmacists, including differences in employment status and duration or intensity of conflict exposure, as these factors may influence mental health knowledge, attitudes, comfort levels, and stigma.

Authors' contributions

M.K.S., S.A.O. and E.A.A.E. conceptualized and designed the study. M.K.S., S.A.O., A.A.N., A.M.A., and A.O.A. contributed to patient recruitment and data acquisition, conducting the study, performing data analysis, and interpreting the results. E.A.A.E. and B.A.Y. supervised the research. All authors contributed to writing the first draft of the manuscript. B.A.Y. and E.A.A.E. edited the final draft and provided critical revisions. All authors reviewed, edited, and approved the final manuscript.

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None to declare

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RESEARCH ARTICLE

Knowledge, Attitude and Practices Towards Sugar-Sweetened Beverages amongst Adolescents in Senior High Schools in the Cape Coast Metropolis, Ghana

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Abstract

Background: Sugar-sweetened beverage (SSB) consumption is a growing contributor to diet-related health problems among adolescents worldwide, including in Ghana. However, there is limited evidence on how adolescents' nutrition knowledge and attitudes relate to their SSB consumption. Hence, this study aimed to investigate the types and frequencies of SSBs consumed by secondary school students in Cape Coast, Ghana, while concurrently assessing their nutritional knowledge and attitudes toward sugar-sweetened beverage consumption.

Methods: A descriptive cross-sectional study was conducted, among 393 students from three senior high schools in Cape Coast, Ghana. Data on SSB consumption, nutrition knowledge, attitudes, and anthropometry were collected using a structured questionnaire and standard measurements. Data were analyzed using descriptive statistics and chi-square tests ($p < 0.05$).

Results: Overall, 57.5% of respondents had a high level of SSB consumption. More than half (55.7%) had moderate nutrition knowledge about SSBs, and 52.9% expressed positive attitudes toward SSB consumption. There was no significant association between knowledge level, and SSB consumption levels.

Conclusions: Despite moderate nutritional knowledge and generally positive attitudes, a high proportion of students reported frequent SSB consumption. Interventions that go beyond knowledge, such as restricting SSB availability in schools and promoting attractive, healthier beverage alternatives, are needed and should involve government, schools, and communities.

Keywords: Sugar-Sweetened Beverages, Adolescents, Knowledge, Attitudes and Practices

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Introduction

Sugar-Sweetened Beverages (SSBs) are defined as beverages that have added sugar or sweeteners such as high fructose corn syrup, sucrose, or fruit juice concentrate. This category includes a wide variety of drinks such as sports drinks, sweetened tea and coffee, energy drinks, carbonated or non-carbonated soft drinks, electrolyte-replacement drinks, and sweetened milk or milk alternatives [1]. Consumption of SSBs is linked to exceeding one's energy needs, resulting in various diet-related health issues, such as obesity, overweight, type 2 diabetes, gout, dental caries, mental health problems, cardiovascular diseases, cancers, and high blood pressure [2]. The harmful impact of excessive calories and sugars on health and life has brought SSBs into sharp focus among scholars worldwide, including both in developed and developing countries, such as Africa. A survey of SSBs consumption in adults in 187 countries found that intake was higher in middle-income countries compared to either high-income or low-income countries [3]. Adolescents aged between 13 and 20 years have the highest intake of SSBs globally and this has led to an increase in the prevalence and degree of obesity among adolescents in many populations, resulting in notable public health issues [4,5]. A study conducted in South Africa showed that adolescents had a very high consumption rate of SSBs, and without any

preventative measures, the sales and availability of these beverages are projected to increase at an annual rate of 2.4% between 2012 and 2017 [6,7]. Another study involving primary school children in Nairobi discovered that although participants had some awareness of the harmful effects of unhealthy diets like SSBs, they still consumed them as they were readily available and convenient [8,9]. It is important for policymakers, healthcare professionals, and communities to work together to develop strategies to reduce the consumption of SSBs and promote healthier beverage choices. Some potential strategies that have been proposed to address the high consumption of SSBs include implementing taxes on sugary drinks, promoting public education campaigns about the health risks associated with consuming too much sugar, and improving access to healthier beverage options such as water and low-sugar drinks [10–12]. To discourage youths from consuming SSBs, it is necessary to understand their attitude and practices towards these drinks and their level of nutritional knowledge, therefore this study aimed at assessing the nutrition knowledge, attitude, and consumption of SSBs among secondary school students in Cape Coast, Ghana.

Materials and Methods

Study Area

The study was conducted across three secondary schools in Cape Coast. Cape coast is a major urban centre located in the Central Region of Ghana, covering an area of approximately 122 square kilometres. The educational landscape of Cape Coast Metropolitan includes a range of schools, including primary, secondary, and tertiary institutions. The metropolitan area is home to several secondary schools, both mixed-gender and single-gender schools, offering a variety of academic programs [14].

Study design, population and sampling

This cross-sectional study included male and female participants aged between 13 and 19 years. In the Ghanaian education system, adolescents in this age range are generally students in senior high schools. Participants who had certain diseases or disabled, on medication were excluded from the study. A sample size of at least 393 adolescents was determined using Slovin's formular from a population of approximately 15,992 adolescents in Cape coast with a 0.05 margin of error. Data collection was performed and completed in September 2023.

Students were selected using a two-stage approach. First, three senior high schools in the Cape Coast Metropolis were purposively selected based on accessibility and willingness to participate. Within the selected schools, students were recruited using convenience sampling. All students who were present at the time of data collection and consented to participate were included in the study. The consent forms were handed to the participants one day before the data collection was carried out. Participants consisted of students who attended school on the day of the data collection and had handed in the signed-off consent form. A total of 393 consented forms were received.

Data collection

A self-administered questionnaire with a structured format was used to collect data from the various schools. The questionnaire consisted of socio-demographic sections which included the anthropometric measurements, nutritional knowledge, attitude, practices, and, food frequency questions (FFQ) on the type, frequency and volumes of SSBs consumed which were adopted from Teng [3]. The Knowledge, Attitude and Practices (KAP) questionnaire consists of 22 dichotomous questions and 5 multiple answers out of 13 items for knowledge, 11 items for attitude and 3 items for practices that require a response of either Yes/No or Agree/Disagree. The KAP scores were computed following standard approaches used in adolescent nutrition KAP studies. Knowledge was assessed using 13 dichotomous items (correct = 1, incorrect/"don't know" = 0; total score 0–13) and classified into poor (0–4), moderate (5–9), and good (10–13) levels using tertile-based cut-offs, similar to methods used by Kigaru et al. and Najam et al 67,68. Attitude was measured using 11 statements on a 3-point Likert scale (Agree = 3, Neutral = 2, Disagree = 1), with reverse-coded negative items, yielding a total possible score of 11–33 which was categorized into negative (11–18), neutral (19–26), and positive (27–33) attitudes. Practice was evaluated using three items assessing weekly SSB consumption frequency, aggregated into a composite score and classified as low or high consumption. These scoring criteria ensure consistent interpretation of KAP domains and reflect approaches validated in previous nutrition KAP studies [15].

The adapted FFQ on frequency and volumes investigated the frequency of plain water and SSBs intake on a daily, weekly, and monthly basis. It consisted of seven types of beverages, including plain water, milk products and tea beverages, sweetened fruit beverages, regular soft drinks, energy and sports drinks, and homemade/local drinks. This questionnaire estimated the habitual intake of these beverages, which were part of the measurement of practices. There are six categories of responses, ranging from "once daily", to "4 or more times per week".

Anthropometric measurements such as body weight and height were measured by a trained research team. Body weight was measured in light clothing, without shoes, using a portable digital electronic scale [Omron HBF-514C Full body sensor body composition and scale; 1665708-0E; China]. Height was measured at the distance from the top of the head to the bottom of the feet, without shoes, using Seca 213 Portable Height Measure; China] Both weight and height were measured twice and the average documented. The BMI was calculated as weight(kg)/height (m2). The classification of BMI-for-age was based on World Health Organization (WHO) criteria) [16].

Statistical analysis

The Statistical Package for Social Science (SPSS) version 21.0 was used for tabulation and analysis of the data. The demographic data was examined using descriptive statistics, and the results were reported as frequency, percentage, mean and standard deviation. The KAP scores were calculated by summing up the participant's

number of correct responses. The frequency of plain water and SSB consumption were described via descriptive statistics, and the results were reported in frequencies and percentages. Association between Knowledge, attitudes and SSB consumption was tested using chi-square tests. All p-values were significant at <0.05.

Ethics

The protocol for the study was reviewed and approved by the Institutional Review Board of the University of Cape Coast, Ghana (UCCIRB/CHAS/2023/118). A written permission to undertake the study was obtained from the Ghana Education Service and the head teachers of the senior high schools. Participation in the study was voluntary and adolescents who agreed to participate in the study were made to thumb print or append their signatures on a participant consent form in the presence of a witness (mostly classmates or class teachers).

Results

Socio-demographic characteristics

The data involved socio-demographic aspects such as gender, age, class level of the participants, as well as the educational background and income of their parent or guardian. The findings in Table 1 showed that out of 393 participants, 53.9%, were male, while 46.1%, were female.

Table 1. Socio-demographic characteristics

	Frequency	Percent
Gender		
Male	212	53.9
Female	181	46.1
Age		
13 - 15	62	15.7
16 - 19	331	84.3
Class		
Form 1	146	37.2
Form 3	247	62.8
Education level of guardians		
None	47	12.0
Primary	10	2.5
Secondary	115	29.3
University	192	48.9
Don't know	29	7.4
Employment status of guardians		
Employed	327	83.2
Unemployed	43	10.9
Retired	23	5.9
Categorical data are presented as percentages.		

For the age distribution, 15.7% of the participants were between the ages of 13 -15 years while 84.3% fell between the ages of 15 – 19. The mean age is 16.83 (+-SD). Based on their class level, most of the participants, constituting about 62.8% of the total, were in Form 3 while 37.2% of the participants were in Form 1. Concerning the educational attainment of their guardian, 12% had guardians with no formal education. A very small percentage, 2.5% had guardians with primary education. About 29.3% had guardians with a secondary level of education. The majority (48.9%) had guardians with a university education. A smaller portion, about 7.4% did not know the education level of their parent or guardian. For the employment status of their guardians, the majority, (83.2%) had guardians who were employed. Almost 11% had guardians who were unemployed and a smaller portion of 5.9% had guardians who were on retirement.

Nutritional status of respondents

The mean weight (SD) was 60.1±11.1 and. the mean height (SD) was 165.4±9.7. The mean BMI-for-age of 1.23 suggests that, on average, most of the participants were within the "Normal" BMI category. The presence of individuals in the "Overweight" (11.5%) and "Obese" (7.1%) categories indicates that there is a portion of the population at risk for weight-related health issues.

Table 2. Nutritional status of respondents

	Mean	SD	Percent
Weight (kg)	60.1	11.1	
Height (cm)	165.4	9. 7	
BMI-for-age	1.2	0.6	
underweight <-2SD			2.5
normal -2SD to 1SD			78.9
overweight 1SD to 2SD			11.5
obese 30+			7.1

Nutritional knowledge level, SSB Consumption, and BMI-for-Age

Table 3 presents the distribution of respondents according to their nutritional knowledge of SSBs, level of SSB consumption, and BMI-for-age by gender. Overall, 26.7% of participants demonstrated poor knowledge of SSBs, 55.7% had moderate knowledge, and 17.6% exhibited good knowledge regarding SSBs and their health effects. When disaggregated by sex, 26.0% of males and 27.6% of females had poor knowledge, while moderate knowledge was observed among 53.3% of males and 58.6% of females. A higher proportion of males (20.8%) demonstrated good knowledge compared to females (13.8%).

Table 3. Distribution of Nutritional Knowledge, SSB Consumption, and BMI-for-Age by Gender (N = 393)

	Both sexes N(%)	Male N(%)	Female N(%)
Level of Knowledge			
Poor knowledge	105(26.7)	55(25.9)	50(27.6)
Moderate knowledge	219(55.7)	113(53.3)	106(58.6)
Good knowledge	69(17.6)	44(20.8)	25(13.8)
Level of SSBs consumption			
Low	167(42.5)	96(45.3)	71(39.2)
High	226(57.5)	116(54.7)	110(61)
BMI-for-age			
underweight <-2SD	10(2.5)	8(3.8)	2(1.1)
normal -2SD to 1SD	310(78.9)	185(87.3)	125(69.1)
overweight 1SD to 2SD	45(11.5)	14(6.6)	31 (17.1)
obese 30+	28(7.1)	5(2.4)	23 (12.7)
Frequency - N, Percentage - %			

Regarding consumption patterns, 57.5% of respondents reported high SSB consumption, whereas 42.5% reported low consumption. The distribution of BMI-for-age categories showed that a greater proportion of males (53.9%) fell within the normal BMI range compared to females (46.1%). Females were more represented in the overweight (17.1% vs. 6.6%) and obese (12.7% vs. 2.4%) categories, while underweight status was more prevalent among males (3.8%) than females (1.1%).

General attitude and Level of Consumption

Table 4 presents respondents' general attitudes toward sugar-sweetened beverages (SSBs) and the level of SSB consumption stratified by gender. Overall, 52.9% of participants demonstrated a positive attitude toward SSB consumption, whereas 47.1% exhibited a negative attitude.

Regarding consumption patterns by gender, 45.3% of males reported low SSB consumption and 54.7% reported high consumption. Among females, 39.2% had low consumption while a higher proportion, 61.0%, reported high consumption. Across the total sample of 393 participants, 42.5% had low SSB consumption and 57.5% had high SSB consumption.

Table 4. Distribution of Nutritional Knowledge, SSB Consumption, and BMI-for-Age by Gender (N = 393)

	Frequency	Percent
General Attitude		
Negative	185	47.1
Positive	208	52.9
Level of SSB consumption		
Low	167	42.5
High	226	57.5

Association between Knowledge, Attitude and SSB Consumption

Table 5 presents the association between participants' level of knowledge regarding sugar-sweetened beverages (SSBs), their general attitude, and their level of SSB consumption. The analysis showed no statistically significant association between knowledge and general attitude toward SSBs (χ^2 test, $p > 0.05$). Similarly, there was no statistically significant association between participants' level of knowledge and their level of SSB consumption (χ^2 test, $p > 0.05$). This shows that variations in nutritional knowledge about SSBs were not significantly related to either attitude toward SSBs or actual consumption patterns within the study population.

Multivariable Logistic Regression Analysis of Predictors of High SSB Consumption

Binary logistic regression analysis showed that none of the sociodemographic or behavioural factors were significant predictors of high SSB consumption. After adjusting for age, sex, parental education, BMI-for-age, nutrition knowledge, and attitude, none of these variables showed statistically significant associations with consumption level (all $p > 0.05$). Students aged 16–19 years were no more likely to consume SSBs than those aged 13–15 years (AOR = 1.14; 95% CI: 0.76–1.72; $p = 0.52$). Similarly, positive attitude toward limiting SSB intake did not predict lower consumption (AOR = 1.05; 95% CI: 0.72–1.55; $p = 0.79$).

Discussion

In interpreting the findings of this study, it is important to recognize that the measure of SSB encompassed a diverse range of seven beverage categories, each with distinct nutritional profiles and implications for adolescent health. Although plain water served as the reference beverage and reflects healthy hydration behaviour, adolescents in many settings often substitute water with caloric alternatives [3]. While milk and tea products offers essential nutrients such as calcium and high-quality protein, flavoured milks and commercially sweetened teas contribute substantial amounts of added sugars comparable to those found in soft drinks [41]. Similarly, sweetened fruit beverages, frequently perceived as healthier choices, are typically formulated with added sugars and provide energy densities similar to regular sodas, thereby contributing significantly to total sugar intake [51]. Energy and sports drinks pose additional risks due to their sugar content and the presence of caffeine and other stimulants, which may exacerbate sleep disturbances, hyperactivity, and cardiovascular strain among adolescents [36]. Homemade or locally prepared drinks which include sweetened porridges, cocoa beverages, hibiscus drinks, and other culturally embedded preparations are typically prepared without standardized recipes, leading to high variability in sugar content and portion size, and are consumed frequently within households [3]. Taken together, these categories illustrate that adolescents are exposed to multiple forms of SSBs beyond commercially packaged soft drinks.

Table 5. Association Between Knowledge, Attitude, and SSB Consumption

	General Attitude				SSB Consumption			
	Negative N(%)	Positive N(%)	χ^2 (df)	p-value	Low N(%)	High N(%)	χ^2 (df)	p-value
Level of Knowledge								
Poor	51 (13.0)	54 (13.7)	0.031	0.535	47 (28.0)	58 (25.7)	0.076	0.134
Moderate	104 (26.5)	115 (29.3)			98 (58.7)	121 (53.5)		
Good	30 (7.6)	39 (9.9)			22 (13.2)	47 (20.8)		
Total	185 (47.1)	208 (52.9)			167 (42.5)	226 (57.5)		
Frequency- N, Percentage- %								

Table 6. Crude and Adjusted Odds Ratios Predicting High SSB Consumption

	OR	95% CI	p-value	AOR	95% CI	p-value
Age (16–19 vs 13–15)	1.09	0.75–1.60	0.62	1.14	0.76–1.72	0.52
Sex (Male vs Female)	1.13	0.78–1.64	0.51	1.09	0.74–1.60	0.67
Parental Education (Higher vs Lower)	1.25	0.86–1.82	0.25	1.21	0.80–1.83	0.37
BMI-for-Age (Non-normal vs Normal)	1.16	0.78–1.72	0.45	1.08	0.71–1.63	0.71
Nutrition Knowledge (Moderate/Good vs Poor)	1.18	0.82–1.72	0.38	1.12	0.78–1.75	0.44
Attitude (Positive vs Negative)	1.07	0.74–1.53	0.73	1.05	0.72–1.55	0.79
Note: OR = Crude Odds Ratio. AOR = Adjusted Odds Ratio. CI = Confidence Interval. Adjusted for age, sex, parental education, BMI-for-age, nutrition knowledge, and attitude.						

Assessment of Adolescents' Knowledge Regarding SSBs

The first objective of the study was to evaluate the knowledge of adolescents regarding SSBs. The level of nutritional knowledge holds significant sway over individuals' dietary habits. In this study, an assessment was conducted to gauge the students' understanding of SSBs. An analysis of composite knowledge scores revealed that, on the whole, the participants possessed a moderate level of knowledge concerning the health consequences associated with SSBs. This finding aligns with prior studies conducted , which reported that most students possessed a moderate level of awareness regarding the detrimental effects of SSBs [19,20,21]. However, these studies did not find any significant correlation between nutritional knowledge and actual SSB consumption patterns. This underscores the critical importance of enhancing awareness among adolescents regarding the health implications of consuming SSBs. Interestingly, no discernible disparities were observed in knowledge levels when considering gender and BMI categories (P>0.05). Consequently, it can be inferred from this findings that factors such as gender, BMI categories, and class year do not exhibit any meaningful association with knowledge levels which is consistent with prior researches conducted [22,23]. Enhancing knowledge regarding the nutritional value of foods can facilitate better dietary choices, equipping individuals with the understanding of how to adopt a healthier diet for improved overall well-being. Achieving this goal necessitates the implementation of clear and easily accessible methods for displaying nutritional information tailored to this age group. Nevertheless, variables like gender, class year, and age groups did not display any significant associations with attitudes toward SSBs (P>0.05). A clear link between socio-demographic factors and nutritional knowledge has not been consistently confirmed but it is imperative to continue increasing awareness among adolescents regarding the health implications of consuming SSBs.

Determination of Adolescents' Attitudes Towards SSBs

The second objective aimed to investigate the attitudes of adolescents towards SSB intake. The findings of this study indicated that more than half of the respondents expressed a positive attitude toward the consumption of SSBs. This observation is in line with the results of a Nigerian study by Fadupin and others [24] among university students, where the majority of students exhibited a positive attitude toward SSBs. A Taiwanese study [25] among high school students similarly established that students with negative attitudes toward SSBs tended to consume fewer SSBs. This also aligns with a study conducted which found that individuals who were heavy users of soft drinks, regardless of the frequency, had more positive general attitudes toward their consumption [26,27],. In terms of the relationship between knowledge and attitudes, the study identified an extremely weak positive linear relationship between the level of knowledge and general attitudes towards SSBs. However, the statistical analysis showed that this correlation was not statistically significant, as the p-value of 0.535 was much higher than the significance level of 0.05. This indicated that the observed correlation between knowledge and attitudes was not strong enough to be considered statistically meaningful. Furthermore, the study revealed gender-based differences in attitudes towards SSB consumption, with males exhibiting a slightly more favorable mean attitude score compared to females. This could be attributed to the higher number of male participants in the study. A similar gender-based difference in SSB consumption attitudes was also noted in a study, where men and younger individuals consumed more regular soft drinks compared to women [29]. However, in this study, no statistically significant difference in general attitudes towards SSB consumption was found between males and females (P=0.572), which contrasts with the findings of Malik et al. [1], who reported that females had more negative attitudes toward the normal sweetness of soft drinks compared to males. Additionally, this study identified a significant difference in general attitudes toward soft drink consumption based on participants' BMI. Normal-weight individuals had more favorable attitudes towards soft drink consumption compared to overweight and obese individuals. These findings are consistent with the findings 32, which found that obese individuals had a higher preference or implicit attitude toward soft drink consumption compared to their non-obese counterparts. These results are supported by evidence showing that although low-energy foods and drinks provide few calories, excessive intake can lead to calorie accumulation, potentially resulting in overweight and obesity [34]. However, it's important to note that this study alone cannot establish a causal relationship between excessive diet soft drink consumption and the development of overweight and obesity; further prospective research is needed to investigate this potential link. Interestingly, the study did not find a significant correlation between knowledge levels and general attitudes toward soft drink consumption (P>0.05), which differs from the findings [35], who reported a weak and negative correlation between knowledge levels and general attitudes toward soft drink consumption (P=0.041). This suggests that individuals with more positive general attitudes toward soft drink consumption may have lower levels of nutrition knowledge.

Consequently, interventions aimed at reducing regular soft drink consumption should be tailored specifically to adolescents.

Investigation of Adolescents' Practices Regarding SSBs

The third objective of this study aimed to investigate the behaviors and practices of adolescents in relation to SSBs. The findings revealed that more than half of the high school students surveyed had a substantial intake of SSBs. This observation is consistent with a study conducted in the United States, which also reported high daily consumption of SSBs among both young adults and children [36]. Similar trends were noted in previous studies [37–39], which found that children had a higher consumption of soft drinks, often more than once per day, compared to adults. Consequently, there is a clear need for robust campaigns targeting this demographic, focusing on excessive or frequent consumption of SSBs. These campaigns could be integrated into the school curriculum or delivered through seminars. Recently, SSBs have been at the center of discussions related to obesity. However, when examining specific beverage consumption patterns, some variations emerge. For instance, a significant proportion of respondents reported consuming carbonated drinks, aligning with study on SSB consumption patterns in the USA, where soda (carbonated drinks) was the most commonly consumed SSB among adolescents [40]. Furthermore, the frequency of consumption revealed thatSSBs like tea and coffee were consumed at least once a day by 18.8% of the respondents. Across all the listed beverages, a volume of 250ml was the most commonly consumed. In interpreting these findings, it is important to consider the range of beverages captured in the study. The questionnaire included plain water, milk products, tea beverages, sweetened fruit drinks, regular soft drinks, energy and sports drinks, and homemade or local beverages. Although most students reported frequent consumption of commercially available soft drinks and sweetened fruit beverages, energy and sports drinks also contributed to overall SSB intake. Homemade beverages such as "sobolo," sweetened cocoa drinks, and locally prepared fruit juices were commonly consumed and may contribute substantial amounts of added sugar despite being perceived as "natural" or healthier options. This variety of beverages likely influences total sugar intake and may explain why consumption remained high even among students with moderate knowledge and positive attitudes. Interestingly, the study found that plain water was the most frequently consumed beverage on a daily basis, a finding consistent with the results of a study conducted by Miller [41], where a majority of participants also reported daily consumption of plain water. It's important to acknowledge that the findings regarding the frequency of SSB consumption were based on self-reports, which may be subject to reporting bias. Therefore, these findings should be interpreted with caution and may not be generalized to other populations of the same age group. High consumption of soft drinks, especially sugar sweetened (regular drinks) have been reported by various studies to have a direct link with diet related health problems [42,43]. SSBs have been implicated as one of the contributory factors to increased body weight and high risk of obesity observed among many population 3,36,42. The high consumption of SSBs observed among the students in this study could be as a result of their perception of SSBs. For example, many of the respondents perceived SSBs as social drinks and it is alarming that the majority consumed SSBs regularly despite their awareness of health implications of excessive consumption of these drinks. The relatively lower consumption frequency of carbonated drinks, fruit drinks, and energy drinks, often limited to at least once a week or less, could be influenced by factors such as affordability, accessibility, and availability.

Relationship Between Demographic Factors and SSB Consumption

The study utilized correlation coefficients to explore the potential links between demographic factors and SSB consumption. Interestingly, the analysis revealed that neither gender nor the class of the participants exhibited any significant associations with SSB consumption levels. This finding contrasts with the results of [44], where a thorough inferential analysis established a notable link between respondents' class and their consumption of SSBs. In stark contrast, a national cross-sectional study conducted among American high school students pointed to a considerable reduction in soda consumption over the years [45]. It's noteworthy that all the participants in our survey were teenagers, with the majority falling in the 16 to 19 age group. Intriguingly, our analysis did not show any association between age and the frequency of SSB consumption. This finding contradicts the results [46] which found a significant correlation between increasing age among respondents and a higher likelihood of frequent SSB consumption. Furthermore, in our bivariate analysis, we could not establish any significant associations between the educational levels or occupations of parents or guardians and the frequency of SSB consumption among the students. This outcome contradicts the findings suggested by Magriplis [47], which supported the notion that adolescents' frequency of SSB consumption is influenced by the educational backgrounds of their parents. With regards to the anthropometric indices among adolescents and young adults, significant association was observed between frequent intake of SSBs and being overweight among the students by many researchers in other countries [48–50]. These reports highlighted that, as a result of plausible physiological mechanism, frequent or excessive intake of SSBs could lead to overweight and obesity, due to imprecise and incomplete compensation for energy consumed in liquid form [51-53]. It was also reported in a national survey data in the United States that, over the past 20 years, there has been an increase in rates of overweight and obesity due to high consumption of carbohydrates, largely in the form of SSBs [54] .

Implications and Recommendations

The findings of this study have several implications for future research and interventions. It is evident that there is a gap between knowledge, attitude, and actual practices regarding SSB consumption among adolescents. Therefore, strategies are needed to bridge this gap and translate positive attitudes into healthier practices. Education on SSBs and their health consequences should be strengthened within school curricula. It is recommended that such strategies take

into account the socioeconomic status of students, with an emphasis on nutritional education about SSBs and healthy eating for those from higher-income families. Additionally, efforts should be made to reinforce the understanding of the adverse effects of high SSB consumption to motivate healthier practices. Reducing the availability of SSBs both in schools and at home can support lifestyle changes and promote healthier beverage choices. Furthermore, Intervention to reduce soft drinks consumption especially sugar sweetened drinks should have theoretical basis as review of the literature shows theory-based interventions of other health behaviour change has substantial effects [54,55]. On this aspect the theory of planned behaviour has been found to be promising in explaining and predicting eating and drinking behaviours [54,55]. Although this study found generally low consumption of soft drinks and low attitudes towards consumption of soft drinks, it sheds some light for policy implication and practices. Adults can still play a significant role in behavioural change of their children and adolescents. This can be implemented through reinforcement of parental rules, knowledge, and skills, since children and adolescents are mostly affected by negative health consequences resulting from excessive soft drink consumption. Another policy implication could be promoting awareness through information campaigns and addressing environmental cues related to soft drink consumption [56,57]. Moreover, soft drink industries should innovate and produce new soft drinks products with regulated amounts of sugars and small size packages to limit intake of sugar and reduce the amount of intake. Also, soft drinks should not form the essential part of the meal and/or can be completely avoided during meals. Alternatively, water and low-fat milk products can replace sugar-rich soft drinks [58].

Before implementing policy measures to reduce soft drinks consumption the government should consider; the prevalence of health effects caused by consumption of soft drinks (such as obesity and cardiovascular diseases) in the target population. The levels of soft drinks intake (which are determined by consumption frequency and amount) in the general population and the existing policy measures which have worked [59]. To check for effectiveness of informative intervention targeting at adolescents' soft drink consumption, their attitude and nutrition knowledge, and long prospective studies are needed. Increase of nutrition knowledge is also necessary since this knowledge is not static, it changes as knowledge on health and diet increase, subjecting dietary recommendations to changes as well [60].

Limitations

While this study provides valuable insights, it is not devoid of limitations. The sample was drawn from a limited number of schools, which may reduce the applicability of the findings to the wider adolescent population, and the use of convenience sampling may have introduced selection bias, as participants were selected based on availability rather than random sampling. As a result, the study sample may not be fully representative of all students, and the findings should therefore be interpreted with caution and may not be generalisable to the wider adolescent population. Additionally, the reliance on self-reported data for beverage intake introduces the possibility of reporting bias, particularly in overweight or obese adolescents. Moreover, the study did not consider various other factors such as dietary habits, physical activity, genetic factors, or environmental influences that could impact body composition. For a more comprehensive understanding of adolescent SSB consumption, future research should incorporate these factors. Furthermore, this study covered numerous variables within a relatively limited timeframe. While this breadth of exploration provides a valid overview of the population, it also hinders in-depth analysis of specific issues. The findings from this study can serve as a foundation for further research aiming to delve deeper into the topics explored herein. Regrettably, due to resource and time constraints, the study's design does not permit the establishment of causality. Longitudinal studies are necessary to ascertain causative relationships. The use of self-administered questionnaires and, consequently, self-reported data, despite its advantages, introduces potential biases, including recall and social desirability biases. Additionally, the absence of accompanying clinical examinations prevents the validation of self-reported findings against objective measurements. Integrating clinical examinations could enhance the perceived validity of the reported results. Therefore, it is essential to consider these limitations when interpreting the study's outcomes.

Conclusion

This study delved into the factors influencing adolescents' knowledge, attitudes, and practices regarding SSBs. The findings revealed that, on the whole, adolescents possessed a moderate level of knowledge about the health implications of SSBs and generally held positive attitudes toward their consumption. However, there was a disconnect between this knowledge and their actual practices, with a significant portion of adolescents consuming substantial quantities of SSBs. Notably, factors such as gender, BMI categories, and class did not significantly impact knowledge, attitudes, or practices related to SSBs. These results underscore the imperative need for targeted interventions aimed at augmenting knowledge and translating these positive attitudes into healthier practices among adolescents. Adolescents unequivocally require nutritional education. While there is a paucity of pre- and post-intervention studies, existing research [60,61], has demonstrated positive outcomes post-intervention. Their study reported increased fruit and vegetable consumption and decreased sweet intake, particularly among females. Among males, a noticeable reduction in soft drink consumption and an increase in fruit and vegetable intake were observed. Nutritional interventions during adolescence have the potential to influence eating behaviours, which can have long-term effects extending into adulthood. Attitudes toward food, especially healthy options, may be influenced by concerns about body image, particularly among adolescent girls. Some students, particularly females, may adopt healthy eating habits not solely due to increased nutritional knowledge but also by emulating their parents' eating habits. Older adolescents tend to assert more

independence in choosing their foods, while younger ones are more likely to follow their parents' eating patterns [61–63]. Government policy interventions can have a substantial impact on controlling SSB consumption and addressing obesity among adolescents and young adults. Continuous campaigns and advertisements highlighting the detrimental effects of excessive SSB consumption should be intensified to discourage students from consuming these beverages. Additionally, the government should consider regulating the production and consumption of SSBs through policy measures to curb the increasing intake and associated health-related harms resulting from their misuse [62].

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Data Availability

Data cannot be shared publicly because it contains sensitive identifying information. However, data are available from the Institutional Review Board of the University of Cape Coast, Ghana (Email: irb@ucc.edu.gh) for researchers who meet the criteria for access to confidential data.

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