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HEALTH SCIENCES INVESTIGATIONS JOURNAL Volume 6 Issue 1

Editors' Choice

Ultrastructural hepatic damage in murine malaria with and without prandial natural cocoa powder and artemether-lumefantrine treatment. Aidoo et al., 2024. Pages 798-807
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About the cover portrait "No Limits"

The painting depicts a woman seeking refuge under her umbrella. The artwork is inspired by the human instinct to shield against obstacles. Read more about the artist's perspective and the editor's view on page 748
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Summary Author Information

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We look forward to hearing from you. Please feel free to contact us if you need any further clarification.

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A Tribute to Our Colleague and Friend,

Professor Phyllis Dako-Gyeke

On behalf of the Editorial Team, I wish to convey my sincerest condolences to Professor Phyllis Dako-Gyeke's family, colleagues and friends. Her volunteer work as an editor and member of the editorial team at the Health Sciences Investigations (HSI) Journal, established in 2019, was instrumental in its growth to become one of the leading journals at the University of Ghana. As an editor and reviewer, Professor Dako-Gyeke played a significant and critical role in assisting the HSI Journal in securing indexing for Africa Journal on Line and Scopus. We are forever grateful for her inspiring input.

Professor Dako-Gyeke, a Social and Behavioural Sciences expert at the University of Ghana School of Public Health, College of Health Sciences, has greatly impacted the scientific and medical community. Throughout her period as an editor of HSI Journal, Professor Dako-Gyeke has demonstrated an unwavering commitment to improving the quality of manuscripts submitted to the HSI Journal for publication. Her pioneering inputs in reviewing and improving the quality of manuscripts submitted for consideration and publication have advanced scientific understanding and translated into practical solutions that have broadened insights into biomedical and clinical research.

Professor Dako-Gyeke has inspired a new generation of healthcare professionals as an educator, supervisor, and mentor. Her dedication to teaching and mentoring young public health graduate Students, Doctors and Researchers has ensured that her legacy will continue through the countless individuals she has trained and influenced. Her approach to editing and reviewing manuscripts and proposals, characterized by empathy, rigour, and a deep commitment to her Students, is a model for all in the biomedical and clinical fields.

Professor Dako-Gyeke joined the Department of Social and Behavioural Sciences in 2009 as a Lecturer and was subsequently promoted to Senior Lecturer in 2014 and Associate Professor in 2020.

Professor Phyllis Dako-Gyeke was the principal investigator for the Regional Training Centre in the Afro region supported by WHO-TDR and the lead for strengthening implementation research capacity across Africa. She was also the Principal Investigator in the Second phase of the TDR programme. She passed away on Tuesday, June 11, 2024.

As we pay tribute to Professor Phyllis Dako-Gyeke, we celebrate her remarkable achievements and the enduring spirit of compassion and excellence she displayed at the Editorial Office. Her legacy will continue to inspire and guide the efforts of those dedicated to improving the HSI Journal and making it one of the world's best biomedical and clinical journals.

May her soul rest in peace.

HSI Health Sciences Investigations Journal

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The HSI Journal Logo

The Logo is an *Adinkra* symbol rendered in the Akan language as ***Nea onnim no sua a, ohu***. It is loosely translated into English as "the one who does not know but learns, gets to know."

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Editorial

Welcome message from the Editor-in-chief

Professor Andrew Anthony Adjei
Email: hsijournal@ug.edu.gh

We gladly welcome readers to Volume 6, Issue 1 of the Health Sciences Investigations (HSI) Journal. Launched in 2020, the HSI Journal has rapidly gained international recognition, attracting global submissions across diverse scientific fields. Notably, our recent Special Edition (Volume 5 Issue 2, 2024) explored the health implications of "galamsey" — a term referring to illegal small-scale gold mining in Ghana. This critically acclaimed issue is a must-read. The HSI Journal is indexed in Elsevier and affiliated journal repositories, including SCOPUS®, and is listed in the African Journals OnLine (AJOL) citation database. The HSI Journal is also pursuing inclusion in other major repositories. Consider the HSI Journal for your next publication. Volume 6 Issue 1 features ten Original Research Articles on diverse topics, each providing valuable insights. The articles cover a range of subjects, including the presence of SARS-CoV-2 in wastewater from hand wash stations, substance use among high school-going adolescents in Ghana, and a rare but significant cause of chest pain in young adult females.

Additionally, the issue investigates contraceptive practices among postpartum women and compares the prevalence of skin disorders among renal and non-renal disease patients. Other studies explore potential sources of novel antimicrobial-producing streptomycetes in Ghana and examine the protective effects of natural cocoa powder against liver damage in malaria infection compared to artemether-lumefantrine. Collectively, these articles offer engaging and informative content for readers. This issue features a top-notch review article analyzing policy strategies in four African nations (Rwanda, Tanzania, South Africa, and Ghana) for achieving Universal Health Coverage. We are also pleased to present two thought-provoking medical case reports. Delve into these articles and share your feedback afterwards – it helps us improve. As Editor-in-Chief, I encourage authors and readers to share their feedback, suggestions, and concerns to help us maintain the HSI Journal's high standards of excellence.

The HSI Journal sincerely thanks the Editorial Board members and reviewers for their invaluable contributions and suggestions in making this special publication possible. We also acknowledge the immense support and guidance the Technical Team, Advisory Board, all authors, and publishers provide.

Acknowledgements
The University of Ghana College of Health Sciences – the copyright owner, patron, and sponsor of the HSI Journal – has always shown a deep interest in the affairs of its constituent institutions. The Journal is indeed grateful to Professor Julius Fobil, the Provost of the College, for his immense support.

Thank you

About the Editor-in-chief

Professor Andrew Anthony Adjei is a Professor of Immunology with over thirty years of biomedical and allied health sciences training and research experience. He is a Fellow of the following: Ghana Academy of Arts and Sciences (FGA), African Academy of Sciences (AAS), Ghana Association of Medical Laboratory Scientists (GAMLS) and African Sciences Institute (ASI). Professor Adjei has been Head of Department, University of Ghana (UG) School of Biomedical and Allied Health Sciences, Deputy Provost, College of Health Sciences (CHS), Director of Research, Innovation and Development (UG), Acting Director, Institutional Research and Planning Office (UG), Coordinator of Research, University of Ghana Medical School (UGMS), Editor-in-Chief, Ghana Journal of Allied Health Sciences, President of Ghana Association of Medical Laboratory Scientists, Project Coordinator, Transdisciplinary Training for Resource Efficiency and Climate Change Adaptation in Africa, Project Coordinator, Building Stronger Universities (Partnership between UG and Universities in Denmark), Project Coordinator, Fogarty Global Health Fellows Training Programme (Partnership between UGMS and University of Morehouse School of Medicine, Atlanta, Georgia, USA), and Project Coordinator, Minority in Health Research Training (Partnership between UGMS and University of Morehouse School of Medicine). Professor Adjei was the immediate past Coordinator of the Worldwide Universities Network and the Australia-Africa Universities Network. Currently, he is the Chairman of the following: Ethics and Protocol Review Committee, CHS Public Lecture Series and Scientific Conference Planning Committee, CHS Newsletter (In Focus), CHS Library Refurbishment Committee, Member of Korle Bu Teaching Hospital Institutional Review Board and the Coordinator, MPhil Programme in Immunology, at the Department of Pathology, UGMS. Professor Adjei is a reviewer of several clinical and biomedical Journals globally. He has served on various UGMS and UG committees and currently serves on both the UG and CHS Academic Boards.

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About the cover

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The use of bright, vibrant colours in the painting creates a sense of energy and movement, drawing the viewer's attention to the woman and her umbrella. The contrast between the cool blues and greens of the woman's attire and the warm reds, browns, golds, and lemons of the background creates a visually striking composition. The semi-abstract style of the painting allows for interpretation and imagination on the part of the viewer.

Tabi Crentsil (2024). "*No Limits"*, a woman finds refuge under her umbrella.

"No Limits"

This painting is inspired by the human instinct to protect against obstacles.

Artist's perspective

The woman's posture and the way she holds the umbrella suggest a sense of protection and self-reliance, while the bold colours and abstract forms add a sense of mystery and intrigue to the scene. The extent to which one may go to shield themselves from impending interference has no limits. Yet those very things shielded otherwise contribute to the endless opportunities that can be derived from such circumstances. Overall, this painting is a beautiful example of art that invites the viewer to appreciate the beauty of colour, form, and composition while also sparking contemplation and interpretation. It is a captivating piece that engages the viewer's senses and emotions, making it a truly valuable work of art.

Take a journey through the painting and identify with the attributes. The rest of the story lies with the viewer.

A production from Tabi Crentsil, 2024

Editors' view

The HSI Journal is captivated by the artwork that graces our page cover. The semi-abstract style invites us to decipher the enigma. What lies beyond the woman's gaze? What stories does her umbrella conceal? The mystery is deliberate - an ode to the unanswered questions that drive our pursuit of knowledge in our fields of study. As health scientists, we grapple with the abstract daily—the unseen pathogens, the molecules in our test tubes, the intricate cellular pathways and the challenges associated with research. Here, the painting mirrors our quest as scientists: to imagine, to unravel, to interpret. Dear readers, as you turn these pages, let the "Umbrella of Resilience" in this "No Limits" artwork embrace you. Let its vibrancy seep into your research, your teaching and your practice. May this painting inspire us to wield our umbrellas with purpose in our endeavours. This painting is a testament to the human spirit's ability to persevere and find solace in the unknown. As we turn the page in our health sciences journal, we are reminded that art has the power to inspire, heal, and connect us in ways that transcend words alone.

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Commentary

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Developing and Growing the 'Ebibiduro' (Natural Medicine) Industry in Ghana: What about Cocoa?

Neils B QUARSHIE

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Keywords: Natural medicine, cocoa, "Ebibiduro", Arthmeter-lumefantrine, Ghana

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Ghana is endowed with a wide range of natural products which are high in therapeutic values. From the shrubs behind our various homes to the bark of the big trees in the deep green forest lies natural compounds with great therapeutic values. Known in the local Akan parlance as 'Ebibiduro', it has been documented that a proportion of the Ghanaian population use these natural medicinal products for their primary healthcare needs and in the management of both communicable and non-communicable diseases [1]. Indeed, Ghanaian have used various parts of plants from Caripa papaya, Moringa oleifera, Hibiscus sabdariffa, Azadirachite indica and others for the treatment of common ailments as well as to ensure perfect wellbeing [1]. The word 'Natural product' used here in this commentary refers to traditional medicines, herbal products, tree barks, as well as food products with medicinal values among others.

Though in the recent past the natural medicine industry in Ghana has been given unprecedented boost regarding policy direction and the rejuvenation of appropriate institutions, yet majority of the natural medicinal preparations used in this country have not undergone appropriate scrutiny and often not approved by the Ghana Food and Drug Board. Whereas in places like China, natural medicine have been well developed and plays a crucial role in health delivery and impact significantly on their economy, the same can't be said about Ghana at the moment. A study carried by Asase in 2023 indicated that the prospects of the medicinal plant trade in Ghana are huge, and if given the necessary attention could lead to a reduction in the national health budget, become a source of foreign and domestic income, as well as create employment and reduce poverty [2]. He further stated that the industry is currently bedeviled with several challenges, such as registration of natural medicinal products and practitioners, a lack of clinical trials for herbal products, standards and quality control issues, shortage of raw plant materials for production, and insufficient scientific research to support traditional claims on the pharmacological effects of medicinal plants.

These assertions are indeed true and needs a careful scrutiny as soon as practicable. It is therefore imperative for Ghana to initiate the process of exploiting our natural resources for health and economic gains. There is the need for government to partner with the private sector in order to execute a well-planned national research and development policy and implementation for the natural medicine industry. In this regard, it has been suggested that the development of our cocoa beans into products with health benefit should take the lead [3]. The use of cocoa power and allied products of the beans in ameliorating disease conditions have been highly touted. The antimalarial activity of cocoa powder had been variously assessed and published [3,4]. In all cases natural cocoa products were found to exhibit significant antimalarial activities. Natural cocoa also possess other medicinal activities: Synopses by WebMD (2023) on their website indicate that the "seed of cocoa is used for infectious intestinal diseases and diarrhea, asthma, bronchitis, and as an expectorant for lung congestion [5]. Furthermore, the seed coat is used for liver,

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bladder, and kidney ailments; diabetes; as a tonic; and as a general remedy. Cocoa butter is also used to treat high cholesterol [5]. Some people apply cocoa butter to the skin to treat wrinkles and to prevent stretch marks during pregnancy [5]. In the pharmaceutical industry, cocoa butter find itself as a base for various ointments and suppositories made by drug companies" [5].

One of the significant health properties of cocoa is highlighted in the current issue of the Health Sciences Investigations journal. The article authored by Aidoo and others demonstrated the therapeutics potency of natural cocoa [6]. The group sought 'ultrastructural evidence for previously demonstrated amelioration of Plasmodial damage in murine hepatic tissue to facilitate comprehension of the phenomenon at the mechanistic level'. Their conclusion was intriguing as it suggest that 'mitigation of liver ultrastructural damage in P. berghei-infected rats given natural cocoa product was better than treatment with Artemether-Lumifantrine (a recommended antimalarial drug for the treatment of uncomplicated malaria in Ghana). The authors linked this occurrence to the anti-inflammatory activity of cocoa. Cocoa is known to contain a variety of chemicals, including antioxidants called flavonoids which are thought to be responsible for the noticed therapeutic benefit [7]. There is the need to exploit these chemical constituents scientifically for the benefit of mankind. For instance, we could concentrate these identified beneficial active constituents into products that could be prescribed as medicines. Ghana is the world's second largest supplier of cocoa beans with the quality of it beans described as the best in the world [8] and must take the lead in this!. A serious research to develop and grow cocoa as a health icon must be vigorously pursued. Meanwhile, campaign or education to encourage people to patronize cocoa products for health benefit must also be pursued. In conclusion, well thought through strategies should be developed or adopted in order to boost the use of natural products such as cocoa products in Ghana's development.

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Original Research Article

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Adequacy of dietary fibre intake among hypertensives in a University Hospital in Accra, Ghana: A cross-sectional study

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Abstract

Background: Dietary fibre, as a component of a healthy diet, has been shown to lower blood pressure and blood lipids and regulate body weight.

Objective: The study aimed to assess dietary fibre intake among people living with hypertension and its association with the socio-demographic characteristics of respondents.

Methods: A cross-sectional study was conducted among 186 participants (82 males, 104 females) attending the University of Ghana Hospital at Legon, Ghana. Dietary fibre intake was assessed using a quantitative food frequency questionnaire that measured food intake over a month. Blood pressure and anthropometric measurements were taken following standard World Health Organisation (WHO) guidelines. Lipid profile values were obtained from each participant's folder.

Results: Participants' median (IQR) dietary fibre intake per day was 14.8 g (2.4 to 38.1). The majority of the participants (88%, n = 165) had low intakes compared to the recommended daily intakes. The analysis revealed no association between either systolic (β = -0.114; p = 0.315) or diastolic blood pressure (β = -0.007; p = 0.947) and dietary fibre intake. The individual predictors indicated that BMI significantly predicted diastolic blood pressure (p = 0.033), and total energy intake significantly predicted systolic blood pressure (p = 0.019). The major source of dietary fibre was a cereal legume mix (tom brown), which contributed 24.5% of fibre to the daily fibre intake.

Conclusion: Reported dietary fibre intake among the study population was below the recommendation for fibre intake according to the United States Food and Drugs Administration Daily Reference Value. Dietary fibre intake was not associated with either systolic or diastolic blood pressure. Nutrition education strategies such as promoting whole meals and consuming plant-based foods should be intensified among people living with hypertension to encourage the consumption of meals rich in fibre.

Keywords: Dietary fibre, hypertension, blood pressure, anthropometry, blood lipids

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INTRODUCTION

Hypertension (blood pressure > 140/90 mm Hg) is a primary cause of cardiovascular morbidity (CVD) and mortality [1]. In 2010, the prevalence of hypertension was estimated to be more than a quarter of the world's population (31.1%), with a higher prevalence in low and middle-income countries [2]. A similar finding was reported by the World Health Organization (WHO), with the highest prevalence observed in the WHO African Region (27%) and the lowest in the WHO Region of the Americas (18%) [3]. This observed situation has been associated with an increase in unhealthy lifestyles such as low intake of fruits and vegetables, high salt and fat intake, sedentary lifestyle, smoking, alcohol consumption, anxiety and stress [4]. In Ghana, the overall prevalence of

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hypertension in 2018 was 30.3%, with a higher prevalence in females than males [5]. The prevalence in the urban areas was higher than the pooled prevalence in the rural areas [5]. Hypertensive patients usually present also with poor glycaemic control and unfavourable lipid profiles compared with the general population [6]. Dietary intake plays a key role in the prevention and management of hypertension and metabolic syndrome. Dietary fibre reduces the glycaemic index of foods and attenuates insulin response by enhancing insulin sensitivity and vascular endothelial function, which has been shown to have a cause-effect relationship with hypertension [7]. Increasing fibre intake by 7 - 15 g/day has been shown to lower blood pressure and reduce the risk of developing hypertension compared to diets lower in fibre [8]. Abatement in non-communicable diseases and cardio-metabolic risk factors have been associated with increased intake of dietary fibre [10-11]. There is a paucity of evidence on dietary fibre intake among hypertensive patients living in Ghana. This study, therefore, assessed dietary fibre intake among individuals with hypertension and the major sources of fibre among the study population.

MATERIALS AND METHODS

Study design and sampling

A hospital-based cross-sectional study was conducted at the diet therapy unit of the University of Ghana Hospital, Legon. Enrolled participants were patients reporting to the diet therapy unit of the University Hospital. The total enumeration technique, which involves selecting all eligible participants at the study site, was used to recruit participants. Daily visits were paid to the hospital, and with the assistance of the dietician, eligible patients 18 years and above diagnosed with hypertension were identified and recruited if they consented and signed a form to participate in the study after it had been explained to them.

Assessment of Dietary Fibre

Dietary fibre intake was assessed using a 31-item quantitative food frequency questionnaire adapted from a similar study [11]. The questionnaire had seven food groups, namely; (1) cereals and grains, (2) roots and tubers, (3) breads, (4) legumes, (5) nuts and seeds, (6)baked products, and (7) fruits and vegetables. Food models and household measures were used to assist in the estimation of portion sizes of usual food intake. Daily dietary fibre intake was calculated using the nutritional analysis software Microdiet (version 3.0, Downlee Systems, UK) and the Ghanaian and West African food composition tables after portion sizes were converted into grams. Dietary fibre intake was classified as either inadequate (< 25 g/day) or adequate (≥ 25 g/day) [12]. The percentage of fibre contribution for each of the foods was generated using Microdiet Nutrition analysis software.

Blood Pressure and Body Composition Measurements

Height was measured to the nearest 0.1 cm with a stadiometer (Omron HBF - 516C, USA). Weight, BMI and visceral fat were assessed using the Omron Body Composition Monitor (Seca 213, Germany). All measurements were taken using standard measurement protocols [13]. Visceral fat was classified as normal (1 - 9), high (10 - 15) and very high (> 15). Blood pressure was measured by a nurse using a calibrated upper arm BP monitor (Omron M2 HEM-7120). Measurements were taken as participants relaxed and sat in a chair (feet on the floor, back supported) for more than 5 minutes before BP measurement. High blood pressure reading was defined as BP readings ≥ 14 0/90 [14]. Three blood pressure measurements were used in the calculation of the patients' average systolic and diastolic pressure. Lipid profile (total cholesterol, total triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein) values were obtained from participants' hospital folders. Clinical laboratory results obtained within the previous three months at the time of data collection were used for the study. Anonymity and confidentiality were ensured throughout the study.

Socio-demographic data

Socioeconomic and demographic information, medical history and physical activity information were collected using a structured questionnaire. Physical activity was categorised as high intensity (vigorous-intensity activity such as jogging, soccer, bicycling, swimming), moderate intensity (brisk walking, lawn mowing, dancing) or light intensity (slow walking, stretching, domestic chores).

Statistical Analysis

Data analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp., Released 2011. Data were presented as median (minimum, maximum), n (%), or mean ± standard deviation. Population characteristics were stratified by fibre intake status using student's t-test, Mann-Whitney U test and chi-square. Respondents were stratified into two groups based on the daily fibre intake of < 25 g/day for low intake or ≥ 25 g per day for high intake. In determining the association between blood pressure and higher fibre intake (≥ 25 g/day), a multilinear regression model was constructed with systolic and diastolic blood pressure values as the dependent variables. The multivariate model was adjusted for age, total caloric intake and BMI.

RESULTS

Of the 186 participants included in the study, 44.1% (n = 82) were males and 55.9% (n = 104) were females. The median age was 58 years. The median BMI was 28.4 kg/m^2, and the median fibre intake was 14.7 g/day. The majority of the respondents (88.7%, n = 165) had a low intake of fibre. Table 1 provides a summary of the clinical and background characteristics of the respondents stratified according to their daily fibre intake. There were no significant differences between the lower and higher fibre intake groups in terms of gender, physical activity, ever visiting the dietitian, marital

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Table 1. Background characteristics and laboratory profile of respondents stratified by the adequacy of fibre intake

	Below recommended fibre intake (< 25 g/day)	At or above recommended fibre intake (≥ 25 g/day)	P-value (CI)
N	165	21	
Age (years)	59 (19 - 83)	57 (19 - 72)	0.011ǂ
Gender			
Male	42.4%	57.1%	0.201 ǂ
Female	57.6%	42.9%	
Marital status			
Single	24.8%	33.3%	0.403 ǂ
Married	75.2%	66.7%	
Employment status %	52.1%	61.9%	0.397 ǂ
	47.9%	38.1%	
Physical activity:(Yes) %	78.8%	66.7%	0.211ǂ
	21.2%	33.3%	
Dietitian Visit: (Yes) %	70.3%	57.1%	0.220 ǂ
No	29.7%	42.9%	
BMI (Kg/m^2)	29.3±5.5	30.5±6.3	0.383 ⊦
Weight (kg)	75.6 (43.8 – 177.9)	78.4 (51.0 – 110.1)	0.231ǂ
Height (cm)	164.4±7.9	166.2±6.2	0.187 (-5.4 – 1.7) ǂ
Visceral fat	11 (2 - 23)	11 (3 - 23)	0.824 ǂ
Systolic Blood Pressure (mmHg)	136.7±15.6	140.0±24.2	0.681 ǂ
Diastolic Blood Pressure(mmHg)	81.4±10.6	88.7±15.4	0.039 ⊦
Total cholesterol	4.9 (2.4 – 10.4)	5.2 (3.2 – 7.9)	0.269 ǂ
Triglyceride	1.2 (0.4 – 4.2)	1.3 (0.6 – 2.1)	0.847 ǂ
HDL	1.3 (2.4 – 24.5)	1.2 (0.8 – 4.8)	0.708 ǂ
LDL	3.0 (0.5 – 7.9)	3.2 (1.7 – 5.3)	0.262 ǂ
Hypertension medication %			
Yes	12.1%	9.5%	0.728 ǂ
No	87.9 %	90.5%	

Data expressed as mean ± SD, median (IQR), or n (%). ǂ Mann-Whitney U test. ǂ chi-square test. ⊦ t-test. HDL: high-density lipoprotein, LDL: low-density lipoprotein, BMI: body mass index. CI: confidence interval

Table 2. Daily nutrient intake of respondents stratified by the adequacy of fibre intake

	Below recommended fibre intake (<25 g/day)	At or above recommended fibre intake (≥ 25 g/day)	P-value
N (%)	165 (88.7)	21 (11.3)	
Caloric Intake (kcal/day)	1698 (445 – 3420)	3092 (1477 – 4809)	0.000 ǂ
Carbohydrate (g/day)	288.1 (65.9 – 556.4)	528.7 (239.4 – 836.8)	0.000 ǂ
Protein (g/day)	39.1 (7.9 – 103.8)	80.2 (32.3 – 142.9)	0.000 ǂ
Fat (g/day)	49.5 (10.7 – 200.2)	84.6 (27.0 – 223.2)	0.015 ǂ

Data expressed as median (IQR). ǂ-Whitney U test

Table 3. Percentage contributions of major food sources of fibre in the diet of participants

Food item	% Contribution
Tombrown	24.5%
Fufu	15.7%
Oats	15.1%
Corn porridge	14.4%
Vegetable Salad	14.4%
Kenkey	13.2%
Waakye	12.9%
Mango	12.7%
Wheat	12.6%

status, BMI, visceral fat, systolic blood pressure, lipid profile and taking a blood pressure medication. Patients in the higher fibre intake group were younger than those in the low fibre intake group. Patients in the low-fibre intake group had a lower mean diastolic blood pressure (81.4 ± 10.6 mmHg) than respondents who consumed more fibre per g/ day (88.7 ± 15.4 mmHg). Daily macronutrient intake, based on the adequacy of dietary fibre intake, is described in Table 2. Patients taking adequate amounts of fibre had higher intakes of total energy, carbohydrate, protein, and fat than did patients who consumed lower than the recommended fibre intake.

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Table 4: Linear regression analysis: diastolic blood pressure and adequate fibre intake (> 25 g/day). Model adjusted for sex, age, total energy intake, and sodium intake

Variables	β	P - value
Fibre intake (g/day)	-0.007	0.947
Sex	-0.056	0.470
Age (years)	-0.145	0.051
Total energy intake (kcal/ day)	0.159	0.160
BMI (Kg/m^2)	0.164	0.033

Table 5: Linear regression analysis: systolic blood pressure and adequate fibre intake (≥ 25 g/ day). Model adjusted for age, total energy intake, and sodium intake.

Variables	β	P - value
Fibre intake (g/day)	-0.114	0.315
Sex	-0.130	0.093
Age (years)	0.052	0.484
Total energy intake (kcal/ day)	0.267	0.019
BMI (Kg/m^2)	-0.114	0.315

A greater proportion of the participants consumed fibre from the cereals and grains, roots and tubers group. Identification of the major sources of fibre among the respondents was generated using the nutrient analysis software MICRODIET. Weanmix (a cereal legume mix), millet porridge, oats, vegetable salad, kenkey (fermented corn dumpling), waakye (rice with black-eyed beans), mango and wheat were identified as the main sources of fibre. Tombrown (roasted corn porridge), which was identified as the highest source of fibre, contributed 24.5% of the overall fibre intake in participants, followed by fufu (pounded plantain and cassava) which contributed 15.7% (Table 3). Although these foods were identified as the major sources of fibre, the portion size consumed by an individual will influence the overall dietary intake. Multiple regression models (Table 4 and Table 5) showed no association between both systolic (p = 0.315) and diastolic blood pressure (p = 0.947) and adequate dietary fibre intake. The individual predictors indicated that BMI significantly predicted diastolic blood pressure (p = 0.033) (Table 4), and total energy intake significantly predicted systolic blood pressure (p = 0.019) (Table 5).

DISCUSSION

This study sought to assess dietary fibre intake among patients living with hypertension. This is based on the premise that dietary fibre has been shown to have numerous health benefits, and it is important for health practitioners to work towards adequate intake of this nutrient in various populations. The present analysis demonstrated no association between dietary fibre intake and blood pressure after adjusting for potential confounders (age, sex, total energy intake and BMI). Alarmingly, the proportion of participants with insufficient daily fibre intake (≤ 25 g) was over 80%. This high level of inadequate dietary fibre is probably responsible for the non-significant association between blood pressure and dietary fibre intake. The p-value was 0.947 for the DBP and 0.315 for the SBP. Dietary fibre is being promoted as an adjunct therapy in the management of high blood pressure due to the benefits it provides [15]. A recent meta-analysis confirmed the role of dietary fibre in reducing the risk and managing cardiometabolic conditions of which hypertension is a part [15]. From this meta-analysis, there was strong evidence to suggest that increasing dietary fibre intake reduces systolic blood pressure by 4.3 mmHg and diastolic blood pressure by 3.1 mmHg. Intake of dietary fibre, especially from grains, lowers both systolic and diastolic blood pressure among mid-life women [16]. The high fibre contribution from tom brown can be attributed to the ingredients used in its preparation. Tom brown is a cereal-legume mixture mostly prepared with whole-grain cereals such as maise, wheat, millet and sorghum. Despite the beneficial role of dietary fibre in hypertension prevention and management, the exact mechanism is not clear. There have been several postulated mechanisms, which include reducing LDL cholesterol and triglyceride, improving the elasticity of blood vessel walls, improving endothelial function and less directly improving insulin sensitivity [15,16]. In sub-Saharan Africa (SSA), maise, sorghum, and wheat serve as major staples for most of the population. The role of maise in the diet of inhabitants of SSA can be compared to that of rice among Asians. [10,17]. In the past few years, strategies adopted in the management of hypertension include incorporating foods rich in fibre. This has influenced dietitians' recommending local staples rich in fibre. Although oat is not a locally produced staple, it appeared among the commonly consumed cereals by participants in this study. This may be due to the perceived health benefits associated with its fibre content. Oats are particularly high in β glucan, a type of dietary fibre with suggested lowering effects on plasma glucose, low-density lipoprotein cholesterol, and systolic and diastolic blood pressure [18,19].

The proportion of participants with insufficient daily fibre intake (≤ 25 g) was over 80%. This result is consistent with studies conducted in other countries, with the majority of study participants having consumption patterns less than the Adequate Intake (AI). A study among Ghanaian migrants in the UK and those resident in Ghana reported a mean dietary fibre intake of 8.3 ± 3.1 vs. 6.7 ± 2.2 g/1,000 kcal, which is below the recommendation of 14 g/1000 kcal/day by the American Diabetes Association [20]. In Ireland, among a population of older adults, median dietary fibre intake was found to be less (18.3 g) than the AI of 25 g [21]. Similarly, in a sample of adults in Spain (age 18 years - 64 years), the mean fibre intake was lower than the recommended (12.59 ± 5.66 g) [22], which is not different from what is reported in Australia (median intake of 20.7

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g) [23] and UK (17.3 ± 6.6 g) [24]. Contrary to these findings, Lie et al. (2018) [10] reported a high intake of dietary fibre (24.9 ± 9.7 g/day) among Ghanaians, although the high intake may be linked to the geographic area (rural community) where most of the locally consumed staples are rich in fibre. A systematic review of obesity in adults and the nutrition transition in Sub–Saharan Africa by Steyn and Mchiza [25] in Cape Town showed that dietary fibre intake had decreased from 20.7 g to 16.7 g as a result of changing dietary patterns. In suburban Chicago, urban Seychelles, and Jamaica, dietary fibre intake was reported to be below 20 g/ day [10]. These findings highlight the need to promote strategies and policies to increase dietary fibre intake. In this current analysis, the high-fibre intake group was also found to have higher energy and macronutrient (carbohydrate, protein, fat) intake compared with those taking in less fibre. This is similar to a study conducted among people living with type 1 diabetes [26]. As a result, the high-fibre group had a higher mean BMI than the lower-fibre intake group. Being younger was significantly associated with higher daily fibre intake, contrary to findings by Seljaket al. [27].

A main limitation of this study is the recall bias associated with the reporting of dietary intakes. The use of the quantitative food frequency questionnaire as the main dietary assessment tool may not accurately reflect the individual's usual intake. Another limitation is the unavailability of the fibre content of some Ghanaian foods, which had to be substituted with similar foods from others. These, however, are established limitations of all current dietary measurement tools that should be acknowledged. Furthermore, the population used consisted of previously diagnosed hypertensive individuals on anti-hypertensive medication, resulting in well-controlled blood pressure, which cannot be solely attributed to their dietary intake. Both exposure and outcome were measured at the same time. Hence, the results are not causal. Also, failure to administer the FFQ over the same period as the clinical laboratory results poses as a limitation in our study

Conclusion

The dietary fibre intake of the majority of the participants in the study was found to be inadequate. Dietary fibre intake was not associated with either diastolic or systolic blood pressure. Larger studies with this population, including but not limited to randomised clinical trials, should be conducted.

DECLARATIONS

Ethical consideration

The study was approved by the College of Health Sciences Ethics and Protocol Review Committee, University of Ghana (Ref No. EPRC/APRIL/2019).

Consent to publish

All authors agreed on the content of the final paper.

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None

Competing Interest

The authors declare no conflict of interest for this paper

Author contributions

MA conceptualised the topic. All authors contributed to analysing the results and writing the manuscript. All the authors approved the final version of the manuscript for publication.

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Availability of data

Data is available upon request to the corresponding author.

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Presence of SARS-CoV-2 in wastewater from handwash stations in selected facilities in Ghana

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Abstract

Background: Following the COVID-19 pandemic, the occurrence of the causative agent, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), in water, has been reported as handwashing was recommended as a non-pharmaceutical tool to limit the spread of the virus. Wastewater from these handwash stations is discarded without proper guidelines and could end up in the environment, serving as a dissemination route for SARS-CoV-2.

Objective: This study investigated the potential role of wastewater from handwash stations in the transmission of SARS-CoV-2 in Ghana.

Methods: A total of 390 water (195 reservoir water and 195 wastewater) samples from handwash stations were collected and analysed from selected schools, commercial banks, and hospitals in Accra, Ghana, between the 13th of June, 2022, and the 25th of August, 2022. Samples were first concentrated using the phase separation method before RNA extraction, and viral nucleic acid was amplified for SARS-CoV-2 detection using RT-PCR (N gene and ORF3a regions). Isolation of SARS-CoV-2 was performed for all 17 samples using VERO E6 cells.

Results: From the RT-PCR analysis, a total of 17 samples (4.4%) were positive for SARS-CoV-2 RNA. All 17 positive samples were wastewater samples. Propagation on Vero E6 cells yielded no cytopathic effect (CPE). Samples from schools had the highest positivity rate (15 out of 17), followed by the hospitals (2 out of 17) and the commercial banks (0 out of 17).

Conclusion: SARS-CoV-2 RNA detected in wastewater has a low likelihood of causing secondary infections in humans; however, the monitoring of SARS-CoV-2 in water matrixes could provide information on viral dynamics in the community.

Keywords: COVID-19, handwash stations, wastewater, reservoir water, GIDC

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INTRODUCTION

SARS-CoV-2 was detected in Ghana on the 12th of March 2020 [1], following its emergence in Wuhan, China, in 2019 [2]. SARS-CoV-2 is highly transmissible, and its transmission mainly occurs through exposure to respiratory droplets of disease carriers generated from sneezing and coughing [3]. Globally, and in Ghana in particular, there was introduction of some non-pharmaceutical measures as part of efforts to curb the spread of the infection. These included wearing face masks, using hand sanitisers, social distancing, a lockdown to restrict movement within some heavily affected parts of the country, a ban on international travel, and handwashing [4]. Over time, some of these restrictions have been eased while others, such as wearing face masks, the use of hand sanitiser and handwashing, are still being practised. Traces of SARS-

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CoV-2 RNA have been detected in different water matrixes [5-7], hence the potential role of water matrixes in the transmission of SARS-CoV-2 in the population. Since the beginning of the COVID-19 pandemic, non-pharmaceutical measures such as handwashing have been recommended to mitigate the spread of the virus. Handwashing is a simple but life-saving practice which sheds the virus down the drain. However, there are no proper guidelines for discarding and treating wastewater generated from these handwash stations, especially in low-income settings where wastewater treatment is inadequate or non-existent. The presence of trace SARS-CoV-2 RNA in water sources and their potential role in disease transmission, coupled with challenges with wastewater treatment in resource-limited settings, emphasises the need to investigate the role of SARS-CoV-2 virus transmission via wastewater. The study determined the presence of SARS-CoV-2 RNA in waste and reservoir water within Accra in the Greater Accra Region of Ghana.

MATERIALS AND METHODS

Study design and sites

This was a cross-sectional study carried out at selected schools, banks, and hospitals in Accra, Ghana, from the 13th of June 2022 to the 25th of August 2022 (Figure 1) based on the high enforcement of hand hygiene as the high human traffic at these sites is heavy. Again, the highest cases of SARS-CoV-2 were recorded in the Greater Accra Region.

Sample collection

Sterile containers were used to collect one litre each of reservoir water (n = 195) used for handwashing and its corresponding raw wastewater (n = 195) from handwash stations. Samples were collected in the mornings and afternoons using the grab sampling technique on each sampling day [8]. Samples were transported at 4℃ immediately to the laboratory and subsequently concentrated using the PEG-dextran phase separation method with slight modifications as described previously by Sharma et al. For each 1-litre sample collected, 500 ml was centrifuged at 1000 g for 20 minutes at 4℃. The supernatants were carefully poured into a 1-liter beaker, and 287 ml of 29% polyethylene glycol (PEG), 39.5 ml of 22% Dextran, and 35 ml of 5 N of NaCl were added. The mixture was stirred vigorously for 1 hour and poured carefully into a separation funnel, mounted on a burette stand in a vertical upright position, and left to stand overnight at 4℃. The funnel was checked for phase separation, and the entire lower phase and interphase were harvested in a drop-wise pattern into a sterile 50 ml centrifuge tube [9].

RNA Extraction and Detection of SARS-CoV-2 using RT-PCR

RNA extraction on the concentrated samples was done using QIAmp RNA extraction kit from Qiagen (QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany) and Real-time Polymerase Chain Reaction (RT-PCR) of SARS-CoV-2 was performed using Veri-Q nCoV-OM (MiCo BioMed Co., Ltd, Gyeonggi, Republic of Korea 13499) detection kit according to manufacturer protocols.

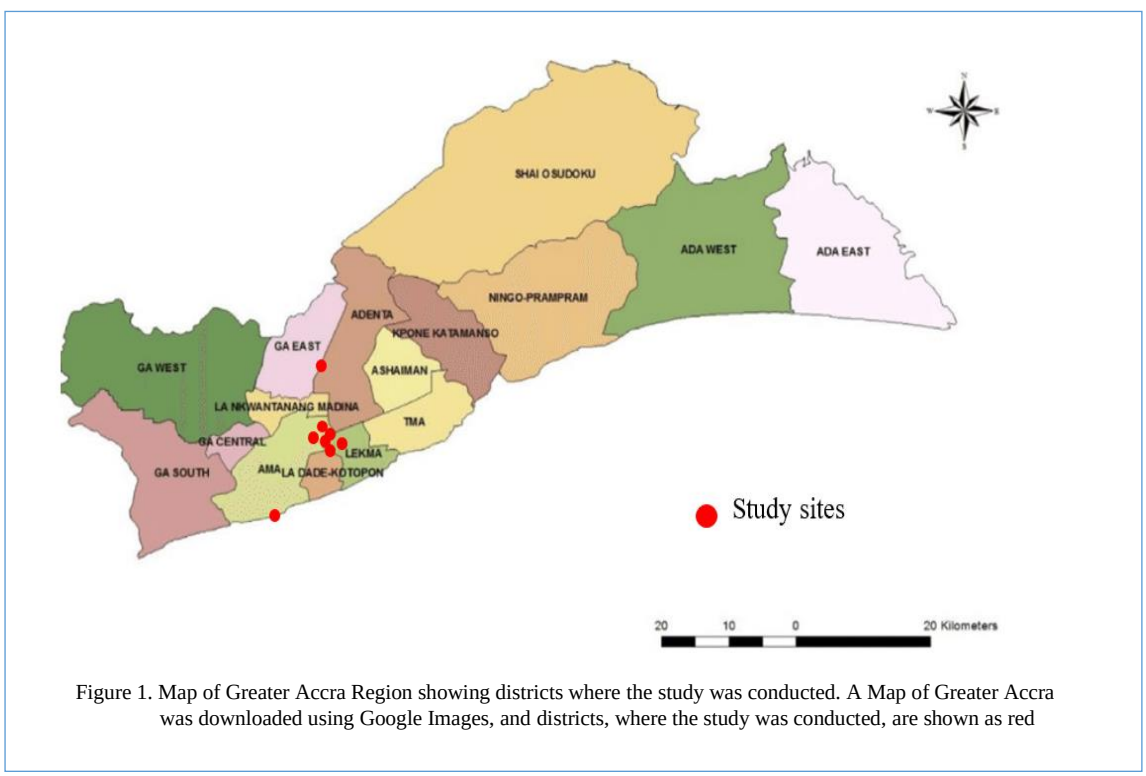

Figure 1. Map of Greater Accra Region showing districts where the study was conducted. A Map of Greater Accra was downloaded using Google Images, and districts, where the study was conducted, are shown as red

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Detection and Cultivation of SARS-CoV-2

Virus isolation was attempted for all positive samples using Vero E6 cells. Cells were cultured in complete Dulbecco Modified Eagle's Medium (cDMEM) as previously described by Rimoldi et al. (2020). The media was changed and replaced with fresh 5 mL DMEM (with no FBS/antibiotic Na-pyruvate and L-Glutamine) before infection attempts. VERO E6 cells were infected using 2 mL of eluent from processed contaminated water samples. Inoculated cells were incubated at 37°C, 5% CO2 atmospheric pressure for 72 - 96 hours. The cells were examined for cytopathic effect (CPE) every 24 hours.

RESULTS

Sampling was carried out in schools, commercial banks, and a COVID-19 treatment centre, Ghana Infectious Disease Centre (GIDC), in the mornings and afternoons of sampling days. A total of 195 samples each were obtained from reservoir and wastewater at hand washing stations, with 196 and 194 samples collected in the mornings and afternoons, respectively. A total of 64 samples were collected from banks, while 68 and 258 samples were from GIDC and schools, respectively. Table 1 shows the distribution of samples collected and the SARS-CoV-2 detection rates. A total of 15 out of 258 (5.8%) samples processed from schools tested positive for SARS-CoV-2 RNA, and two samples from the GIDC tested positive (2.9%) for SARS-CoV-2 RNA. None of the samples collected from the banks tested positive for SARS-CoV-2 (Table 1). It was observed that more samples collected in the morning were positive for SARS-CoV-2 RNA than those collected in the afternoon (p = 0.011). Of the 196 samples collected in the morning, 7.1% (n = 14) tested positive for SARS-CoV-2 RNA, and 1.5% (n = 3/194) tested positive for SARS-CoV-2 RNA for samples collected in the afternoon (Table 1). Again, a significant association was observed between sample types and SARS-CoV-2 positivity, with contamination occurring only in wastewater samples (8.7%, p = 0.001). However, we were not able to recover the virus from all RNA-positive samples. There was a significant difference in the SARS-CoV-2 positivity rate between samples collected from the schools in the morning and afternoon (Figure 2). Samples collected from the schools in the morning (12 out of 130, representing 9.2%) had a higher SARS-CoV-2 detection rate compared to samples collected from the schools in the afternoon (3 out of 128, representing 2.3%) (Table 1). There was no significant association between SARS-CoV-2 detection at GIDC and sampling time (Figure 2). Out of 34 samples collected in the morning at GIDC, 5.9% (n = 2) tested positive for SARS-CoV-2. None of the samples collected in the afternoon tested positive for SARS-CoV-2.

Table 1. Distribution of SARS-CoV-2 positivity rates by sample characteristics

Characteristics		SARS-CoV-2		
Sample site	Samples Processed	Number of positive samples	%	p-value
Schools	258	15	5.8%	0.103
*GIDC	68	2	2.9%	
Banks	64	0	0%	
Sample time				
Morning	196	14	7.1%	0.011
Afternoon	194	3	1.5%	
Sample type				
Reservoir water	195	0	0%	0.001
Wastewater	195	17	8.7%	
Total	390	17	4.4%	

*GIDC: COVID-19 treatment centre

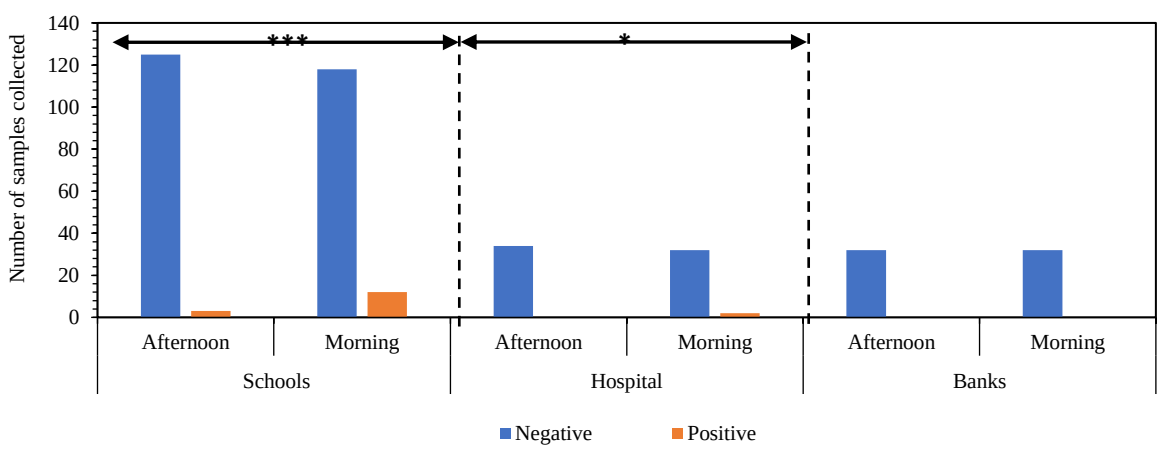

Figure 2. Detection of SARS-CoV-2 RNA based on sampling time at various study sites

*** denotes p-value < 0.001 and * denotes p-value > 0.1

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No sample from the bank tested positive for SAR-CoV-2 in the morning or in the afternoon (Table 1).

DISCUSSION

In this study, we sought to detect the presence of SARS-CoV-2 in water from handwash stations in selected facilities in the Greater Accra Region of Ghana. Since the beginning of the pandemic, SARS-CoV-2 has been detected in various water sources, particularly in wastewater. The COVID-19 pandemic in Ghana has been largely driven by cases from the Greater Accra region. It was imperative to determine this for Ghana. We collected samples from selected schools, a COVID-19 treatment centre and banks. Throughout the study, we collected a total of 390 samples, 190 from wastewater and their corresponding reservoir water. From this, the SARS-CoV-2 detection rate was 4.4 % (n = 17/390). We detected SARS-CoV-2 RNA in wastewater but not in reservoir water from handwash stations in Ghana. However, a viable virus was not recovered from cell cultures. The detection of SARS-CoV-2 RNA in wastewater is in line with growing evidence across the world [5-6,10-11], where SARS-CoV-2 has been detected in water matrixes. Additionally, the null detection of SARS-CoV-2 RNA in reservoir water reported in this study is similar to findings reported by Rimoldi et al. and Rosiles-González et al. [11-12]. This has several implications for public health and surveillance. The sampling site categories indicated that wastewater samples from schools had the highest SARS-CoV-2 RNA detection rate (5.8%), followed by the hospitals (2.9%), with samples from the banks recording no SARS-CoV-2 RNA [Table 1].

The COVID-19 vaccination program in Ghana currently covers people aged 15 and older, and most children in basic schools fall below this age bracket [13]. The lack of COVID-19 vaccination in most basic school children could explain why SARS-CoV-2 RNA was detected in high numbers in samples from schools. In accordance with these findings, an increase in COVID-19 cases involving children in Accra (18% to 20%) was reported in June 2022 [14]. This increase was related to outbreaks in schools in Accra, Ghana [15]. The significant association ($p < 0.001$) between samples collected in the morning and afternoon at the schools could be due to students' handwashing habits at specific times of the day rather than the temperature of the environment. Although studies have found that coronaviruses are affected by temperature [16-17], it was observed during the period of this study that there were high interactions and high compliance to hand hygiene among students in the morning, during morning break periods, compared with the afternoon in the schools even though compliance levels were not measured.

Hospitals are at the forefront of the treatment of diseases, including COVID-19. It is therefore not surprising that SARS-CoV-2 RNA was detected in samples from the hospital, albeit at a low level (2.9%) (Table 1) despite rigorous disinfection exercises. Interestingly, SARS-CoV-2 was detected in wastewater in the morning at the hospital but not in the afternoon. Considering that this hospital is a COVID-19 treatment centre, such low-level detection was interesting. It underscores the importance of the disinfection regime at the facility.

The null detection of SARS-CoV-2 RNA in samples processed from the commercial banks could be attributed to the combined use of alcohol hand rubs and handwash stations used in hand hygiene in all banks captured in this study. The synergetic action of the alcohol hand rubs and handwashing at the handwash stations could be accounting for the elimination of viral RNA in wastewater generated from these handwash stations. COVID-19 cases in the Ghanaian populace have generally remained low as compared to other countries, and with 28.7% of the total population estimated to be fully vaccinated [14], the low numbers of SARS-CoV-2 RNA detected are in conformance with the general case burden in the country. The inability to isolate viable, infective SARS-CoV-2 virus from culture even though SARS-CoV-2 RNA was amplified on RT-PCR suggests that surfactants used in handwashing are effective in disrupting virion structure and inactivating the virus; thus, SARS-CoV-2 RNA detected in wastewater has a low likelihood to retain its infectivity before contact cause infections in humans.

Conclusion

This study found that while SARS-CoV-2 RNA may be present in wastewater, it may not pose a significant risk for transmission through this route. Nevertheless, continued surveillance of wastewater samples is essential in tracking the prevalence of the virus and identifying potential outbreaks. Vaccination of children under 15 years in Ghana should be considered and implemented since the data illustrates that there was higher positivity at the basic schools (children between 4 - 16 years) compared to the other study sites.

DECLARATION

Ethical consideration

The study was approved by the Scientific and Technical Committee of the Noguchi Memorial Institute for Medical Research: STC Paper 6(4) 2021-22 (the 25th of May, 2022)

Consent to publish

All authors agreed to the content of the final paper.

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Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

Conceptualisation: IAA, MAAP; Funding acquisition: IAA, MAAP; Methodology: IAA, MAAP, KWCS, MOA, ID, NAAN; Resources: IAA; Investigation: MOA, ID, EOD; Data curation: MOA, ID; Data analysis: MOA, MAAP, IAA; Manuscript development: MOA, IAA, MAAP; Manuscript review and editing: MOA, IAA, MAAP, KWCS, NAAN, JN, EOD, JOC; Supervision: IAA, MAAP

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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Dermatological manifestations among patients with chronic kidney disease attending the renal clinic and dialysis unit of a tertiary hospital in Ghana

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Abstract

Background: Cutaneous manifestations occurring in patients with chronic kidney disease (CKD) can indicate systemic problems such as metabolic abnormalities that have significant morbidity and mortality risks. Most studies on this subject have involved patients with end-stage kidney disease (ESKD) with skin manifestations.

Objective: The study aimed to determine the prevalence of dermatological manifestations amongst persons with CKD attending the Korle-Bu Teaching Hospital as compared with the prevalence in non-renal patients.

Methods: A cross-sectional study was used to determine the prevalence of skin diseases among chronic kidney disease (CKD) (renal) and non-renal patients from January 2016 to June 2016. Each patient was assessed using a full medical history, physical examination and a full dermatological examination of skin, nails, and hair. Data was entered into Epi info and analysed with SPSS Version 18. Descriptive statistics was used in the analysis.

Results: The prevalence of dermatological disorders was 95.2% in the renal patients compared to only 5.6% in the non-renal patients. The most common mucocutaneous disorder in renal patients was pallor (72.4%, n = 105), followed by xerosis (58.1%, n = 86), then pruritus (22.1%, n = 32). The most common nail disorder was half and half nails (66.3%, n = 55), followed by brown nails (10.8%, n = 32) and onycholysis (9.6%, n = 8). The most common hair abnormality was sparse scalp hair loss (44.4%, n = 16), sparse body hair loss (33.3%, n = 12), and diffuse scalp hair loss (13.9%, n = 5).

Conclusion: The prevalence of skin disorders was higher in CKD patients than in patients without renal disease. Dermatological manifestations are an important component of CKD symptomatology, and healthcare providers should aim to recognise, diagnose, and manage them to improve patient outcomes.

Keywords: Chronic kidney disease, renal dysfunction, dialysis, dermatological manifestations.

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INTRODUCTION

Chronic kidney disease (CKD) is a pathophysiologic condition that results in a decrease in quantitative and qualitative activities of the nephrons, subsequently leading to end-stage kidney disease (ESKD) [1]. CKD is defined as either kidney damage or glomerular filtration rate (GFR) less than 60 ml/min/1.73 m^2 for greater than three months, where kidney damage refers to pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies [2-4]. The effects of CKD are varied and complex. Notably, uraemia may manifest as a dysfunction of multiple organs, including the mucocutaneous tissues, hair, and nails [5]. In addition, conditions associated with renal replacement therapy are fraught with numerous and often relatively unique cutaneous disorders. Skin diseases may coincidently co-exist with other medical illnesses or be specific markers or manifestations of underlying systemic disease. Some of these systemic diseases that can be suspected through

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cutaneous manifestations include CKD, endocrine disorders such as thyrotoxicosis, lymphomas, nutritional deficiencies, and HIV/AIDS [6].

A wide variety of skin diseases occur in CKD patients. With an almost 100% prevalence in the dialysis population, skin disorders are frequently the subject of patient complaints [7-9]. In a study by Pico et al., all CKD patients had at least one or more dermatological manifestations, while another study noticed skin changes in 79% of CKD patients. Malkud et al. and Sultan et al. observed that 50% - 100% of CKD patients had at least one skin manifestation [9,10]. In addition, a study by Falodun et al. quoted the prevalence of skin diseases in CKD as 89.1% [11]. The significant risk factor for these skin diseases in the CKD population is underlying renal disease. Skin disorders have a considerable negative effect on a patient's quality of life [12-14]. Such skin problems can induce serious discomfort, anxiety, depression and sleeping disorders and have an overall negative effect on the mental and physical well-being of such patients [3,15,16]. Treatment of skin diseases is based on the type of skin disease, its pathophysiology and degree of skin involvement. Although the majority of skin disorders seen in CKD are relatively benign, a few rare ones have the potential to cause serious morbidity and mortality. Early recognition of these dermatological manifestations of CKD and prompt treatment can dramatically alter the clinical course, prolong life and even save the lives of such patients [12,17,18].

There is currently a paucity of information on dermatological manifestations of CKD in West Africa and Ghanaian patients, in particular with CKD [11,19]. Although patients visiting kidney disease clinics report dermatological disorders, the conditions have not been related to CKD. The aim of the study was to determine the prevalence of dermatological manifestations amongst persons with CKD attending the Korle-Bu Teaching Hospital as compared with the prevalence in non-renal patients.

MATERIALS AND METHODS

Study design and sites

The research was a hospital-based cross-sectional study that included renal patients at the renal clinic and dialysis units and non-renal patients at the orthopaedics outpatients of the Korle Bu Teaching Hospital in Accra from January 2016 to June 2016.

Sample size and sampling technique

Non-renal patients were included in the study to compare the prevalence of skin diseases in the two groups. A renal patient is defined as having CKD if there is evidence in medical records of a clinical diagnosis of CKD and an eGFR of less than 60 ml/min/1.73 m^2 over the last three months with or without sonographic evidence of pathologic kidney anatomy (i.e., shrunken echogenic kidneys or polycystic kidney disease). All chronic kidney disease patients on maintenance haemodialysis at the Korle-Bu Teaching Hospital and aged at least 18 years during the study period were eligible for inclusion in the study. Patients with dermatological disorders which were secondary to other systemic conditions such as diabetes or connective tissue diseases and not directly or indirectly due to CKD and patients with dermatological disorders prior to the diagnosis of chronic kidney disease were excluded. Two independent skin specialists assessed any existing dermatological disorders, and an agreement of both findings was used to exclude patients with skin disorders attributed to other chronic conditions aside from kidney disease. In circumstances where there was a disagreement between the two specialists, a senior/consultant specialist was consulted to make a final decision on whether to include or exclude the patient in context.

Using a reported prevalence of skin disorders in CKD of 89.1%, as stated in a study by Falodun et al. [11], the sample size of one hundred and forty-five (145) was obtained using the Cochrane formula [8]. Participants were sampled consecutively from the dialysis unit, renal outpatient clinic, and medical inpatient wards until the sample size was obtained. An average of 10 patients were recruited weekly from each site.

Research Instrument

A standardised questionnaire was used to collect data on sociodemographic (age, sex, occupation), clinical characteristics (duration of CKD diagnosis, eGFR, duration on haemodialysis) and physical skin examination findings of the study participants. Data on eGFR was obtained from the medical records of the patient, whilst data on the start of dialysis and duration of hemodialysis collaborated with a records book kept at the dialysis unit, which contained the date of starting dialysis and for how long an individual has been on haemodialysis. The same eGFR equation was used in both renal and non-renal cases. Questionnaires were administered by trained research assistants.

The non-renal patients for the study were patients attending the orthopaedic trauma outpatient clinic during the study period, aged 18 years and above for both sexes, with no underlying chronic systemic disease, including CKD (patients with eGFR > 89 ml/min/1.73 m^2). The selection of non-renal patients was done by consecutive sampling over the study period. These non-renal patients were taken through pre and post-test counselling on recruitment days before screening for Hepatitis B, C and HIV using rapid diagnostic test (RDT) kits, as these are routine tests for patients with CKD. The results of the participants' tests were discussed with them. Blood samples were also taken to determine eGFR. Participants were then examined for any dermatological disorder in a secure consulting room with an attendant chaperone. Skin biopsy, culture and sensitivity for bacterial infections, gram stain, potassium hydroxide mount and fungal culture were done to confirm the diagnosis in doubtful situations.

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Data Analysis

Data was entered into the Statistical Package for Social Sciences SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0 Chicago: SPSS Inc. for analysis using mainly descriptive statistics.

RESULTS

One hundred and forty-five patients from the renal clinic and dialysis units were included, and 148 non-renal patients were screened from the orthopaedic outpatient unit. Two patients were hepatitis B positive, and one was HIV positive. Therefore, the three were excluded from the study. The socio-demographics of the renal and non-renal patients were not significantly different. However, the majority (41.7%, n = 61) of the renal patients were unemployed, compared to the 20.7% (n = 30) of non-renal patients (p = 0. 000) (Table 1). The mean (± standard deviation) age among patients with CKD was 48.2 ± 15.5 years, and that among non-renal patients was 52.2 ± 16.9 years. Regarding the duration of CKD from the time of diagnosis, 38.2% (n = 55) had the condition between zero to six months, 28.5% (n = 41) were between 6 - 12 months, 10.4% (n = 15) were between 1 – 2 years, 9% (n = 13) between 2 - 3 years, 7.6% (n = 11) between 3 to 5 years, 4.2% (n = 6) between 5 to 10 years and 2.1% (n = 3) had had duration of CKD from the time of diagnosis being greater than ten years. A greater proportion of the renal patients (57.3%, n = 83) were on haemodialysis, and 72.3% (n = 105) had been on haemodialysis between zero and one year (Figure 1). Each patient had at least one complaint. The commonest complaint was bipedal oedema (50.7%, n = 74). Palpitations and easy fatigability followed this with 41.9% (n = 61) and 41.2% (n = 60) respectively (Figure 2). The majority of the patients had more than one associated risk

Table 1. Socio demographic characteristics of study participants

Characteristics	Renal patients n(%)	Non-renal patients n(%)
Age ranges (years)	18-83	18-82
Gender		
Male	83 (57.2)	59 (40.7)
Female	62 (42.8)	86 (59.3)
Nationality		
Ghanaian	136 (93.8)	143 (98.6)
Non-Ghanaian	9 (6.2)	2 (1.4)
Ethnicity		
Ga/Dangme	26 (18.4)	46 (31.7)
Ewe	35(24.8)	34 (23.4)
Akan	60 (42.6)	50 (34.5)
Hausa	18 (12.8)	12 (8.3)
Others	2 (14)	3 (2.1)
Educational Background		
None	5 (3.5)	5 (3.5)
Primary	7 (4.9)	22 (15.2)
Middle school/JSH	66 (46.2)	73 (50.3)
SHS	38 (26.6)	35 (24.1)
Tertiary	26 (18.2)	10 (6.9)
Post-graduate	1 (0.7)	
Religion		
Christian	128 (88.3)	105 (72.4)
Muslim	16 (11)	30 (20.7)
Others	1 (0.7)	10 (6.9)
Marital Status		
Married	95 (67.4)	80 (55.2)
Single	34 (24.1)	38 (26.2)
Divorced	3 (2.1)	13 (8.9)
Separated	8 (5.7)	10 (6.9)
Others	1(0.7)	4 (2.8)
Employment status		
Employee	22(15.8)	70 (48.3)
Private formal	9 (6.5)	20 (13.8)
Self employed	50 (30.6)	25 (17.2)
Unemployed	58 (41.7)	30 (20.7)

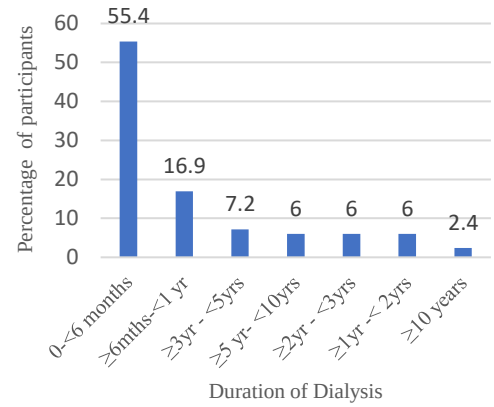

Figure 1. Duration of haemodialysis

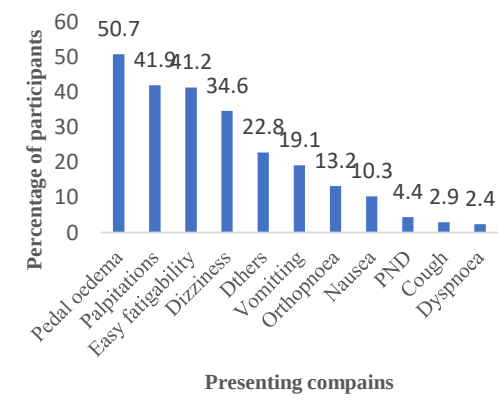

Figure 2. Complaints of renal patients. PND: paroxysmal nocturnal dyspnea.

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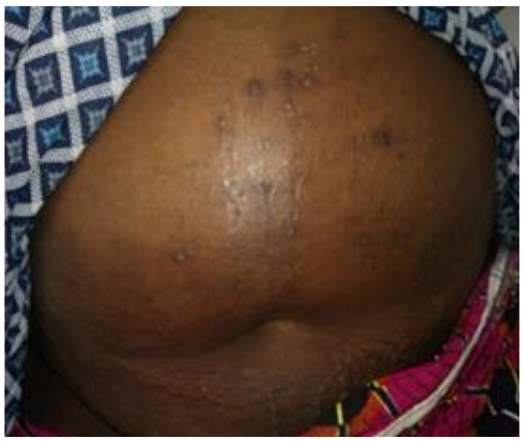
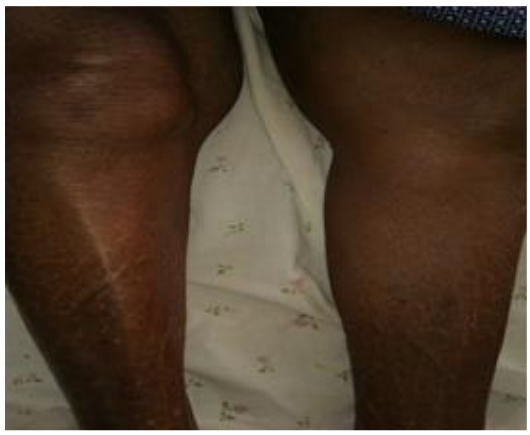
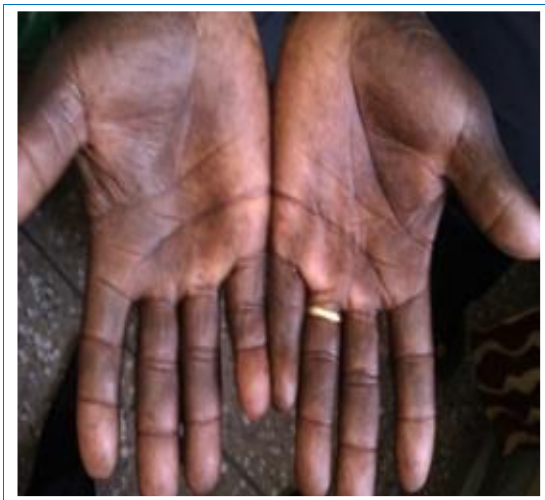


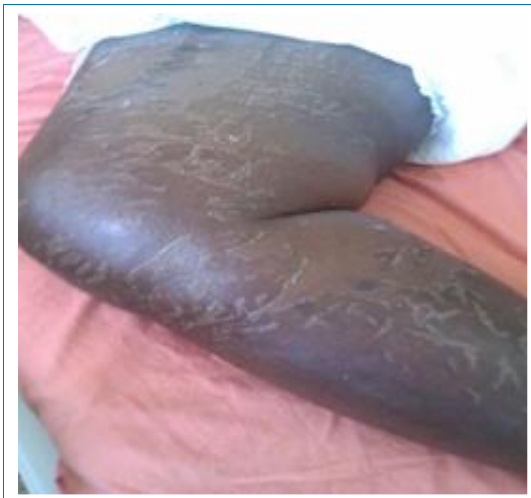
A. Pruritus with excoriation marks



B. Xerosis with ichthyosiform changes



C. Hyperpigmentation of the palms



D. Scaling of the limb

Figure 3. Mucocutaneous presentations of renal diseases

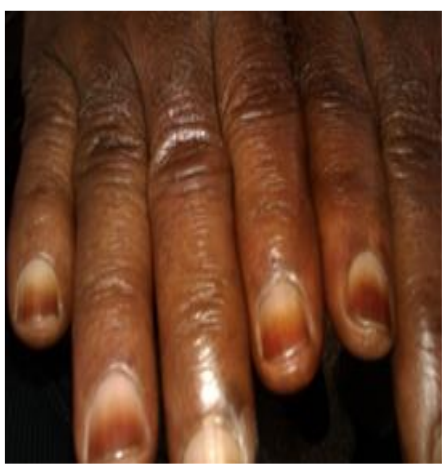


Figure 4. Lindsay's half and half nails

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factor; Hypertension was the most common risk factor, accounting for 83.2% (n = 121), followed by diabetes, accounting for 31.4% (n = 45). Mucocutaneous presentations were the predominant dermatological complications (Figure 3).

About 95.2% (n = 138) of the study participants exhibited at least one of the mucocutaneous presentations. Pallor, xerosis and pruritus were the most presenting manifestations (Table 2). A smaller proportion of 25.5% (n = 37), had at least one hair manifestation suggestive of CKD: 43.2% (n = 16) patients had sparse scalp hair, 32.4% (n = 12) had sparse body hair, 13.5% (n = 5) had diffuse scalp hair, 8.1% (n = 3) had dry lusterless hair and 2.7% (n = 1) had dandruff. The majority of the dermatological manifestations were observed among patients with ESKD (eGFR < 15 ml/min/1.73 m^2). Comparatively, only 5.6% (n = 8) of the non-renal study participants had dermatological manifestations, and these included three patients with Acne Vulgaris, two with Pityriasis Versicolor, and one each having scabies, Tinea cruris, and Paederus dermatitis.

Table 2. Distribution of dermatological conditions among case group

Mucocutaneous complications	Frequency (n = 145)	Percentage (%)
Pallor	105	72.4
Xerosis	86	58.1
Pruritus	32	22.1
Hyperpigmentation	29	20,0
Scaling	29	20.0
Ichthyosis	24	16.6
Excoriations	16	11.3
Seborrhoeic dermatitis	10	6.9
Planar warts	4	2.7
Coated tongue	3	2.1
Acne vulgaris	3	2.1
Jaundice	3	2.1
Uremic frost	2	1.4
Idiopathic guttate hypomelanosis	2	1.4
Pityriasis vesicolor	2	1.4
Petechiae	1	0.7
Ecchymosis	1	0.7
Bullous dermatosis	1	0.7
Xerostomia	1	0.7
Acneiform eruption	1	0.7
Bacterial folliculitis	1	0.7
Papular dermatosis	1	0.7
Plantar keratoderma	1	0.7
Pruritic papular eruption	1	0.7
Milaria crystalina	1	0.7
Tinea corporis	1	0.7

Table 3. Nail changes among case group

Nail changes	Frequency (n = 83)	Percentage (%)
Lindsay's half and half	55	66.3
Brown nail	9	10.8
Onycholysis	8	9.6
Sunungal hyperkeratosis	7	8.4
Leukonychia	4	4.8
Mees's lines	3	3.6
Clubbing	3	3.6
Koilonychia	2	2.4
Beau lines	1	1.2

DISCUSSION

The effects of CKD are varied, and patients with CKD present with various dermatological manifestations. In the current study, 95.2% of the 145 renal patients had at least one mucocutaneous lesion as compared to 5.6% (n = 8) of non-renal patients at the KBTH. This is comparable with a study by Khanna et al. in which 96% of CKD patients had at least one dermatological manifestation [20]. Some studies have reported that all CKD patients had at least one or more dermatological manifestations [7,21], while Bencini et al. [22] noticed skin changes in 79% of CKD patients. In this study, there were 57.2% (n = 83) males. This is similar to findings from other studies that reported 66%, 72% and 65.3% of their study participants to be male [5,23,24]. These studies show a male predominance that might reflect the fact that risk factors for CKD, such as hypertension and smoking, are commoner in males [24]. Differences in health-seeking behaviours of males and females might also play a role in the observed differences as women usually seek medical help earlier and could manage risk factors to mitigate the development of the disease or its complications.

Duration of CKD from the time of diagnosis ranged from one day to over ten years. The majority of patients, 38.2%, were diagnosed within the six months of the study period. A study reported that the duration of CKD ranged from 3 to 60 months [24]. Patients with a longer duration of CKD are more likely to have complications of CKD, including cutaneous manifestations [9]. Over half (57.3%, n = 83) of the present study participants were on maintenance haemodialysis. The majority (55.4%, n = 80) had been on haemodialysis for six months or less, and about 2.4% (n = 3) were on haemodialysis for over ten years. In the study by Sultan [10], the total duration of haemodialysis ranged from 0.08 - 20 years, meaning patients in question were on haemodialysis for a much longer period. Patients on haemodialysis are known to develop cutaneous manifestations ranging from infections to malignancies [9]. Masmoudi et al. [17] and Headley et al. [25] also found that prolonged hemodialysis was associated with cutaneous changes. All the present study participants had at least one complaint. The most common complaint was bipedal oedema [50.7%], which was lower than the 88.7% reported by Amoako et al. in Kumasi, Ghana [26]. The reason for the differences observed is unclear and may be due to the differences in patient management in the two centres and

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the geographical location of the two regional capitals. One is coastal, and the other is hinterland, but the population is expected to be similar in the same country. Further research is needed to clarify the difference. Pallor was the most common physical finding, accounting for 76.1% (n = 110). In a study by Sultan et al., 45% of CKD patients had pallor [10]. Also, in a study by Falodun et al., only 2.5% had pallor [11]. These figures suggest our patients were more anaemic, and we conjecture that nutritional deficiency of iron, hookworm infestation, and the disease process could probably explain this.

Xerosis was the most common mucocutaneous manifestation, accounting for 58.1% (n = 84), followed by pruritus at 22.1% (n = 32), hyperpigmentation and scaling accounting for 20% (n = 29), and ichthyosis at 16.6% (n = 24). In a study by Udayakumar et al. [28], 79% of CRF patients on chronic hemodialysis had xerosis. Also, in a study by Khanna et al. [20], 72% of cases had xerosis as the most common complaint, while Sultan et al. [10] reported xerosis in 54% of patients. The differences may be due to climatic conditions prevailing in these countries, hydration of the skin, duration of haemodialysis, and individual skin physiology.

Hypertension was the most common risk factor, accounting for 83.2% (n = 121), followed by diabetes, accounting for 31.4% (n = 45), and then chronic glomerulonephritis (7.3%, n = 11). This was comparable to a study by Sultan et al. [10] in which hypertension was the commonest risk factor, accounting for 60%, followed by diabetes (14%) and then obstructive uropathy (7%). However, in a study by Khanna et al. [20], chronic glomerulonephritis (45%) was the most common risk factor, followed by diabetic Nephropathy (22%), then hypertension (12%). Also, Khan R et al. reported hypertension as the second most common risk factor for CKD [21]. Hypertension is recognised as an important cause of chronic renal failure in outpatients as well as in inpatients in Africa [22,23]. In a 6-year study of 3632 patients with ESRD, based on South African dialysis and transplant registry statistics, hypertension was reported to be the cause of ESRD in 4.3% of White people, 34.6% of Black people, 20.9% of mixed ethnic groups and 13.8% of Indians [29]. The differences in variability of risk factors could probably be explained by the geographic locations in which the studies were conducted. Also, ethnicity seems to play a major role in hypertension.

Our study had a few limitations, such as the majority of patients were on haemodialysis for about a year, which was not a long enough period to observe all dermatological manifestations. Secondly, previous treatment for skin conditions was not taken into account as these could have been dermatological manifestations of CKD. However, our study compares with studies done elsewhere, confirms previous results, and establishes new findings. The biochemical markers of the stages of the diseases and the progression of the skin manifestation are the focus of our subsequent study.

Conclusion

This study has established that cutaneous lesions are a common presentation amongst patients with CKD, as 95.2% (n = 138) of the renal patients had at least one dermatological manifestation. The majority of dermatological manifestations were prevalent amongst stage 5 (eGFR < 15 ml/min/1.73 m^2) kidney disease patients. Patients with ESRD may present with an array of skin abnormalities that need to be diagnosed and managed appropriately.

DECLARATIONS

Ethical consideration

The study protocol was approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana. Protocol Identification CHS-Et/M:8-p4.12014-2015. Written informed consent was obtained from all study participants.

Consent to publish

All authors agreed on the content of the final paper.

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None

Competing Interest

The authors declare no conflict of interest

Author contributionS

All authors contributed equally to the study conceptualisation, design, data collection, analysis, drafting and finalisation of the manuscript.

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Availability of data

Data is available upon request to the corresponding author

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Substance use among high school-going adolescents, Northern Region, Ghana

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Abstract

Background: Substance use among adolescents in Ghana remains underreported despite its consequences. There is limited information on the prevalence of alcohol, cigarette, and other drug use among adolescents in the northern region of Ghana.

Objective: This study assessed adolescent substance use in nine senior high schools in the Northern Region of Ghana to inform evidence-based decision-making.

Methods: We conducted a school-based cross-sectional study to assess the prevalence of substance use and the factors that influence its occurrence among randomly sampled adolescents in the Northern region of Ghana. A semi-structured questionnaire was used to collect data on their background characteristics and use of substances. Binary logistic regression was used to determine the association between substance use and adolescents' characteristics at the 5% significance level.

Results: Of the 403 adolescents studied from nine senior high schools, the average age was 17.7 years (SD = 1.01), with 52.9% (n = 213) being male. The lifetime use of any substance was 62.3% [95% CI: 57.35 - 67.03]. Smoking status, alcohol intake, and illicit drug use were 6.2% (n = 25/403), 5.0% (n = 20/403), and 62.3% (n = 251/403), respectively. Substance use by peers (aOR = 2.07, 95% CI: 1.16 - 3.68), sexual activity (aOR = 1.81, 95% CI: 1.08 - 3.03), sexual relationships (aOR = 1.66, 95% CI: 1.03-2.69), social media use (aOR = 2.13, 95% CI: 1.36 - 3.36), and smartphone use (aOR = 2.89, 95% CI: 1.65 - 5.07) were significantly associated with substance use.

Conclusion: This study revealed an alarming rate of substance use among adolescents in the Northern Region. Peer use of drugs, sexual activity, sexual relationships, and use of smartphones and social media were factors identified to influence substance use. The research team organised educational and sensitisation programmes for the selected high schools. The findings could influence the development or enhancement of school policies related to substance use prevention, detection, and disciplinary actions by the Ghana Education Service (GES).

Keywords: Illicit drugs, substance use, Northern Region, adolescents, hgh School

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INTRODUCTION

Substance abuse refers to the harmful or hazardous use of psychoactive substances, including alcohol and illicit drugs [1]. Substance use among adolescents is a growing major issue of public health concern in both developed and developing countries worldwide [2]. Adolescents commonly use alcohol, cigarettes, marijuana, khat, and recreational drugs (cocaine, heroin, and tramadol). According to the United Nations Office on Drugs and Crime (UNODC) World Drug Report 2022, around 284 million people aged 15 – 64 years used drugs worldwide in 2020, a 26 percent increase over the previous decade [3]. Substance use is a key cause of disability-adjusted life years (DALY) among adolescents [4]. The DALY due to substance use in Africa has been estimated to be more than twice that of high-income countries [4,5]. A

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recently published systematic review found that the overall prevalence of 'any substance use' among adolescents in sub-Saharan Africa was 41.6%, with alcohol and tobacco being the highest prevailing substances (40.8% and 45.6%, respectively) across the continent compared to any other substance use [6,7]. Substance use among adolescents is influenced by various factors, including childhood trauma and adverse experiences such as physical, emotional and sexual abuse [8-11]. Other demographic and socioeconomic risk factors include male sex of younger age (10 – 19 years), lower education grades, adolescents from divorced parents, unemployed or fully employed mothers, and private school attendance [12-15]. In Ghana, substance use among adolescents remains underreported despite its reported consequences. According to the Narcotic Control Board (NACOB), about fifty thousand (50,000) people in Ghana, particularly the youth, abuse drugs, out of which 35,000 are students from junior/senior high schools and tertiary institutions. The three northern regions account for more than 50% of the reported usage [16]. Health professionals working at state-funded psychiatric hospitals estimate that about 10% of inpatients and between 20% to 30% of outpatient cases are linked to substance use and abuse in Ghana [17]. In recent studies conducted among Ghanaian adolescents, 24% - 48.8% of adolescents have ever used a substance, with alcohol and tramadol being the most commonly used substances [18,19].

Despite the major public health care crisis caused by the prevalence of alcohol, cigarette, and other drug use among adolescents, few studies have focused specifically on the problem in the northern region of Ghana. This study assessed the prevalence of substance use among adolescents in senior high schools in the Northern Region of Ghana.

MATERIALS AND METHODS

Study design and setting

The study was conducted among school-going adolescents in Ghana's Northern Region. The Northern Region is one of the 16 regions of Ghana with 16 administrative districts. The capital of this region is the Tamale metropolitan area. The region shares boundaries with the North East Region to the north, to the east by the Eastern Ghana Togo border, to the south by the Oti Region, and to the west by the Savanna Region. The region's current population is 2,310,939, with over 25% adolescents. There are more than 20 public senior high schools in the Northern Region, which are classified into categories A, B, and C according to the recently updated 2020 school register released by the Ghana Education Service. These senior high schools are designed to offer student lessons at three levels: SS1, SS2, and SS3. We conducted a school-based cross-sectional study to assess the prevalence of substance use and the factors that influence its occurrence among adolescents in the Northern Region of Ghana. A semi-structured questionnaire was used to collect data on the background characteristics and use of substances from nine selected senior high schools in the region.

Study population and Sample size estimation

The study population included all individuals aged 10 – 19 years attending senior high schools in the Northern Region of Ghana. Students in exchange programmes from other regions or countries were excluded from the study. A study conducted in Woreta High School revealed 47.9% substance use among adolescents [15]. Using P (proportion of adolescents who use substances) of 47.9%, a 95% Confidence level, and m (margin of error) of 5%, we estimated a sample size of 400. A stratified sampling approach was used to select senior high schools for the study. The schools were kept in their naturally occurring academic strata, such as Category A, Category B and Category C. Three schools were randomly sampled from a list of the schools in each category. A proportional allocation using the student population of the selected schools was used to determine the number of students selected from each school. At each school, the school list obtained from the school administration was used as the sampling frame for systematic random sampling of students.

Data collection

Data were collected using a semi-structured questionnaire adapted from the Community That Care (CTC) Youth Survey' for adolescent substance use and problem behaviours [20]. The questionnaires were prepared and administered in English. This tool was adapted and modified for the study. The questionnaires collected information on adolescents' sociodemographic characteristics, such as age, sex, grade, caregiver's age, sex, marital status, and educational level. Data were also collected on adolescents' exposure to substances, such as having either parent using substances, having friends who use substances, knowing where to get the substances, and being sent to buy the substances. Data were collected on whether participants had ever used a substance, the type of substance used, the frequency of use, and the source of the substance. Trained research assistants from the University for Development Studies collected the data. Data was collected from April to May 2023.

Statistical analysis

Data were entered into Microsoft Excel 2017, cleaned, and imported into Stata Statistical Software: Release 2017 (Version 15.1) College Station, TX:StataCorp LLC for statistical analysis. Categorical variables such as sex, grade, caregiver's sex, marital status, and educational level were summarised as frequencies and proportions. Continuous variables such as age were summarised as means and standard deviations. Logistic regression analysis was used to test the association between substance use and the various adolescent characteristics. Both crude and adjusted logistic regression analyses were performed at the 5% significance level. Robust standard errors were used to adjust for clustering in both the crude and adjusted

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analyses, with the category of school as the main clustering variable.

RESULTS

A total of 403 adolescents from nine senior high schools participated in the study. The average age of the adolescents was 17.7 ± 1 years, and more than half, 52.9% (n = 213), were males. More than two-thirds, 82.4% (n = 332), were Muslims, with almost half, 47.4% (n = 191), in their third year. The majority, 57.3% (n = 231) of the adolescents, were from nuclear families, with almost all, 97.0% (n = 391), admitting they have a good relationship with their parents (Table 1). Out of the 403 adolescents studied, 7.9% (n = 32) of the adolescents reported their parents either smoke, take alcohol or use illicit drugs, whereas 21.8% (n = 88) stated their friends smoke, take alcohol or use illicit drugs. Most (72.0%, n = 290/403) adolescents use social media, and 81.6% (n = 329/403) use smartphones. Less than one-third, 27.8% (n = 112/403) and 46.9% (n = 189/403) of the adolescents were sexually active and had a sexual relationship, respectively (Table 2). Out of the 403 adolescents studied, 62.3% (95% CI: 57.35 - 67.03) use substances. About 6.2% (n = 25/403) and 5.0% (n = 20/403)

Table 1. Socio-demographic characteristics of studied Adolescents, Northern Region, 2023

Variables	Frequency (n = 403)	Percentages (%)
Sex		
Female	190	47.2
Male	213	52.8
Age (mean, SD) years	17.7 (1.0)	
Religion		
Christianity	69	17.1
Islam	332	82.4
Traditionalist	2	0.5
Level		
First	93	23.1
Second	119	29.5
Third	191	47.4
Educational level (Caregiver)		
No formal education	214	53.1
Elementary	48	11.9
Secondary	91	22.6
Tertiary	50	12.4
Place of residence		
Rural	199	49.4
Urban	204	50.6
Type of family		
Extended	172	42.7
Nuclear	231	57.3
Relations with parents		
Bad	12	3.0
Good	391	97.0
Living with both parents		
No	117	29.0
Yes	286	71.0

Table 2. Individual-level characteristics of adolescents studied, Northern Region, 2023

Variables	Frequency (n = 403)	Percentages (%)
Substance use (caregiver)		
Non-user	371	92.1
User	32	7.9
Peer influence		
No	315	78.2
Yes	88	21.8
Drugs without prescription		
No	36	8.9
Yes	367	91.1
Social media use		
Non-user	113	28.0
User	290	72.0
Smartphone ownership		
Does not own	74	18.4
Own	329	81.6
Sexually active		
Not active	291	72.2
Active	112	27.8
Consequences of substance use		
Not aware	218	54.1
Aware	185	45.9
Sexual relationship		
No	214	53.1
Yes	189	46.9
Education on substances		
No education	217	53.9
Educated	186	46.1
Personal room		
No	226	56.1
Yes	177	43.9

Table 3: Substance Use among studied Adolescents, Northern Region, 2023

Variables	Frequency (n = 403)	Percentages (%)
Substance use		
Non-user	152	37.7
User	251	62.3
Smoking		
Non-smoker	378	93.8
Smoker	25	6.2
Alcohol intake		
No	383	95.0
Yes	20	5.0
Illicit drug use		
Non-user	152	37.7
User	251	62.3
Drugs used		
Raphenol	24	22.2
Diazepam	13	12.0
Bernalin	15	13.9
Tramadol	56	51.9
Subtotal	108	100.0

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Table 4. Logistic regression analysis for factors associated with substance use among studied adolescents, Northern Region, 2023

Variables	Substance Use		COR (95%CI)	P - value	AOR (95%CI)	P - value
	No n (%)	Yes n (%)				
Residence						
Rural	85 (42.71)	114 (57.29)	1.00		1.00	
Urban	67 (32.84)	137 (67.16)	1.52 (1.02 2.28)	0.041	1.45 (0.93 2.24)	0.098
Sex						
Female	76 (40.00)	114 (60.00)	1.00		1.00	
Male	76 (35.68)	137 (64.32)	1.20 (0.80 1.79)	0.372	1.49 (0.97 2.30)	0.071
Level						
First	38 (40.86)	55 (59.14)	1.00		1.00	
Second	36 (30.25)	83 (69.75)	1.59 (0.90 2.81)	0.109	1.72 (0.95 3.11)	0.073
Third	78 (40.84)	113 (59.16)	1.00 (0.60 1.65)	0.997	1.05 (0.62 1.77)	0.850
Educational level (Caregiver)						
No formal education	93 (43.46)	121 (56.54)	1.00		1.00	
Elementary/secondary	40 (28.78)	99 (71.22)	1.90 (1.21 3.00)	0.006	1.76 (1.09 2.83)	0.020
Tertiary	19 (38.00)	31 (62.00)	1.25 (0.67 2.35)	0.483	1.05 (0.54 2.05)	0.881
Substance use (Caregiver)						
No	142 (38.27)	229 (61.73)	1.00		1.00	
Yes	10 (31.25)	22 (68.75)	1.36 (0.63 2.96)	0.433	1.33 (0.57 3.16)	0.505
Peer influence						
No	132 (41.90)	183 (58.10)	1.00		1.00	
Yes	20 (22.73)	68 (77.27)	2.45 (1.42 4.23)	0.001	2.07 (1.16 3.68)	0.014**
Drugs without prescription						
No	16 (44.44)	20 (55.56)	1.00		1.00	
Yes	136 (37.06)	231 (62.94)	1.36 (0.68 2.71)	0.384	1.53 (0.76 3.10)	0.235
Social media						
No	56 (49.56)	57 (50.44)	1.00		1.00	
Yes	96 (33.10)	194 (66.90)	1.99 (1.28 3.09)	0.002	2.13 (1.36 3.36)	0.001**
Smartphone use						
No	42 (56.76)	32 (43.24)	1.00		1.00	
Yes	110 (33.43)	219 (66.57)	2.61 (1.56 4.36)	0.001	2.89 (1.65 5.07)	0.001**
Sexually active						
No	122 (41.92)	169 (58.08)	1.00		1.00	
Yes	30 (26.79)	82 (73.21)	1.97 (1.22 3.18)	0.005	1.81 (1.08 3.03)	0.024**
Consequences of substance use						
No	95 (43.58)	123 (56.42)	1.00		1.00	
Yes	57 (30.81)	128 (69.19)	1.73 (1.15 2.61)	0.009	1.47 (0.94 2.30)	0.087
Sexual relationship						
No	95 (44.39)	119 (55.61)	1.00		1.00	
Yes	57 (30.16)	132 (69.84)	1.85 (1.23 2.78)	0.003	1.66 (1.03 2.69)	0.037**
Received Education on substance use						
No	102 (47.00)	115 (53.00)	1.00		1.00	
Yes	50 (26.88)	136 (73.12)	2.41 (1.58 3.67)	0.001	2.16 (1.19 3.94)	0.012
Age			0.82 (0.67 1.00)	0.060	0.86 (0.69 1.07)	0.179

smoked and took alcohol, respectively. More than half (62.3%, n = 251/403) of the study participants use illicit drugs, with tramadol being the most used at 51.9% (n = 56/108) (Table 3). After controlling for the effect of sex, age, residence, caregiver educational level and type of family, substance use by peers (aOR = 2.1, 95% CI: 1.16 - 3.68), being sexually active (aOR = 1.8, 95% CI: 1.08 - 3.03), being in a sexual relationship (aOR = 1.7, 95% CI: 1.03 - 2.69), use of social media (aOR = 2.1, 95% CI: 1.36 - 3.36) and having a smartphone (aOR = 2.9, 95% CI: 1.65 - 5.07) were significantly associated with substance use among the adolescents (Table 4).

DISCUSSION

We assessed the prevalence of substance use and the factors that influence its occurrence among randomly sampled adolescents in the Northern Region of Ghana. The current

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study reports that more than half of high school adolescents in the Northern Region of Ghana were involved in substance use. Our findings are comparable to the prevalence of substance use of 65.7% [21] and 69.3% [22] reported in southeastern Nigeria. However, a much lower prevalence of 17.3% [23] and 32.9% [24] of substance use in similar studies have been reported in Nigeria and 30% by Ahmadi & Hasani among Iranian high school students. The variations between the prevalence in our study and others could be explained by the differences in the cultural background of the study populations, access to, and availability/variety of abusable substances. Age differences may also account for prevalence differences, where more than 80% of the students in Iranian high schools were between 15 – 18 years compared to the 13 – 18 years in Ghana. The high substance use among adolescents implies that these adolescents stand the risk of experiencing difficulties concentrating and have memory problems, as well as a decline in overall cognitive function, leading to lower academic achievements. This will negatively impact the breed of the next generation in Ghana.

Also, these children risk mental health disorders, including anxiety, depression, and other mood disorders, which could increase the investment by the government in the health sector. The study also revealed a prevalence of smoking and alcohol intake of less than one-tenth. The reported prevalence of smoking in our study was much lower than the 11.3% reported by the Global Youth Tobacco Survey [26] and 19.3% reported in a systematic review that estimated the global prevalence of tobacco use among adolescents [27]. However, the reported smoking prevalence in this study was higher than previously reported in Ghana, which was 3.2% [28]. The prevalence of smoking estimated in the current study is in tandem with the projected decrease in smoking prevalence in the general population to 3% in Ghana by 2025 [29]. The lifetime alcohol use reported by Oppong Asante and Kugbey was 11.1%, which is higher than that reported in the current study [30]. Even though smoking and alcohol use exist among students, our findings, in comparison with past reports, suggest a decline in smoking and alcohol use [31,32]. Although Ghana's legal drinking age has long been 18 years, it is evident that young drinkers can still purchase and have access to alcohol.

In our study, tramadol was the most used substance among high school pupils who abused substances. The high use of tramadol among these adolescents is a social canker, and if not controlled, will expose the next generation of adults to mental and social health consequences. The prevalence reported is, however, inconsistent with a lower prevalence of 6.3% tramadol use, which was reported in a similar study in Nigeria [33]. The disparity in the findings could be attributed to the methods used in the assessment. Our study assessed the lifetime use of these substances, whereas the Nigerian study assessed the current use of these substances. Our findings revealed that peer influence was a major factor for substance use, as the likelihood of adolescents who are being influenced by their peers using a substance was twice that of adolescents who are not being influenced by peer pressure. This finding suggests that young people are susceptible to peer pressure and often turn to substance use to gain acceptance. This corroborates the findings of studies that reported peer influence as a significant predictor of substance use [34,35]. Adolescents who were sexually active and in sexual relationships were almost twice as likely to be involved in substance use compared to their counterparts who were not sexually active or involved in a sexual relationship. Similar to our findings, a study on substance use and risky sexual behaviours among street-connected children and youth aged 8 – 19 years in Accra, Ghana, revealed a significant association between the use of marijuana and smoking cigarettes with being sexually active [10]. Another study reported that the possible reason for substance use among sexually active adolescents may include the facilitation of a sexual encounter (i.e., to lower sexual inhibitions and increase self-esteem and confidence) [37].

Our study further revealed that high school students' social media use and smartphone ownership are significantly linked to substance use. Students who used social media were 2-fold more likely to engage in substance use while owning a smartphone was likely to cause approximately a 3-fold increase in substance use among adolescents. Similar to our finding, a study to ascertain the relationship between social media use and substance use among middle and high-school-aged youth in the United States of America reported that the number of social media sites used was significantly associated with higher odds of ever using a substance and multiple substances. Additionally, there was a strong correlation between accessing social media regularly - once an hour or more and a person's likelihood of having used marijuana, e-cigarettes, any substance, or multiple substances [38].

These findings suggest that adolescents in high school are exposed to addictive drugs, such as alcohol and smoking, through social media and other Internet platforms. This evokes the need to develop effective interventions to deal with the issue and protect adolescents and young people from the negative consequences of these social media platforms. In ensuring a reduction in the high prevalence of substance use by adolescents in senior high schools in the region, the research team organised educational and sensitisation programs for 15 selected high schools in the region. The program was organised in collaboration with Ghana Educational Service and Parent Teacher Associations. Adolescents were educated on substance use and the consequences involved in its usage. Adolescents were also presented with various avenues through which they could seek help in times of psychological need instead of resorting to substances. The importance of school-based psychologists in the Ghana Educational System was made paramount with presentations from three psychologists. Our study had some limitations that should be considered when interpreting the findings. Underreporting of sensitive

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questions cannot be ruled out. Furthermore, because this was a cross-sectional study, causal relationships could not be established. Additionally, no further questions were asked in this study about alcohol, smoking, and sexual habits among students, as the aim was to determine the prevalence of these behaviours; however, further inquiries might have uncovered details regarding other risk factors.

Conclusion

Substance use among high school adolescents in the Northern Region of Ghana is high. Factors such as peer use of drugs, sexual activity, sexual relationships, and the use of smartphones and social media were associated with increased substance use among adolescents. The identified predictors could be targeted and modified through educational and sensitisation programmes to reduce the use of substances. This study should inform the development or enhancement of school policies related to substance use prevention, detection, and disciplinary actions by the Ghana Education Service (GES).

DECLARATIONS

Ethical consideration

The study's clearance was obtained from the Ghana Health Services Ethics Review Committee (GHS-ERC-021/02/23). Permission was obtained from the Northern Regional Education Circuit (NREC). Permission was sought from the leadership of the selected senior high schools before data collection. Written informed consent was obtained from the participants' caregivers. Signed assent was also obtained from the pupil before collecting data.

Consent to publish

All authors agreed on the content of the final paper.

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None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

All authors participated in conceptualising and designing the study. Data was curated by AGM and IY. Data was analysed by AGM, MO and IY. The initial manuscript was drafted by AGM and YAK, but all authors made significant intellectual contributions to the final manuscript. All authors read and approved the final manuscript.

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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HIV in Sunyani and peri-urban areas is associated with previous history of syphilis infection and multiple sexual partners: A cross-sectional study

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Abstract

Background: The Bono Region of Ghana has consistently recorded high HIV prevalence over the years, with an average prevalence of 3.4% in the recent HIV survey. Sunyani, the capital region of Bono, recorded an HIV prevalence of 3.8% higher than the HIV Sentinel Survey average.

Objective: This study assessed the factors associated with the high HIV prevalence in the Sunyani municipality of the Bono Region of Ghana.

Methods: Participants accessing HIV counselling and testing services were enrolled from three hospitals in the Sunyani Municipality using a cross-sectional design from March to August 2018 and were interviewed using a structured questionnaire. Descriptive and logistic regression analyses were used to identify factors associated with HIV prevalence at $p < 0.05$ with a 95% confidence interval.

Results: The mean age of respondents was 30.8 ± 8.5 years. The prevalence of HIV among participants was 10.3% (95% CI: 7.82 - 13.54). HIV prevalence per sub-districts ranged from 0 to 11.9%, with New Dormaa recording the highest at 11.9% (CI: 5.7 - 23.1). Almost 28% (n = 13/46) of the HIV-positive participants reside in districts outside the Sunyani Municipality. Participants with a previous history of syphilis were six times more likely to be HIV positive compared with those with vaginal or urethral discharges (aOR = 6.39, 95% CI: 4.14 - 9.78), and those with multiple sex partners have three times increased odds of contracting HIV compared with a single partner (aOR = 3.33, 95% CI: 1.16 - 9.55).

Conclusion: The prevalence of HIV in Sunyani municipality was significantly associated with multiple partners and a previous history of syphilis infection.

Keywords: HIV, AIDS, syphilis, Ghana

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INTRODUCTION

According to the World Health Organization, approximately 60% of people living with HIV (PLWH) in the world reside in Sub-Saharan Africa [1]. Though HIV morbidity and mortality have declined in the world by 49% and 61%, respectively, sub-Saharan Africa continues to be the most affected region in the world [1]. The median HIV prevalence for 2019 in Ghana was 2.0%, an increase from the 2015 prevalence of 1.8% and 2014 prevalence of 1.6% [2], a situation which indicates an increase in HIV prevalence in the country over the past five years. HIV prevalence at regional levels ranged from 0.8% in the North East region of Ghana to 3.4% in the Bono region [2]. Sunyani has a fluctuating HIV prevalence, which almost stabilised at 2.0% in 2012 and 2013. However, HIV prevalence in Sunyani steadily increased to 5% in 2014, which is double the Bono regional prevalence of 2.6% [3]. Before 2016, there had been a steady decrease to 3.8% in 2015 and 2019. However, the prevalence in Sunyani was double the then Brong Ahafo regional

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prevalence of 1.7% [3]. According to the Ghana National HIV Sentinel Site Survey report, 2016 and 2019, Sunyani was one of the thirteen (13) sites that did not change in prevalence over the previous years [3].

Despite all the interventions put in place by the Ghana National AIDS/STI Control Programme to reduce the prevalence of HIV in Ghana, HIV continues to be a Public Health threat affecting many Ghanaians, with females primarily infected [4]. Large proportions of new HIV cases are recorded every year, and these are not evenly distributed per geographic location. Though HIV prevalence decreased from 4.2% in 2016 to 3% in 2017, Sunyani tends to record HIV prevalence (3.8%) above the Bono Region regional prevalence of 3.4 % and the HIV Sentinel Survey average of 2.0% [2]. Several studies have revealed that unprotected sex, contracting another sexually transmitted infection (STI), sharing contaminated needles and syringes, accidental needle stick injuries, breast milk, the unfavourable economic position of women, rate of rape, inconsistent condom use and alcohol consumption are known factors associated with HIV transmission [5,6,7,8]. The National AIDS/STI Control Programme has an overall goal of reducing the impact and effect of HIV with interventions such as “know your HIV status”, free “Prevention of Mother-to-Child Transmission”, “condom promotion and distribution”, “blood safety”, “HIV Exposure Prevention in the Health care and other settings”. In the face of all these interventions by the National AIDS/STI Control Program, the prevalence of HIV in the Sunyani Municipality over the past years has been consistently above the Bono Regional prevalence. Barring any move by the regional and district Health Directorate to identify and determine the underlying cause of the increase since 2017, this could lead to a further increase in HIV prevalence in the Sunyani municipality. Identifying the factors that drive this unusually high prevalence of HIV will help in designing targeted interventions for implementation in the Sunyani municipality. This study identified factors contributing to the fluctuating trend in HIV prevalence over the past years in the Sunyani Municipality and its surrounding towns.

MATERIALS AND METHODS

Study design and sites

Sunyani is the capital of the Bono Region of Ghana. There are 31 health facilities and 34 community-based health planning services (CHPS) Zones in the Sunyani Municipality, consisting of 18 prevention of mother to child transmission (PMTCT) sites. Four out of the 31 municipal health facilities provide voluntary HIV testing and counselling services, including regional and municipal hospitals. The study was conducted in three health facilities, namely the Regional Hospital, the Municipal Hospital and the Seventh Day Adventist (SDA) Hospital, which were identified as having recorded the highest number of HIV-positive cases in the Sunyani Municipality. A facility-based cross-sectional study was conducted among participants accessing HIV testing and counselling services in the Sunyani Municipality of Ghana in 2018. Data on patients’ demographic characteristics, knowledge of HIV, transmission risk factors and HIV prevention were collected and analysed to determine factors associated with HIV prevalence in the area.

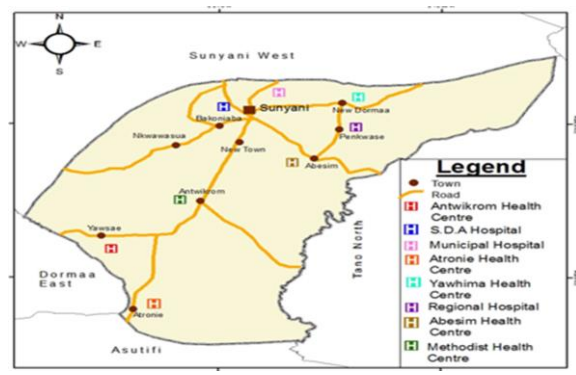

Figure 1. Map of Sunyani and surrounding areas (adopted from Williams Kumi, Research Gates.net)

Table 1. Sample selection from facilities

Hospital	Number tested for HIV	Proportional sample
Regional Hospital	3428	187
Municipal Hospital	2538	139
SDA Hospital	2178	119
Total	8144	445

Study participants

Participants who were 19 years and above accessing HIV testing and counselling services at the three facilities were included in the study. They were proportionally and randomly sampled from each of the facilities depending on the total number of participants who tested for HIV at each of the facilities.

Sampling method and sample size determination

Using the Cochrane formula of sample size estimation, a total sample size of 445 was determined for the study. The minimum sample size was determined using an estimated HIV prevalence of 4.2% [3], a desired level of precision of +/- 5% and a confidence interval of 95%. Three hospitals in the municipality were selected for the study. The hospitals with the highest number of HIV tests for 2017 were selected. The three health facilities offering HIV counselling and testing services in the municipality were included in determining the proportion of HIV tests done in 2017. Samples were then proportionately allocated to the hospitals based on their estimated annual data on the number of HIV tests conducted. All clients accessing HIV testing and counselling services in the three hospitals were

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selected and consented to the study until the required sample size was met.

Data collection instrument

An interviewer-assisted structured questionnaire was used to collect the data. Face-to-face interviews were conducted with participants accessing HIV counselling and testing services in the three selected hospitals. The data collection tool captured the participants' socio-demographic characteristics, including age, sex, educational level, occupational status, marital status, and religion. Also, data on HIV risk factors were captured to include the previous history of sexually transmitted infections (STI), type of STI previously contracted, the previous history of TB, the number of sexual partners, alcohol use, smoking status, and condom use.

Statistical Analysis

Data were entered into Microsoft Excel (state the version year) and imported into Stata Statistical Software: Release 15. *StataCorp LLC,2017* for cleaning and analysis. Descriptive statistics were done by running frequencies and proportions. Continuous variables such as age were summarised into mean. Age was then categorised into four years intervals according to the Ghana HIV Sentinel Site Report [3]. Pearson chi-square test was done to determine the significant difference between HIV prevalence and the categorical variable, and it was presented as a contingency table with p values. Fisher's exact test was done for variables with frequencies less than 5. A simple logistic analysis was conducted, and variables with a p-value < 0.05 were considered to be statistically significant. Significant variables and variables which the literature has determined are associated with HIV infection were modelled in a multiple logistics regression to determine factors that are statistically significantly associated with HIV infection. The results were presented in a two by two table to display frequencies, percentages, crude and adjusted odds ratios at 95% confidence interval and p values. Statistical significance was set at $p < 0.05$.

The team obtained ethical clearance and approval from the Ghana Health Service Ethics Review Committee (GHS-ERC: 082/12/18). Permissions were obtained from the three health facilities before the commencement of the study. Participants' confidentiality and privacy were safeguarded during questionnaire administration. Each respondent was assigned a code that could be traced to the registration number in the HIV counselling and testing register.

RESULTS

Table 2 shows the background characteristics of the respondents who participated in the study. The ages of participants ranged from 19 - 66 years, with a mean age of 30.8 years (SD ± 8.5 years). The mean age for males was 30.3 years (SD ± 7.9 years), while that of females was 34.2 (SD ± 10.8 years). Most participants (89.7%, n = 381/445) were Christians compared to Muslims (13, n = 64/445). Out of the 445 participants who participated in the study, 10.3% (n = 46) tested positive for HIV (Table 2). HIV Prevalence per sub municipal in Sunyani Municipality. Table 3 shows the proportion of HIV-positive participants per the number

Table 2. Background characteristics of study participants

Variable	Number (N)	Frequency (%)
Age in years (M±SD)	30.8 (±8.5)	
Age group (years)		
<=19	15	3.4
20-24	65	14.6
25-29	143	32.1
30-34	117	26.3
35-39	56	12.6
40-44	17	3.8
45+	32	7.2
Age for Female (M±SD)	34.2 (SD ±10.8)	
Age for male (M±SD)	30.3 (SD ±7.9)	
Sex		
Female	382	85.8
Male	63	14.2
Education		
No Education	30	6.7
Primary	24	5.4
JSS	112	25.2
MSLC	15	3.4
SEC/TEC	84	18.9
Tertiary	180	40.5
Status of Occupation		
Unemployed	17	3.8
Employed	156	35.1
Self Employed	230	51.7
Student	42	9.4
Marital Status		
Married	305	68.5
Single	100	22.5
Separated	16	3.6
Cohabiting	24	5.4
Religion		
Muslim	64	14.4
Christian	381	85.6
HIV Test Results		
Reactive	46	10.3
Non-Reactive	399	89.7

Table 3. Proportion of HIV reactive clients per sub district in Sunyani

Sub District	Total (N)	HIV positive n (%)	95% CI
Sunyani Municipal	445	46 (10.3)	7.8 - 13.5
Abesim	52	6(11.5)	5.2 - 23.6
Antwikrom	6	0	0
New Dormaa	59	7 (11.9)	5.7 - 23.1
Newtown/Bakoniaba	67	5 (7.4)	3.1 - 16.8
Penkwase	39	2 (5.1)	1.3 - 18.7
Sunyani Central	82	8 (9.7)	4.9 - 18.4
Sunyani West	84	5 (5.6)	2.3 - 12.9
Outside Sunyani	56	13(23.2)	13.9 - 36.2

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of residents in their sub-districts. Out of the 46 who tested positive for HIV, the majority of them (13) live outside Sunyani Municipal. Among the subgroup living outside Sunyani, the HIV prevalence recorded was 23.2% (95% CI: 13.9 - 36.2). Table 4 is a univariate and multivariate logistic regression analysis testing the association between factors associated with HIV prevalence. In this table, participants' age, education level, history of STI, type of STI previously

Table 4. Logistic Regression Analysis of Factors Influencing HIV Prevalence in Sunyani

				Unadjusted			Adjusted		
Variable	N	Reactive n (%)	Non-Reactive n (%)	OR	95% CI	P value	OR	95% CI	P value
Age group						<0.001*			0.012*
<=19 (Ref)	15	1 (6.7)	14 (93.3)	1.00			1.00		
20-24	65	3 (4.6)	62 (95.4)	0.68	0.06-7.01	0.744	0.08	0.002-2.59	0.154
25-29	143	10 (6.9)	133(93.1)	1.05	0.13-8.84	0.962	0.38	0.02-9.19	0.551
30-34	117	13 (11.1)	104 (88.9)	1.75	0.21-14.2	0.603	0.23	0.009-5.89	0.377
35-39	56	5 (8.9)	51 (91.1)	1.37	0.15-12.7	0.148	0.22	0.007-7.29	0.397
40-44	17	4 (23.5)	13 (76.5)	4.31	0.42-43.7	0.217	0.81	0.01-63.98	0.927
45 and above	32	10 (31.3)	22 (68.8)	6.36	0.73-10.29	0.093	0.65	0.01-33.34	0.829
Sex[c]						0.508			0.723
Female (Ref)	382	38 (9.9)	344 (90.1)	1.00			1.00		
Male	63	8 (12.7)	55 (87.3)	1.32	0.58-2.97	0.508	2.38	0.25-22.59	0.449
Educational level[c]						<0.001*			0.922
No Education (Ref)	30	7 (23.3)	23 (76.7)	1.00			1.00		
Primary	24	4 (16.7)	20 (83.3)	0.66	0.17-2.58	0.547	1.46	0.1-20.89	0.78
JSS	112	14 (12.5)	98 (87.5)	0.47	0.17-1.29	0.144	0.16	0.01-1.79	0.137
MSLC	15	4 (26.7)	11 (73.3)	1.19	0.29-4.96	0.806	2.46	0.06-103.02	0.636
SEC/TECH	84	12 (14.3)	72 (85.7)	0.55	0.19-1.56	0.258	3.22	0.296-34.99	0.337
Tertiary	180	5 (2.8)	175 (97.2)	0.09	0.03-0.32	<0.001*	0.27	0.016-4.52	0.359
STI						0.004*			<0.001*
Never had STI	155	25 (16.1)	130 (83.9)	1.00			1.00		
Ever had STI	290	21 (7.2)	269 (92.8)	2.46	1.33-4.56	0.004*	0.00	0.0001-0.048	<0.001*
STI type						<0.001*			0.123
Vaginal discharges (Ref)	117	17 (14.5)	100 (85.5)	1.00			1.00		
Syphilis	29	16 (55.2)	13 (44.8)	7.23	2.96-17.71	<0.001*	6.39	4.14-9.78	0.035*
Gonorrhea	31	13 (41.9)	18 (58.1)	4.24	1.76-10.24	0.001*	1.58	0.21-2.09	0.661
TB						0.036*			0.262
Never had TB(Ref)	20	5 (25)	15 (75)	1.00			1.00		
Ever had TB	425	41 (9.7)	384 (90.4)	3.12	1.07-9.03	0.036*	3.39	0.27-42.01	0.342
Sexual activeness						0.028*			0.666
Never had Sex (Ref)	33	7 (21.2)	26 (78.8)	1.00			1.00		
Active in the past month	346	22 (6.4)	324 (93.6)	0.25	0.09-0.65	0.004*	0.22	0.04-0.108	0.108
Not active past 1month	66	17 (25.8)	49 (74.2)	1.29	0.47-3.5	0.619	0.83	0.09-7.18	0.868
Number of partners						0.025			0.025*
One (Ref)	364	28 (7.7)	336 (92.3)	1			1.00		
Two or more	81	18 (22.2)	63 (77.8)	3.43	1.79-6.57	<0.001*	3.33	1.16-9.55	0.025*
HIV Testing						0.001*			0.03*
Never tested	377	31 98.2)	346 (91.7)	1.00			1.00		
Ever tested	68	15 (22.1)	53 (77.9)	0.32	0.16-0.63	0.001*	0.33	0.08-1.35	0.124
Referral						0.001*			0.581
Diagnostic HIV(Ref)	71	12 (16.9)	59 (83.1)	1.00			1.00		
Walk in VCT	128	20 (15.6)	103(84.4)	0.91	0.42-1.99	0.814	2.12	0.38-3.98	0.395
PMTCT	246	14 (5.7)	232 (94.3)	0.29	0.13-0.68	0.004*	3.60	0.67-19.65	0.139

Known Confounders, *: Statistically significant, Ref: Reference, VCT: Voluntary Counseling and Testing, PMTCT: Prevention of Mother to Child Transmission

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contracted, history of tuberculosis, sexual activeness, number of sexual partners, history of testing for HIV, and participants' referral for HIV testing were found to be associated with HIV at the univariate analysis ($p < 0.05$). In the multivariate analysis, participants' age, history of STI, number of sexual partners, and history of testing for HIV remained statistically significantly associated with HIV. Participants who had a past history of STI were about three times more likely to be infected with HIV as compared to those who had no previous history of STI (cOR 2.46 95% CI: 1.33 - 4.56).

Participants who had a past history of syphilis had seven times higher odds of being infected with HIV as compared to those with a previous history of vaginal/urethral discharges (cOR: 7.23, 95% CI: 2.96 - 17.71). These factors remained significantly associated in the multivariate analysis (aOR: 6.39, 95% CI: 4.14 - 9.78). Similarly, participants with a previous history of gonorrhoea were more likely to be infected with HIV (cOR: 4.24, 95% CI: 1.76 - 10.24). HIV infection were associated with multiple sexual partners. Participants with two or more partners were about three times more likely to be infected with HIV infection compared to those with only one partner (cOR: 3.43, 95% CI: 1.79 - 6.57). This association remained independently significant (aOR: 3.33, 95% CI: 1.16 - 9.55) in the multivariate analysis when age, sex, educational level, previous history of STI, type of STI, previous history of tuberculosis, sexual activeness, and HIV testing were adjusted.

DISCUSSION

This study demonstrated an HIV prevalence of 10.3% in the Sunyani municipality in 2018 and that previous history of syphilis infection and multiple sexual partners were significantly associated with HIV infection. HIV prevalence of 10.3% in the Sunyani Municipality in 2018 was high compared with the National HIV Sentinel Survey reported a prevalence of 4.2% [3]. The 2019 HIV Sentinel survey reported an HIV prevalence of 3.4% in the Bono region, which is the highest prevalence among all the 16 regions in the country [2]. There was a higher proportion of HIV-positive participants aged between 25 - 34 years and also among those above 45 years old in this study, and this is consistent with previous WHO findings, which indicate that HIV is common among younger adults [8]. The recent HIV report also put the highest proportion between the 40 - 44 age group, although the 25 - 34 age group is still high [2]. The findings from this study are a useful report in the fight against HIV in terms of targeting prevention messages in light of the UNAIDS 95 – 95 - 95 target goal of significantly reducing HIV incidence by 2030 [1]. There was evidence in this study that the majority of the participants who tested positive were sexually active in the previous month prior to the commencement of the study. Several reports indicated that sexually active men and women have a higher prevalence of HIV compared to sexually inactive people [9,10,11]. This study has demonstrated that a previous history of sexually transmitted infections (STI) was significantly associated with HIV transmission. This finding corroborates several reports which associated STI with HIV infection [12,13]. Vaginal microbes, independently and synergistically [12], enhance HIV RNA shedding into genital secretions [13] and increase HIV susceptibility by 4.5 fold [14]. For instance, in Brazil, a study with participants with a previous history of sexually transmitted infections such as syphilis and gonorrhoea had a higher risk of being infected with HIV [15]. Thus, sexually transmitted infections such as gonorrhoea, chlamydia, syphilis, and others could facilitate HIV transmission by breaking the lining of the genital tract, causing ulcers, and making it easier for the HIV in the genital secretions to slip through during sexual intercourse. In this study, syphilis infection, compared with gonorrhoea and vaginal discharges, was found to be significantly associated with HIV infection in the adjusted regression model. Therefore confirming earlier reports of syphilis and HIV transmission overlap [16].

HIV prevention strategies by HIV advocacy groups and the National HIV/AIDS Control Programmes must continue to include STI prevention strategies through messages targeting less risky sexual behaviours and regular and proper condom use during sexual intercourse in the Sunyani municipality. Similarly, several studies have indicated that having unprotected sex and inconsistent condom use are known factors associated with STIs and HIV infections [5,6,7]. These messages must be consistent as we target the UNAIDS 95 – 95 - 95 goals. Having multiple sexual partners was significantly associated with HIV infection among the participants in this study. This supports previous studies which indicated that multiple sexual partners and a history of genital ulcers among men in the past 12 months were associated with HIV infection [17,18,19]. Having multiple sexual partners is a contributory factor to having a sexually transmitted infection because, with multiple partners, the probability of meeting a partner with an STI infection increases. Generally, people who indulge in risky sexual behaviour, such as having multiple sex partners, do not use protective condoms [19]. Having an STI increases the chance of getting infected with HIV since a previous history of STI indicates that unprotected sex has occurred. STIs are associated with inflammations, histiocytes and lymphocytes, and HIV RNA shedding into genital secretions as well as fluids of sex canals [18].

The authors of this study, therefore, recommend that HIV control strategies, such as repeat testing for HIV and STI, should be reinforced, especially among those who have multiple partners within the Sunyani Municipality. Although in Ghana, every pregnant woman is tested at antenatal registration and at 34 weeks of pregnancy to screen for HIV during pregnancy, several Know Your HIV Status (KYS) campaigns need to be organised in the Sunyani Municipality. Limitations in this study were possibly participants' recall bias and perception of stigma associated with HIV. Recall bias was anticipated to some

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extent since clients had to remember their previous history of sexually transmitted infection, previous history of tuberculosis, previous testing for HIV, and previous use of condoms. This error was, however, minimised by using a local calendar listing the main national and religious events and other timelines as well as symptoms of the infections.

Conclusion

The proportion of those who tested positive for HIV in Sunyani Municipality was 10.3%. Out of the 46 HIV cases, 13 of them, representing 28%, reside in districts far away from the Sunyani Municipality. The factors that were significantly associated with HIV prevalence in the Sunyani and peri-urban areas in this study were previous history of syphilis infection and multiple sexual partners. Based on the findings from this study, the following recommendations are made: effective prevention interventions such as health education and behavioural change communication should be targeted and sustained. In addition, the Know Your HIV status campaign should be prioritised in high-burdened sub-districts as well as low-burdened sub-districts to sustain HIV control.

DECLARATIONS

Ethical consideration

This study received ethical approval from the Ghana Health Service Ethics Review Committee (GHS-ERC: 082/12/18), and permission was granted by the participating health facilities before the commencement of the study.

Consent to publish

All authors agreed on the content of the final paper.

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None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

KAW made contributions to the design, acquisition, and analysis, including interpretation of the data and drafting of the manuscript. RB and FA contributed to the manuscript's drafting, data interpretation, and critical review. BS contributed to the design, drafting, and interpretation of the data, as well as critically reviewing the manuscript for intellectual content. All authors read and approved the final manuscript.

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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Early phase bioprospecting and phenotypic characterisation of streptomycetes in Greater Accra

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Abstract

Background: Microbial life forms, including streptomycetes, are an important source of natural products obtained from bioprospecting.

Objective: The study aimed to provide baseline data on the phenotypic diversity of streptomycetes from soils in Greater Accra and to determine the suitability of the selected areas for bioprospecting of potentially novel antimicrobial-producing strains

Methods: Twenty-one soil samples were collected from 7 areas in Greater Accra. Streptomycetes were cultured on Oatmeal Agar for sporulation. Spores were subcultured onto a variety of media to ascertain the colony morphology of the strains. Extracts of pure isolates were obtained via submerged cultures. The antimicrobial activity of the extracts was determined against clinical bacteria using the agar well diffusion method and categorised by their antimicrobial inhibition halo diameter.

Results: A total of 15 phenotypically proven diverse strains of streptomycetes were recovered from the soil samples. Two of the isolates were antimicrobial producers. Tryptone Soya Broth extract of *Streptomyces sp*. V1 showed good efficacy (++) against *Pseudomonas aeruginosa* (22 ± 2 mm) whereas *Streptomyces sp*. W2 showed good efficacy (++) against *Escherichia coli* (20 ± 8 mm) and moderate efficacy (+) against *Pseudomonas aeruginosa* (13 ± 2 mm).

Conclusion: Soils from different areas in Ghana may be potential sources of the next novel antimicrobial-producing streptomycetes.

Keywords: Bioprospecting, antimicrobial, phenotypic, streptomycetes, Ghana

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INTRODUCTION

Bioprospecting entails exploring biological material for commercially valuable genetic and biochemical properties [1]. Microbial life forms, including streptomycetes, are an important source of natural products obtained from bioprospecting activities. Natural products, for many years, have been the starting point of drug discovery. Streptomycetes are the most predominant, comprising almost 70% of the actinomyces [2,3] and account for 1 - 20% of soil microflora [4]. Streptomycetes can be found in terrestrial and marine soils, water bodies, and decaying organic matter [2,5]. Their ability to produce a plethora of secondary metabolites has been exploited in the pharmaceutical industry to produce medically important drugs used in the treatment of infections [6], cancer [7], and diseases associated with the immune system [8]. Most classes of antibiotics currently in existence were discovered in the golden era (1950 - 1970s) [9]. Unfortunately, the increasing use of antibiotics in both humans and animals has led to the development of resistant bacterial pathogens. Traditionally, bioactive compounds were discovered

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through conventional bioprospecting based on the isolation of potential producers and screening their extracts in a variety of bioassays [10]. With time, interest in the bioprospecting of streptomycetes waned because the bioactive compounds produced by most of these were not in sufficient quantities [11], coupled with consistent rediscovery of already known compounds [10].

Subsequently, multiple approaches, such as ribosome engineering, metabolic engineering, rational-based drug design, bio- and chemoinformatics, and omics, have been explored to obtain a diversity of products from novel secondary metabolites isolated from streptomycetes [10,12]. Currently, the increasing emergence of antibiotic resistance and its widespread nature are motivating a continuous search for new compounds. Considering that the "omic" approaches of creating novel compounds rely on a target base compound, it is still preferable to be able to isolate and cultivate an actual host secondary metabolite compound. Furthermore, it is also clear that several natural products cannot be produced under standard laboratory conditions [10] and hence should be isolated from the environment. Owing to the remarkable success of finding therapeutic secondary metabolites from streptomycetes [6,8], there has been a renewed interest in bioprospecting of streptomycetes [11]. In Ghana, few studies on the bioprospecting of streptomycetes have suggested that careful bioprospecting of streptomycetes from soils may lead to the discovery of novel antimicrobial-producing strains [13,14]. To achieve this, phenotyping, although preliminary, is a vital requirement to characterise the new species of streptomycetes that may be isolated [15]. This study aimed to provide baseline data on the phenotypic diversity of streptomycetes from soils in Greater Accra and to determine the suitability of the selected areas for bioprospecting of potentially novel antimicrobial-producing strains.

MATERIALS AND METHODS

Sample size and sampling technique

Soil samples were collected from seven (7) different locations, all in the Greater Accra Region of Ghana. These sites included seashores, lakesides, farmlands, and medical dumpsites. Soil samples were collected from the ground surrounding Ashaiman farmlands, Pantang dumpsite, Korle Bu dumpsite, Jamestown seashore, Dansoman seashore, Alison Green lakeside, and Ashale Botwe lakeside. For each site, the soil was collected at three separate 25 m^2 plots laying 10 m apart to obtain a representative sample of the area [16]. For each plot, the soil was sampled from 8 different points along the perimeter of each 25 m^2 area. The upper layer of the soil was removed, and the samples were collected from 5-20 cm depth. The samples from each plot were placed into three separate labelled sterile containers, giving a total of 21 composite samples. The geolocation of each plot was captured from the centre of the plot using the Ghana Post GPS software. The samples were transported to the Microbiology Laboratory of the Science Laboratory Technology Department of Accra Technical University for further analysis. Soil samples were air-dried at room temperature for 10 - 15 days [5]. Samples were processed under aseptic conditions. For each collected sample, 1 g of the soil was suspended in 9 ml of sterile distilled water and then pre-heated for 6 min at 55°C to reduce non-spore-forming bacteria. Each soil suspension was serially diluted in sterile normal saline (0.85%) up to 10 - 5 dilutions. Aliquots of 1 ml of the 10 - 2 and 10 - 3 dilutions were spread evenly over the surface of modified Starch Casein Agar (SCA) (1.2 g soluble starch,1.8 g Agar, 0.036 g Powdered milk, 0.6 mL of soil extract, 120 mL of water powdered milk) and Luria-Bertani Agar. Cultures were incubated aerobically at 28 - 30°C for 7 - 21 days [4].

Morphological characterisation of the streptomycetes colonies was done by following the methods given in the International Streptomycetes Project [17]. Colonies from the same site appearing on the same culture media that were morphologically indistinguishable were treated as being the same. Streptomycetes colonies were confirmed by microscopic examination as filamentous branching Gram-positive bacteria [4]. Isolates identified as streptomycetes were subcultured on Oatmeal Agar (0.80 g oat powder, 0.06 g agar, 20 ml water). The cultures were incubated for seven days at 28°C to 30°C. After growth, the spores were harvested and stored in cryotubes at -20°C as 25% glycerol stocks [18]. Streptomycetes spores from glycerol stocks were sub-cultured onto Mueller-Hinton Agar, Luria-Bertani Agar, Oatmeal Agar, Soil Extract Agar, Tryptone Soya Agar, Nutrient Agar, and Tryptone Glucose Yeast Agar. These were incubated at 28°C to 30°C and monitored for seven days for the occurrence of growth [18]. The colonies were described based on pigmentation, margin, and elevation. Streptomycetes spores were inoculated on Oatmeal Agar and incubated at 28°C to 30°C for the occurrence of growth. Colonies were aseptically cut from the culture and inoculated into Luria-Bertani Broth and Tryptone Soya Broth (TSB). These, alongside uninoculated Tryptone Soya Broth and Luria-Bertani Broth to be used as negative controls, were incubated at 28°C to 30°C for seven days. The broth cultures were centrifuged at 2100 rpm for 10 minutes. The supernatants were filtered and kept at -20°C until ready for testing.

Antimicrobial activity screening was determined by the agar well diffusion method [5] using previously characterised *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli* obtained from the Microbiology Laboratory of Accra Technical University, Department of Science Laboratory Technology. Gentamicin disks were included as positive controls. Negative controls comprised uninoculated Tryptone Soya Broth and Luria-Bertani Broth. Also, each extract was tested against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 control strains. After incubation, the agar plates were examined, and the zones of inhibition were measured. The test for each extract was performed in triplicates. The antimicrobial activity of the active extracts

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was categorised by the antimicrobial inhibition halo (AIH) diameter as described by Leal et al. (2020) [19]. An inhibition halo of 0 mm was categorised as non-effective (-). An inhibition halo > 0 mm but < AIH was categorised as having moderate efficacy (+). An inhibition halo > AIH but < two-fold AIH was categorised as having a good efficacy (++). An inhibition halo > two-fold AIH was categorised as having strong efficacy (+++).

Data analysis

StataCorp. 2015. Stata *Statistical Software:* Release 14 (StataMP 14). College Station, TX: StataCorp LLC was used to determine the frequency of the various phenotypic variants of streptomycetes isolates, the mean and standard deviation of the zones of inhibition produced by the extracts, and an unpaired T-test to determine whether there was any significant difference between the means of the zones of inhibition produced by the extracts and the reference antimicrobial disks. The level of statistical significance was set at $p < 0.05$.

RESULTS

Overall, 15 distinct streptomycetes were isolated from the 7 study sites (Table 1). The greatest occurrence of streptomycetes was in soil samples collected from Dansoman seashore (3) and Alison's Green lakeside (3). These were followed by Pantang dumpsite (2), Korle Bu dump site (2), Jamestown seashore (2), and Ashale-Botwe lakeside (2). The lowest occurrence was in soil samples collected from Ashaiman farmlands (1). All the isolated streptomycetes (100%) were able to utilise glucose and citrate (Figure 1). The frequency of gas producers (61.5%) and H_2S (56.3%) producers was greater than that of non-producers. The frequency of catalase-positive strains (43.8%), sucrose fermenters (30%), lactose fermenters (30%), and oxidase-positive strains (15.4%) was less than strains that did not produce these reactions. There were no urease producers (0%) among the streptomycetes isolates. A broader range of colours was presented by streptomycetes on Nutrient Agar (5/5) than Soil Extract Agar (4/5), Oatmeal Agar (3/5), Tryptone Yeast Glucose Agar (3/5), Mueller-Hinton Agar (3/5), Luria-Bertani Agar (3/5), and Tryptone Soya Agar (2/5) (Figure 2). These were white, black, brown, pink, and different shades of green and yellow. White was the most prominent colour presented by streptomycetes on Luria-Bertani Agar (85.7%), Tryptone Soya Agar (84.6%), Tryptone Glucose Yeast Agar (78.6%), Mueller-Hinton Agar (61.5%), Nutrient Agar (61.5%), and Soil Extract Agar (42.9%). Green was the most prominent colour presented on Oatmeal Agar (61.5%). Pink (7.1%) was a rare colour, and it was presented only on Luria-Bertani Agar. Colonies presented flat, raised, convex, and umbonate elevations on the culture media (Figure 3). All the elevations were presented by colonies on Tryptone Glucose Yeast Agar (4/4) and Luria-Bertani Agar (4/4) whereas some were presented by colonies on Mueller-Hinton Agar (3/4), Tryptone Soya Agar (3/4), Nutrient Agar (3/4), Oatmeal Agar (2/4), and Soil Extract Agar (2/4). Flat colonies were presented on the complete range of culture media used (7/7). This was followed by umbonate (5/7), convex (5/7), and raised (4/7) colonies. Flat colonies were prominent on Soil Extract Agar (85.7%), Nutrient Agar (69.2%), Tryptone Soya Agar (61.5%), Mueller-Hinton Agar (46.2%), and Tryptone Glucose Yeast Agar (42.9%). Raised colonies were prominent on Oatmeal Agar (77.8%).

Table 1. Streptomycetes isolated at study sites

Site	No. of Streptomycetes Isolates	Isolate Codes
Ashaiman farmlands	1	*Streptomycetes* sp. PTC2
Pantang dumpsite	2	*Streptomyces* sp. PD1 *Streptomyces* sp. PD2
Korle Bu dumpsite	2	*Streptomyces* sp. C2W *Streptomyces* sp. B2B
Dansoman seashore	3	*Streptomyces* sp. DSP01 *Streptomyces* sp. DSW01 *Streptomyces* sp. DSY01
Jamestown seashore	2	*Streptomyces* sp. V1 *Streptomyces* sp. W2
Alison's Green lakeside	3	*Streptomyces* sp. S1W3 *Streptomyces* sp. S2PT *Streptomyces* sp. S3P0
Ashale-Botwe lakeside	2	*Streptomyces* sp. PT1W *Streptomyces* sp. PT3P

Colonies presented entire, filiform, and undulate margins on all the culture media (Figure 4). The most prominent colony margin presented was undulate on Tryptone Glucose Yeast Agar (78.6%), Luria-Bertani Agar (64.3%), Mueller-Hinton Agar (53.9%), Nutrient Agar (53.9%), and Tryptone Soya Agar (46.2%). Filiform margin colonies were prominent on Oatmeal Agar (76.9%), and entire margin colonies were prominent on Soil Extract Agar (57.1%). On Luria Bertani Agar, the colony forms were irregular (92.9%) or circular (7.1%). On Oatmeal Agar, the colony forms were filamentous (61.5%), circular (30.8%), or irregular (7.7%). On soil Extract Agar, the colony forms were irregular (57.1%) or circular (42.9%). On Tryptone Soya Agar, the colony forms were irregular (53.9%) or circular (46.2%). On Mueller-Hinton Agar, the colony forms were irregular (76.9%) or circular (23.1%). On Nutrient Agar, the colony forms were irregular (61.5%) or circular (38.5%). On Tryptone Glucose Yeast Extract Agar, the colony forms were irregular (71.4%) or circular (28.6%). Overall, 5 (33.3%) of the isolates showed antimicrobial activity for metabolites extracted in Tryptone Soya Broth (TSB), and 3 (20.0%) showed antimicrobial activity for metabolites extracted in Luria-Bertani Broth (Table 2). These were Streptomyces sp.V1, *Streptomyces sp.*W2, *Streptomyces sp.*PT3, *Streptomyces sp.*PT1, and *Streptomyces sp.*S2. Against *Pseudomonas aeruginosa*, the mean zone of inhibition for *Streptomyces sp.*V1TSB extract

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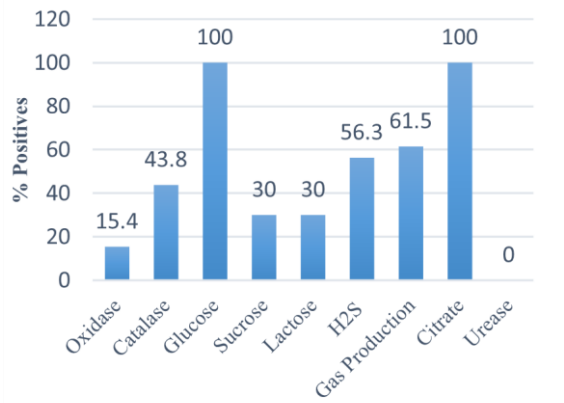

Figure 1. Biochemical identities of streptomycetes isolates

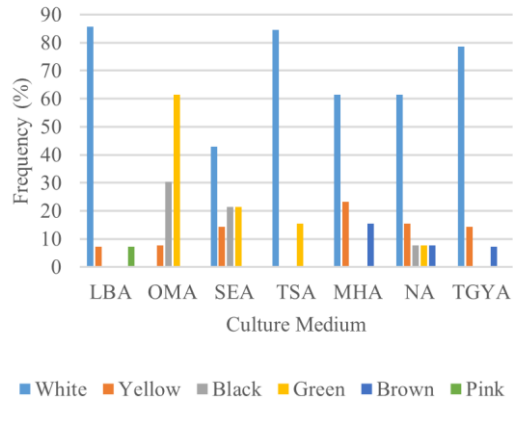

Figure 2. Colony pigmentations expressed by streptomycetes isolates on various culture media

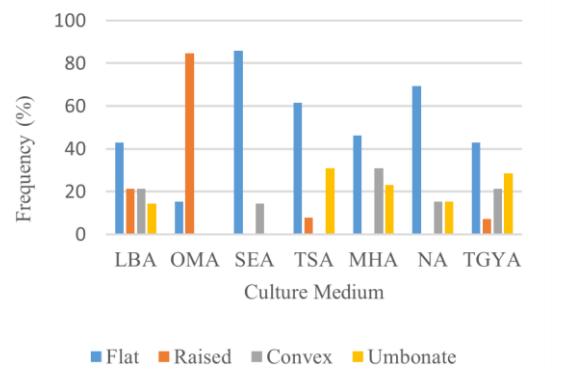

Figure 3. Colony elevations presented by streptomycetes isolates on various culture media.

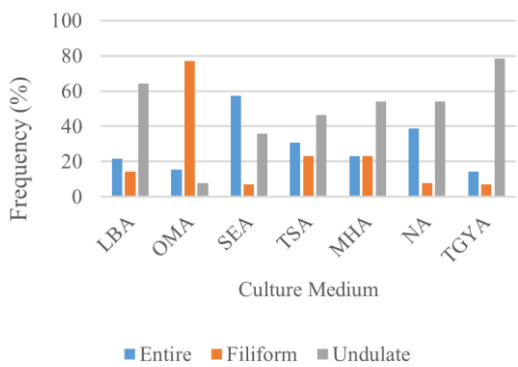

Figure 4. Colony margins presented by streptomycetes isolates on various culture media.

Luria-Bertani Agar (LBA), Oatmeal Agar (OMA), Soil Extract Agar (SEA), Tryptone Soya Agar (TSA), Mueller-Hinton Agar (MHA), Nutrient Agar (NA), and Tryptone Glucose Yeast Agar (TGYA).

Table 2. Antimicrobial activities of active extracts against isolates that showed susceptibility.

Test Isolate	Zone of Inhibition (mm)						
	V1 TSB	W2 TSB	V1 LBB	PT3 TSB	PT1 LBB	PT1 TSB	S2 LBB
P. aeruginosa	22 ± 2	13 ± 2	-	-	-	-	-
E. coli	-	20 ± 8	-	-	-	-	-
S. aureus ATCC 29213	-	-	12 ± 0	0 ± 0	11 ± 1	0 ± 0	14 ± 0
E. coli ATCC 25922	-	-	10 ± 0	10 ± 1	9 ± 0	10 ± 0	12 ± 0

*Tryptone Soya Broth (TSB), Luria-Bertani Broth (LBB

Table 3. Antimicrobial inhibition halo categorization of extracts

	TSB Extracts	
Gentamicin Reference Zone	Streptomyces sp. V1	Streptomyces sp. W2
Staphylococcus aureus (10 mm)	-	-
Escherichia coli (16 mm)	-	++
Pseudomonas aeruginosa (20 mm)	++	+
Salmonella typhi (7 mm)	-	-

*Non effective (-), moderate efficacy (+), Good efficacy (++), Strong efficacy (+++)

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(22 ± 2 mm) was not significantly greater (p = 0.2500) than the gentamicin reference zone of inhibition (20 mm). Also, the mean zone of inhibition for *Streptomyces sp.*W2 TSB extract (13 ± 2 mm) was not significantly less (p = 0.9114) than the gentamicin reference zone of inhibition (20 mm). For *Escherichia coli*, the mean zone of inhibition for *Streptomyces sp.*W2 (20 ± 2 mm) was not significantly greater (p = 0.3524) than the gentamicin reference zone of inhibition (16 mm). For the control strains, *Streptomyces sp.* S2 LBB extract had a greater inhibitory effect (14 ± 0 mm) on *S. aureus* ATCC 29213 than *Streptomyces sp.* V1 LBB extract (12 ± 0 mm) and *Streptomyces sp*. PT1 LBB extract (11 ± 1 mm). Also, *Streptomyces sp.* S2 LBB extract (12 ± 0 mm) had a greater inhibitory effect on E. coli ATCC 25922 than *Streptomyces sp.* V1 LBB extract (10 ± 0 mm), *Streptomyces sp.* PT1 (10 ± 0 mm), *Streptomyces sp.* PT3 TSB extract, and *Streptomyces sp.* PT1 LBB extract (9 ± 0 mm). The TSB extract of *Streptomyces sp.*V1 showed good efficacy (++) against *Pseudomonas aeruginosa* but was not effective (-) against the other tested clinical bacteria (Table 3). The TSB extract of *Streptomyces sp.*W2 showed good efficacy against *Escherichia coli* (++) and was moderately effective against *Pseudomonas aeruginosa* but was not effective (-) against the other tested clinical bacteria.

DISCUSSION

Owing to the remarkable success of secondary metabolites from streptomycetes as a drug source, there is a continued search for strains that may possess antimicrobial activity. This study aimed to further highlight Ghana as an area for the isolation of potent antibiotic-producing streptomycetes. Careful exploration of strains with antibiotic activity can lead to the discovery of new drug targets. Statistically, there is a 1 in 30,000 or 40,000 success rate in bioprospecting of natural products [1]. Therefore, five antimicrobial producers of 15 streptomycetes isolates are a significantly rare occurrence. This supports the bioprospecting potential of areas in Ghana, such as the coastal lines. Similarly, the antimicrobial-producing *Streptomyces sp.*1S1 [20] isolated from the Southern coast of the Red also has its origins in the seashore. The short-line biochemical test conducted in this study was able to differentiate between the morphologically distinct forms of streptomycetes strains from the different sampling sites, suggestive of the occurrence of a diversity of strains and the potential of the area for bioprospecting. The frequency of streptomycetes isolated from the different ecologies, that is, seashore, lakeside, dumpsite, and farmland, differed slightly. However, the majority were isolated from soils sampled from moist environments such as the seashore and lakeside, and this is not surprising given that streptomycetes thrive in moist environments [21,6]. Similarly, secondary metabolite-producing streptomycetes have been mostly isolated from soil and marine environments, as reported in a review by Lacey and Rutledge [22].

A limited number of media was used for the primary isolation, but these supported the growth of different strains. Using a combination and more advanced media formulations such as the International Streptomyces Project (ISP) 2 and 4 [23,4] may enhance the chances of isolating a greater diversity of streptomycetes, some of which may potentially be novel antimicrobial-producing strains. Growth capacity on the different culture media suggests that although most streptomycetes sporulate well on Oatmeal Agar [18], the growth rate is relatively slower. Therefore, other generally available media which promote quicker growth, for instance, Mueller-Hinton and Nutrient Agar, can be considered when sporulation is not the objective. Oatmeal Agar also promoted variation in pigmentation of the streptomycetes; hence, it may be good for preliminary identification. The composition of the growth medium may play a significant role in determining the culture character of streptomycetes as the colony morphology varied from one medium to the next. Colony morphology is a manifestation of the physiological processes that occur in the different strains because of the unique combination of factors in the medium that are available to the streptomycetes. Apart from pigmentation, streptomycetes isolate expressed variations in form, elevation, and margin on the different culture media used. Adeyemo et al. (2021) reported similar variations for *Streptomyces sp.* SUI and *Streptomyces sp.* SW72IV, and *Streptomyces sp.* SW72 VII is grown on various ISP media, Starch Casein Agar, and Nutrient Agar. These showed textures ranging from smooth and shiny to rough and dry, aerial colours such as whitish, cream, tan, and ivory, as well as poor or luxurious growth on different media [24].

Depending on the objective, a medium can be chosen to influence the nature of growth required when studying streptomycetes. From a bioprospecting perspective, culture media, which may appear to be appropriate for the subculturing of streptomycetes, may not necessarily be suitable for isolation. Luria-Bertani Agar was good for isolating streptomycetes from the different sampling sites but did not perform as well as others when spores were subcultured onto these other media. This supports the concept of manipulating the production of secondary metabolites by changing the composition of the media [25]. Antimicrobial metabolites were produced in either Tryptone Soya Broth, Luria-Bertani Broth, or both, depending on the source of the streptomycetes. Therefore, the choice of media for work on streptomycetes should be informed by the purpose or objective of the work. Ideally, for improving yields, be it for spore production or extraction of secondary metabolites, a range of media should be used to determine the suitability of each culture media for the strain of interest. This will reduce the time of waiting and improve yields in subsequent works once the most appropriate medium for the strain in question has been established. Working with a limited number of broth fermentation media may have contributed to the low number of antimicrobial-producing strains (33.3%) identified among the isolates. It is known that changing the media can switch on various biocryptic genes in

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streptomycetes [26] and enhance their antimicrobial production. It has been previously reported that some streptomycetes isolated from the valley of Taza in Morocco produced antimicrobial substances in Bennett medium (50%), Starch Casein medium (> 45%), and Mueller-Hinton medium (> 13%) [27]. Therefore, even the strains from the other sites that did not demonstrate any inhibitory activity against the test bacteria may possess the potential for antimicrobial production beyond the normal screening process.

Conclusion

Soils from different areas are potential sources of the next novel antimicrobial-producing streptomycetes. These streptomycetes may be morphologically diverse but may also share some colony features, which vary depending on the culture media in which their growth occurs. A range of both agar and broth culture media should be used for culture and isolation of streptomycetes during early phase bioprospecting endeavours as a means of enhancing chances of isolation and improving identification of antimicrobial-producers. Further work should be carried out on the streptomycetes isolates that were identified as antimicrobial producers by purifying the extracts for testing to ascertain the actual potencies. Also, the identified antimicrobial-producing strains should be genotyped to establish the possibility of their novelty, and the extracts of these strains should be characterised to identify the bioactive components responsible for their antimicrobial activities.

DECLARATIONS

Ethical consideration

There are no ethical issues.

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

REA conceived the idea. REA and DAP developed the methodology. LS, BNB, EOK, KE, AAA, PDH, NA, KNANB, VS, MA, RAY, FSA, RMA, and SO were involved in the sample collection. Laboratory analysis and drafting of the manuscript were done by all authors.

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Availability of data

Data for this work is available upon request to the corresponding author.

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Uptake and correlates of long-acting reversible contraceptive use among post-partum women in Ledzokuku-Krowor municipality in Ghana: A facility-based cross-sectional study

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Abstract

Background: The unmet need for post-partum family planning globally is around 65%, but in sub-Saharan Africa (SSA), it ranges between 25 and 96%, and 84.5% in Ghana. Long-acting reversible contraceptive (LARC) methods can optimally space births and markedly reduce the high levels of unwanted and or risky pregnancies as well as maternal and child mortality and morbidity. Although they are the most effective, easy, and safe to use after delivery, reports from the Ledzokuku-Krowor Municipality Assembly (LEKMA) show the prevalence of LARC methods is very low among post-partum women.

Objective: This study aimed to determine the prevalence of LARC uptake and assess factors associated with the use of LARC methods in the LEKMA of Ghana.

Methods: A facility-based cross-sectional study using a probability proportionate to size sampling approach was employed to recruit post-partum women within 12 months of delivering a live baby and accessing child welfare clinics in the Government-run health facilities in LEKMA. Secondly, a semi-structured interview guide was administered to sexual and reproductive health workers. The multivariable binary logistic regression model with a robust standard error was used to determine correlates of LARC use.

Results: A total of 406 post-partum women were enrolled in the study. Only 4.1% (n = 16) used LARC, although 59.7% (n = 242) of post-partum mothers had access to LARC methods. The results from the multivariable binary logistic regression model showed adequate knowledge of LARC (adjusted odds ratio = 4.88, 95% CI: 2.64, 26.79, $p < 0.05$), and age was associated with the odds of uptake of LARC. Interviews with facility managers and other health workers revealed that barriers to uptake of LARC methods include fear of side effects, lack of spousal support, misconceptions about LARC, and lack of adequate knowledge of providers.

Conclusion: There is a high unmet need for contraception among post-partum women. Even though uptake of LARC in the post-partum period in LEKMA is low, it is relatively high among women with adequate knowledge of LARC and in unions (presumably in stable relationships or marriages). Ghana Health Service and the Ministry of Health should make LARC affordable and easily assessable through efficient distribution at the community health post and adequately build the capacity of providers through institutionalised training. There is a need for stakeholders to intensify community-level education geared towards mitigating the barriers to uptake, including myths and misconceptions, and promoting the possible benefits of the use of LARC services.

Keywords: Long-acting, reversible, contraceptive, post-partum, family, planning

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INTRODUCTION

According to the Royal College of Obstetricians and Gynaecologists report, 2015, it has been estimated that globally, around 222 million women would like to prevent or delay pregnancy but have no access to contraception. The unmet need for family planning is greatest in the post-partum period and represents one of the biggest opportunities to address the challenge of unmet need for family planning [1]. Women normally become susceptible to pregnancy before initiating contraception

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after delivery and may not be aware of the risk of pregnancy before the return of menses[2]. The post-partum period has been identified as a time when the unmet need for contraception is particularly high in developing countries. Also, post-partum women who do not receive LARC immediately are reported to be at an increased risk of repeat pregnancy [3]. Creating wider access to LARC, the most effective contraceptive, can substantially reduce the high levels of maternal mortality and morbidity, as well as unwanted pregnancies and unsafe abortion. In Ghana, Demographic and Health Survey (DHS) data shows that the proportion of women with post-partum amenorrhea dropped from 96% in the first two months after birth to 21% at 12 - 15 months [4]. Thus, the proportion of such women with unmet needs does not take into account women who are considered to have no risks of getting pregnant and, hence, do not need contraception.

Focusing on post-partum family planning (PPFP) is particularly essential in Ghana and West Africa as the length of post-partum insusceptibility keeps declining. This could lead to an increasing proportion of women being at risk of unwanted pregnancy if quality PPFP services are unavailable or inaccessible. Among the numerous family planning methods, LARCs, particularly copper IUDs and hormonal implants, are the most efficacious and cost-effective in the long term [5]. Their failure rates of less than one percent compared to 4.8% for pills makes them superior [6]. The contraceptive failure rate among participants using pills, patches, or rings was 4.55 per 100 participant-years, as compared with 0.27 among participants using long-acting reversible methods [7]. The LARC methods are thus twenty times more effective in preventing unintended pregnancies than other methods [5]. It has a continuation rate of more than 80% and does not require ongoing user effort [8]. Above all, almost all women are eligible [8].

Intrauterine devices (IUDs) are suitable for most post-partum women and can be inserted within 10 minutes of delivery, 48 hours post-partum, and intra-cesarean with a very low expulsion rate [9]. The IUD is an ideal post-partum method because it does not interfere with lactation, facilitates adequate birth spacing, and does not require repeat healthcare visits for contraceptive refills [10]. Additionally, among the numerous family planning methods, LARCs, particularly copper IUDs and hormonal implants, are the most efficacious and cost-effective in the long term [5]. Despite these advantages of LARC, in Ledzokuku-Krowor Municipal Assembly (LEKMA), an urban district in the Greater Accra Region of Ghana, out of 4,824 deliveries in 2015, only 23.3% (n = 1,125) opted for any post-delivery contraceptive. Of the 1,125 contraceptive users, only 2.6% (n = 126) accessed LARC methods.

The purpose of this study is to investigate the major determinants, including motivators and barriers, to the uptake of LARC among post-partum mothers (0 - 12 months) in the LEKMA.

MATERIALS AND METHODS

Study design and sites

The study area (LEKMA) is one of 16 districts in the Greater Accra Region of Ghana. It has a total population of 273,743, projected from the 2010 population of 227,932 (projected from the 2010 census) (GSS, 2014). The study was conducted at three public health facilities, namely, LEKMA Hospital, LEKMA Polyclinic, and Teshie Community Clinic. These facilities were selected because they are the only facilities that offer LARC services in the municipality. A facility-based cross-sectional study design was employed to determine the prevalence and correlates of LARC use as a post-partum family planning method among women of reproductive age in LEKMA.

The target population for this study was all post-partum mothers 0 - 12 months who either delivered or accessed postnatal care in any of the three public health facilities in LEKMA for the period of September to November 2017. The study assessed the level of knowledge and use of LARCs among post-partum women and how respondents' background characteristics influenced them. A structured questionnaire was administered to consenting post-partum women who delivered or attended postnatal care at the three facilities during the period of the study and were enrolled in the sample when they met the inclusion criteria. Systematic random sampling was used to select participants from all three facilities to ensure that the sample fairly represents the target population.

Sample size

The primary objective of the study was to estimate the uptake of LARC. The sample size calculation also allowed for the analysis of risk factors independently associated with the uptake of LARC. The sample size was performed using Cochran's sample size formula for one proportion. In a review of the uptake of LARC among women in low- and middle-income countries (LMIC), Harrison and Goldenberg (2017) found that not more than 15% of post-partum women use LARCThe study further assumed a 95% confidence interval, 4% precision, and a non-response rate of 34%.

The estimated sample size was 412 post-partum mothers 0 - 12 months who either delivered or accessed postnatal care in any of the three public health facilities. Using probability proportionate to the number of deliveries in each facility, a total of 299, 35, and 78 women were respectively interviewed at LEKMA Hospital, LEKMA Polyclinic, and Teshie Community Clinic. Among post-partum women assessing postnatal care from LEKMA, the under-listed formed the basis for inclusion in the study.

- A participant was aged 15 to 49 years
- Had delivered a live baby during the last 12 months
- Accessed postnatal care in any of the three public health facilities at LEKMA
- Has no history of tubal ligation or hysterectomy
- The current partner has not had a vasectomy

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Outcome measure

The outcome measure of interest in this study was the uptake of LARC post-partum at the time of the study. It is a binary indicator coded 0 and 1 for non-use and uptake of LARC, respectively.

Independent variables

The following indicators were studied based on the review of literature on LARC uptake: age of the woman, parity, education level, place of residence, marital status, religion, employment, and knowledge of LARC. A respondent was said to have adequate knowledge of LARC if she answered "Yes" to at least 6 of the following 8 knowledge questions: (1) Do you know IUDs can be inserted for post-partum mothers? (2) Do you know Implanon can be inserted for post-partum mothers? (3) Do you know that you can have an IUD inserted directly (10 minutes - 24 hours) after childbirth? (4) Do you know that you can have Implanon inserted weeks after childbirth? (5) Do you know that IUDs give protection for up to 10 years? (6) Do you know that Implanon gives protection for up to 3 years? (7) Do you know that IUD can be removed any time you want to get pregnant? (8) Do you know that Implanon can be removed any time you want to get pregnant?

Statistical analysis

The uptake of LARC was summarised using proportions. In bivariate analyses, Fisher's exact test or Pearson's chi-squared test, as appropriate, was used to examine the associations between each uptake and background characteristics. The 95% confidence interval for the outcome measure was calculated using logit-transformed confidence intervals. A multivariable binary logistic regression model was used to identify factors independently associated with the uptake of LARC. A model was first fitted for each potential explanatory variable to construct a parsimonious model using all the potential explanatory variables. In this model, each variable was a candidate for inclusion in the full model if the p-value for association with uptake was 0.2 or less when considered individually. Variables were then removed from the model if the p-value for the likelihood ratio test was more than 0.2, provided removal did not change the coefficients of variables in the model by more than 10%. Some categories of explanatory variables were combined as appropriate to ensure sufficient numbers for the analyses. All analyses were performed using Stata 15 MP (StataCorp, College Station, Texas, USA).

RESULTS

A total of 406 post-partum women who gave birth or were accessing postnatal care from LEKMA Hospital, LEKMA Polyclinic, and Teshie Community Clinic participated in the study. The average age of the post-partum women was 27 years (interquartile range [IQR]: 25, 30). About half (47%, n = 191) of the mothers had at least secondary education and two-thirds (64%, n = 259) of the respondents resided in Teshie. Eight in every ten post-partum mothers were in a union (married, 47%, n = 190; or cohabiting, 33%, n = 133). The distribution of the background characteristics can be found in Table 1. Table 2 shows the bivariate analysis of factors associated with the uptake of LAR. The bivariate analysis demonstrated that uptake of LARC was highest (16.1%, 95% CI: 6.8 - 33.8) among post-partum mothers of the oldest age group (35 - 44). It was also relatively high among younger post-partum mothers aged 20 - 24 (8%, CI: 3.6 - 16.8) and 16 - 19 (4.6%, 95% CI: 0.6 -27.2). Use declined to less than 2% among post-partum mothers 25 - 29 (1.7%, 95% CI: 0.5 - 5.2) and 30 - 34 (1.1%, 95% CI: 0.2 - 7.5). Though no statistically significant relationship was found, LARC use increased with the number of children alive. LARC use increased from 2.6% (95% CI: 1.2, 5.6) among post-partum women with one child to 8.7% (95% CI: 2.1 - 29.7) among post-partum mothers with at least four children in the bivariate analysis. In bivariate analysis, the use of LARC according to the educational attainment of post-partum mothers suggested the highest LARC users were post-partum women with no education (12.5%, 95% CI: 3.0 - 39.9). Post-partum

Table 1. Characteristics of the study participants

Background characteristics	Number of post-partum women (% of total)
Age (Years)	
Median (IQR)	27 (25, 30)
16-19	24 (6)
20-24	75 (18)
25-29	181 (45)
30-34	94 (23)
35-44	32 (8)
Education	
None	16 (4)
Primary	62 (15)
Middle/JSS	137 (34)
Secondary+	191 (47)
Residence	
Nungua	106 (26)
Teshie	259 (64)
Other	41 (10)
Marital status	
Never married	59 (15)
Living together	133 (33)
Married	190 (47)
Divorced/Separated/Widowed	23 (6)
Religion	
Catholic	27 (7)
Anglican	13 (3)
Methodist	8 (2)
Presbyterian	53 (13)
Pentecostal/Charismatic	258 (64)
Witness/SDA	10 (2)
Islam	17 (4)
Non-religious	5 (1)
Other	15 (4)
Employment status	
Employed	287 (71)
Unemployed	119 (29)
Total	406 (100)

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mothers with no education constitute only 4% of the sample, which could explain this unexpected relationship. Among post-partum mothers who were educated, the highest use was among post-partum mothers with middle/JSS education (6.7%, 95% CI: 3.5 - 12.5), lower (2.2%, 95% CI: 3.5 - 12.5) for mothers with secondary or higher education and least among those with primary education (1.6%, 95% CI: 0.8 - 5.7). From the bivariate analysis, the highest uptake of LARC (5.3%, 95% CI: 1.3 - 19.1) was among post-partum women from other places of residence compared to post-partum mothers in Teshie (5.1%, 95% CI: 3.0 - 8.7) and Nungua (1%, 95%, CI: 0.1 - 6.6]). LARC use was higher among post-partum women who were in marital unions. Uptake among post-partum mothers in marital unions was 4.5% (95%, CI: 2.7 - 7.5) compared to uptake of 2.4% (95% CI: 0.6 - 9.3) among post-partum mothers who were not in marital unions. LARC use among post-partum women showed higher use (17.7%, 95% CI: 5.6 - 43.7) among Muslim mothers compared to post-partum mothers who were either Christian (3.4%, 95% CI: 1.9 - 5.8) or non-religion/other' (5%, 95% CI: 0.7 - 29.4). No variation in uptake of LARC according to employment status was observed in the bivariate analysis. Uptake was nearly the same for employed (4.3%, 95% CI: 2.2 - 7.0) and unemployed (4%, 95% CI: 1.8 - 9.9) post-partum women.

Table 3 shows the results of the multivariable analysis of factors associated with uptake of LARC. The results showed that the odds of use of LARC were significantly associated with adequate knowledge. Post-partum mothers with adequate knowledge of LARC were almost five times more likely to use LARC compared to those without adequate knowledge. While the effect of adequate knowledge on the use of LARC was not significant in bivariate analysis, it attained statistical significance in multivariate analysis when other background factors were

Table 2. Uptake (current use) of LARC by background characteristics

Characteristics	Number of post-partum women	Number (%) currently using LARC	95%CI	Fisher's exact p-value
Adequate knowledge of LARC				
No	127	2 (1.6)	[0.3, 6.1]	0.104
Yes	268	14 (5.2)	[3.1, 8.6]	
Age of child (months)				
<6	320	14 (4.4)	[2.6, 7.3]	1.000
6+	63	2 (3.2)	[0.7, 12.0]	
Age of mother (Years)				
16-19	22	1 (4.6)	[0.6, 27.2]	0.001
20-24	75	6 (8)	[3.6, 16.8]	
25-29	176	3 (1.7)	[0.5, 5.2]	
30-34	91	1 (1.1)	[0.2, 7.5]	
35-44	31	5 (16.1)	[6.8, 33.8]	
Number of children alive				
1	233	6 (2.6)	[1.2, 5.6]	0.12
2-3	139	8 (5.8)	[2.9, 11.1]	
4+	23	2 (8.7)	[2.1, 29.7]	
Education				
None	16	2 (12.5)	[3.0, 39.9]	0.039
Primary	61	1 (1.6)	[0.2, 11.0]	
Middle/JSS	134	9 (6.7)	[3.5, 12.5]	
Secondary+	184	4 (2.2)	[0.8, 5.7]	
Residence				
Nungua	104	1 (1)	[0.1, 6.6]	0.137
Teshie	253	13 (5.1)	[3.0, 8.7]	
Other	38	2 (5.3)	[1.3, 19.1]	
Marital status				
Living together/Married	312	14 (4.5)	[2.7, 7.5	0.541
Never/Divorced/Seperated/Widowed	82	2 (2.4)	[0.6, 9.3]	
Religion				
Christianity	358	12 (3.4)	[1.9, 5.8]	0.024
Islam	17	3 (17.7)	[5.6, 43.7]	
Non-religious/Other	20	1 (5)	[0.7, 29.4]	
Employment status				
Employed	278	11 (4)	[2.2, 7.0]	1.000
Unemployed	117	5 (4.3)	[1.8, 9.9]	
Total	395	16 (4.1)	[2.4, 6.5]	

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Table 3. Table 3 shows the results of the multivariable analysis of factors associated with uptake of LARC

Factors	Crude OR [95%CI]	Unadjusted p-value	Adjusted OR [95%CI]	LR adjusted p-value
Adequate knowledge of LARC				
No	1	0.063	1	0.041
Yes	3.44 [0.77, 15.39]		4.88 [2.64, 26.79]	
Age (Years)				
16-19	1	0.004	1	0.014
20-24	1.83 [0.21, 16.04]		2.93 [0.30, 28.31]	
25-29	0.36 [0.04, 3.66]		0.46 [0.04, 4.97]	
30-34	0.23 [0.01, 3.88]		0.22 [0.01, 3.95]	
35-44	4.04 [0.44, 37.28]		2.34 [0.23, 23.88]	
Number of children alive				
1	1	0.184		
2-3	2.31 [0.78, 6.80]			
4+	3.60 [0.68, 18.98]			
Education				
None/Primary	1	0.132	1	0.083
Middle/JSS	1.78 [0.47, 6.77]		1.12 [0.24, 5.17]	
Secondary+	0.55 [0.12, 2.51]		0.26 [0.04, 1.70]	
Residence				
Nungua	1	0.106	1	0.080
Teshie	5.58 [0.72, 43.21]		5.67 [0.69, 46.21]	
Other	5.72 [0.50, 65.02]		11.36 [0.85, 151.68]	
Marital status				
Living together/Married	1	0.376		
Never/Divorced/Separated/Widowed	0.53 [0.12, 2.39]			
Religion				
Christianity	1	0.079	1	0.122
Islam	6.18 [1.56, 24.40]		5.94 [1.23, 28.70]	
Non-religious/Other	1.52 [0.19, 12.29]		0.88 [0.09, 8.34]	
Employment status				
Employed	1	0.885		
Unemployed	1.08 [0.37, 3.19]			

taken into account (p = 0.041). When the effect of age was examined alongside other background characteristics in multivariate analysis, the pattern of use remained the same as in the bivariate analysis. The odds of use were higher among young mothers 20 - 24 years (aOR = 2.9, 95% CI: 0.30 - 28.31) and the oldest post-partum mothers (35 - 44 years) (2.3, 95% CI: 0.23 - 23.88). The odds of use were lower for the remaining age groups relative to post-partum mothers aged 16 - 19 years. This relationship attained statistical significance in both bivariate analysis (p = 0.001) and multivariate analysis (adjusted p = 0.014).

Multivariable analysis indicates higher odds of use among post-partum mothers with middle/JSS education who were 12% more likely to use LARC. The odds of LARC uptake were lower among mothers with at least secondary education (0.26, 95% CI: 0.04 - 1.70). The observed relationship between education and LARC among post-partum women in LEKMA is significant when only the effect of education on LARC is examined at the bivariate level; it was not statistically significant when the net effect of all background characteristics was considered in multivariable analysis. The odds of use increased by six times for post-partum women who were Muslim and almost two times for non-religious/other religious categories compared to being Christian in the multivariate analysis. The relationship was significant when only the effect of religion was considered in the bivariate analysis but did not attain statistical significance when the net effect of all background characteristics was considered in the multivariate analysis. The multivariable analysis indicated the odds of LARC use were lower (0.5, 95% CI: 0.12 - 2.39) among post-partum women not in marital unions, albeit insignificant.

The synthesis of the key informant interviews involving health workers revealed that some of the barriers to LARC uptake among post-partum women include side effects such as bleeding after Implanon insertion and some misconceptions about LARC such as IUD travelling to other parts of the body after insertion and barrenness after use. Again, the health workers indicated that irregular menses and lack of spousal support also hinder uptake.

DISCUSSION

This study estimated the prevalence of LARC use among post-partum women and identified factors associated with the uptake of LARC. Despite the high perceived need for contraception in the post-partum period because of the

benefits and the generally positive attitude towards LARC, the use is still very low among post-partum women in LEKMA. Only 4% of post-partum women used LARC despite an adequate knowledge rate of 68%. This finding shows a lack of association between intention and healthy behaviour and is consistent with the evidence of the large disparity between demand for and use of contraception (LARC) throughout Ghana and West Africa [11]. The low uptake of LARC among post-partum women reflects both the low uptake of family planning in the post-partum period and the low use of LARC in Ghana. For instance, less than one percent (0.8%) of women in Ghana aged 15 - 49 use IUDs, and 5.2% use implants [4]. Harrison Goldenberg [12] reviewed published literature in low- and middle-income countries (LMIC) about LARC use, and a summary of their findings demonstrates that LARC use varies.

Overall, less than 15% of post-partum women in low and middle-income countries (LMIC) used LARC during the post-partum period. They found that in Nairobi, Kenya's urban slums, LARC methods were the least used during the first year post-partum. Also, only 4% of women opted for implants, and even fewer chose IUDs. In Ethiopia, only 1.8% of those currently using contraception had adopted an IUD, and 0.2% chose an implant. Higher user rates were, however, observed in Malawi (14%). Harrison and Goldenberg, in the same study, observed that studies from Southeast Asia (SEA) show utilisation rates are similar to Sub-Saharan Africa: in Pakistan, not more than 5% of post-partum women chose a LARC, and in India, 2.9% of post-partum women used IUD with none using implants. The low uptake of post-partum family planning observed in LEKMA is, therefore, a pervasive phenomenon that cuts across different countries and regions of the world.

The low use of LARC among post-partum women is partly an outcome of the low use of family planning in the post-partum period in general. For example, Rutaremwa, Kabagenyi [13] found that slightly more than a quarter (28%) of Ugandan women aged 15 - 49 used a modern method of contraception in the post-partum period, but since their study was not limited to only LARC, it follows that use of LARC will be much lower. Also, Gebreselassie and Rutstein [14], in a comparative study among four countries (Kenya, Indonesia, the Dominican Republic, and Peru), discovered that 20% to 40% of mothers in the post-partum period did not use any contraceptive method after they became susceptible to pregnancy (two in five women in Kenya, one in four in the Dominican Republic, and one in five in Indonesia and Peru). It was found that among sampled post-partum women who had brought their children for the second dose of measles vaccine between 18 and 24 months in a County referral hospital in rural Kenya, post-partum family planning was relatively high. More than four in five (86.3%) of these women used contraceptives within one year of delivery. However, the use of LARC was lower: LARC: 24.1% for IUD and 14.2% for Implanon. Thus, even where family planning use is high in the post-partum period, the use of LARC methods is generally lower. This situation presents the opportunity to increase LARC use in the post-partum period because these women may not be opposed to family planning (because they may already be using a short-acting method). Therefore, making LARC methods available in health facilities and addressing challenges related to access and misconceptions has the potential to increase LARC uptake among post-partum women. Among the background factors used in this study, knowledge of LARC and the age of post-partum women are the factors significantly associated with LARC use among post-partum women in LEKMA. Education and religion have a significant association in the bivariate analysis but do not attain statistical significance at the multivariate level. However, the lack of statistical significance for most variables when the multivariate analysis is applied may be due to the sample and/or the low uptake of post-partum family planning (number of cases with the outcome of interest - the dependent variable).

LARC uptake is the behaviour of interest that is to be influenced when the theory of planned behaviour (TPB) is applied. In this case, adequate knowledge and age are the two background factors that directly affect the behaviour of LARC uptake. Knowledge was found to be significantly associated with the use of family planning among post-partum women in LEKMA. Post-partum women with adequate knowledge of LARC were more likely to use any of the methods than those without adequate knowledge. Generally, adequate knowledge dispels myths associated with family planning in general and LARC in particular. One recognised inhibitor of the use of family planning services, even where services are available, is the lack of knowledge. This finding is consistent with several studies in sub-Saharan Africa that have shown an association between knowledge and family planning, although the measurement of knowledge differed among different researchers[15]. The study by Anguzu Tweheyo [15] realised knowledge and attitudes towards the use of LARC influenced use among women in the Lubaga division of Kampala district in Uganda. These studies used women's mass media exposure as a proxy for knowledge of post-partum family planning, and they found a significant association between knowledge of post-partum family planning and uptake.

One unique feature of this study, however, is the direct measurement and use of adequate knowledge as a predictor of LARC use. This finding from the application of the theory in this study reveals that knowledge is one of the background factors that can directly affect behaviour change (LARC uptake). This is particularly relevant in Sub-Saharan Africa, with low contraceptive prevalence and a high unmet need for family planning. This implies that uptake of LARC in LEKMA can be raised by increasing knowledge of post-partum women through the provision of IEC on post-partum family planning and the use of LARC, and this should be implemented in LEKMA and the rest of the country because uptake of LARC in the post-partum period significantly reduces unintended and closely spaced

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pregnancies and attendant complications. LARC was also significantly the highest among the younger age group (20 - 24) and higher among post-partum mothers of the oldest age group (35 - 44) compared to post-partum mothers of the youngest age group (16 - 19). It was expected that women of older ages and of higher parity would be more inclined to use LARC or stop births. However, because of cultural issues related to adopting permanent methods, these women would rather use LARC due to the longer protection provided. This is demonstrated by the high likelihood of LARC uptake among older mothers compared to young mothers. This finding is consistent with that of Gebreselassie and Rutstein [14], who found increased use of PPFP among older mothers (35+) in Indonesia, the Dominican Republic, Peru, and Kenya in a four-country study. These findings suggest that behavioural change can be achieved when attitudes are predisposed to dynamism, as in the case of young post-partum mothers, or when driven by need, as with older post-partum mothers. However, a contrasting finding was made in Uganda by Rutaremwa et al. (2015) between age and LARC, where the increase in age significantly reduced the use of LARC.

The higher odds of use among relatively young mothers (20 - 24) in LEKMA could be due to higher educational attainment among these mothers, which is known to affect LARC use positively. The odds of use among post-partum mothers aged 25 - 29 years and 30 - 34 years are lower than among teenage mothers, and this is surprising because adolescents are known to be among the categories with the highest unmet needs. The reason for this result is not immediately apparent and requires further investigation. The remaining independent variables (number of children alive, education, residence, marital status, and religion) were not found to be statistically significant. Whilst the number of children alive did not attain significance with post-partum LARC use, its effect was in the expected direction, with the likelihood of use increasing with the number of children alive. At higher parity, mothers are expected to reach desired family sizes and hence have higher motivation to limit childbearing. The lack of significant association between education and LARC uptake in the post-partum period, as well as the negative relationship with higher education, is not supported by many studies. The general expectation and common finding is a positive association between education and LARC [16,17].

Conclusion

In this study, there was a low uptake of LARC among post-partum women in LEKMA despite relatively high access to LARC services. The age of the post-partum mother and knowledge of LARC contributed significantly to the use of LARC among post-partum women. The government should institutionalise the capacity-building of service providers and collaborate with civil society organisations to intensify community-level education to increase the uptake of LARC.

DECLARATIONS

Ethical consideration

The study protocol was approved by the Ghana Health Service Ethical Review Committee. (GHS-ERC: 04/03/2017), permission was sought from the Greater Accra Regional Health Directorate and head supervisors at the research sites. Women were moved to a private space, where the purpose, objectives, and significance of the study were explained to them. Any possible risks associated with the study were explained to them. They were assured of confidentiality, privacy, and anonymity. It was explained to them that their identities and the information they provided would remain confidential and not be shared with anyone. Any other concerns of respondents regarding the study were answered. A signed informed consent sheet was obtained from those who participated.

Consent to publish

All authors agreed on the content of the final paper.

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None

Competing Interest

No conflict of interest was reported by the authors.

Author contribution

LAA conceived the idea, led the field data collection, and wrote the first draft of the manuscript. SB and DD reviewed the statistical methods. KT, DA, SB, ETM, DD and AMD contributed to the write-up of the introduction, the literature review, discussion of the findings, interpretation, and revision of the manuscript drafts, and supported the data validation and data management. All authors have access to the data and have read and approved the final manuscript.

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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Ultrastructural hepatic damage in murine malaria with and without prandial natural cocoa powder and artemether-lumefantrine treatment

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Abstract

Background: There is paucity of data on the potential benefit of cocoa in reducing oxidative stress and inflammation in the pathophysiology of plasmodial infection and its associated damage to liver tissues

Objective: This study compared hepatocyte ultrastructural integrity in rodent malaria treated with aqueous natural cocoa powder (NCP) ingestion with and without artemether-lumefantrine (AL).

Methods: Twenty-four Sprague-Dawley rats were randomly assigned to groups. Every rat was inoculated with 0.2 ml of parasitised blood containing 1 x 10^5 *P. berghei* (NK 65) parasites per microlitre to induce malaria infection. Subsequently, group 1 rats were given 2% (weight/volume) aqueous NCP; group 2 rats were given 2% NCP and 0.6 ml of 4.0 mg/kg AL. Group 3 rats received only the same dose of AL as group 2, whilst group 4 rats were given neither NCP nor AL. NCP and AL were administered by oral gavage once daily. Liver tissue harvested from euthanised and perfusion-fixed rats was processed for transmission electron microscopic examination. Hepatic tissue damage was quantitatively assessed using design-based stereology. Ultrastructural variables assessed were sinusoidal diameters, sinusoidal endothelial wall thickness, volume density of Kupffer cells, and perisinusoidal microvilli.

Results: As per the study variables, liver damage in group 1 rats was significantly extenuated compared with rats in group 2, group 3, and group 4. Serum biochemical markers assayed indicated statistically lower levels of aspartate transaminase (AST) and alanine transaminase (ALT) in groups 1,2 and 3 rats compared to group 4 rats. Inferably, mitigation of liver ultrastructural damage in *P. berghei*-infected rats given NCP was better than treatment with AL and putatively attributable to the anti-inflammatory activity of cocoa evidenced by significantly lower serum transaminases.

Conclusion: The evidence shows that damage to the ultrastructural liver morphology in murine malaria was significantly mitigated by daily ingestion of NCP compared with AL treatment with respect to hepatic sinusoidal endothelial thickness and density of hepatic microvilli despite parasitaemia being comparable to untreated control rats

Keywords: Hepatoprotection, cocoa, murine, plasmodial infection, stereology

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INTRODUCTION

The 2019 factsheet of the US Center for Disease Control and Prevention (CDC) on Malaria's Impact Worldwide [1] cites the World Health Organization's World (WHO) Malaria Report 2017 that malaria is one of the most severe public health problems worldwide. Furthermore, nearly half the world's population lives in areas at risk of malaria transmission, and it is the leading cause of death and disease in many developing countries. Drug resistance of plasmodium presents an enduring challenge for the global curtailment of the malaria scourge [2]. This necessitates a continued search for novel treatment options, of which nutraceuticals such as cocoa hold promise [3]. Despite strong reservations about the realistic transfer of laboratory studies on rodent malaria to clinical trials, there remains wide recognition of the important contribution of animal models in malaria research [4-6]. Extensive literature on the pathophysiology

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of malaria [7-12] indicates that the plasmodium parasites first damage infected erythrocytes directly and then trigger a chain reaction of nonspecific inflammatory processes, which elicit host immunological responses that further aggravate the inflammatory reactions. In rodent malaria infection, *plasmodium berghei* sporozoites reach the liver either by the portal vein or hepatic artery and migrate through several hepatocytes, causing cellular damage [13]. The parasites eventually invade one hepatocyte, where they multiply and differentiate into thousands of merozoites, which subsequently attack and damage red blood cells. *Plasmodium berghei* infection results in chronic oxidative stress, and tissue damage is a known cause of reactive oxygen species [14-16]. Cocoa is a rich source of dietary flavonoids, which have a potent antioxidative capacity [3,17-20]. Literature abounds on the anti-inflammatory actions of dietary flavonoids in general and specifically cocoa [21-23]. Given the known role of oxidative stress and inflammation in the pathophysiology of plasmodial infection and its associated damage to liver tissues [24] vis-à-vis the antioxidative and anti-inflammatory benefit of cocoa, this study compared hepatocyte ultrastructural integrity in rodent malaria treated with aqueous natural cocoa powder (NCP) ingestion with and without artemether-lumefantrine (AL). Findings will contribute to elucidating the mechanism(s) undergirding anecdotal elimination of clinical malaria in people who drink NCP daily as a beverage and offer empirical support for NCP as a diet-mediated antimalarial prophylaxis [3]. Moreover, clues will be offered to understand the mechanism(s) by which prandial NCP affords hepatoprotective benefit in rodent malaria [18,19] and alcoholic toxicity [20].

MATERIALS AND METHODS

Study design, participants and samples

This study was approved by the Committee on Research, Publications, and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medicine and Dentistry, and the Institutional Animal Care and Use Committee (IACUC) of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana.

Animals and parasites

Twenty-four male Sprague Dawley rats aged 6 - 8 weeks and weighing between 180 g - 200 g were kept under laboratory conditions with an ambient temperature of 28°C, relative humidity of 70% ± 4%, 12-hour light and dark cycle and 24-hour natural ventilation at the University of Ghana, Medical School Animal House, Korle Bu, Accra, Ghana. The rats were kept in four different cages with dimensions of 30 cm x 22 cm x 16 cm (length x breadth x height, respectively) after 7-day acclimatisation from the animal holding unit. The animals were fed rat chow from Ghana Agro Food Company (GAFCO, Tema, Ghana) and given autoclaved tap water every morning. Procedures involving the care and use of animals conformed to local institutional guidelines and complied with national and international guidelines for the use of animals in biomedical research. *P. berghei* (NK65) was donated by the Immunology Department of the NMIMR, Ghana. The parasites in (infected) rat erythrocytes at a concentration of 1 x 10^5 were suspended in sodium citrate and stored in liquid nitrogen.

Experimental protocol

The 24 rats were randomly assigned by lottery to four (4) groups of six (6) animals in four cages. Once weekly, before and during the experiment, the body weights of rats were recorded. Regardless of group, each rat in the study was inoculated with 0.2 mL of parasitised blood containing 1 x 10^5 *P. berghei* (NK 65) parasites per µl of blood. Rats in group 1 (G1) were given free access to and voluntarily drank 2% (weight/volume) aqueous NCP via feeding bottle ad libitum for 14 days. Rats in group 2 (G2) similarly had access to and freely drank 2% (weight/volume) aqueous NCP ad libitum for 14 days. G2 rats were additionally administered 0.6 ml of 20 mg/120 mg AL (Coartem, Novartis Pharma AG, Basel, Switzerland) by oral gavage once daily on days 4 and 5 post-inoculation. Rats in group 3 (G3) were not given NCP but water ad libitum for 14 days, and the same dose of AL was given on the same days as for G2 rats. To harmonise stress associated with oral gavage, G1 rats were also given NCP via this route besides their voluntary drinking on the days that the AL oral gavage was administered to G2 and G3. Rats in group 4 (G4) served as negative control and were inoculated with the same concentration and volume of parasites but were neither given NCP nor AL. Rats from each group were coded with picric acid on specific body parts for easy identification. The treatment lasted for 14 days, during which all rats were fed the same standard.

Preparation of 2%(w/v) unsweetened NCP

NCP was prepared as previously described [19] with 2 g of commercially obtained NCP (GoodFood®, KEL Kakawa Co. Ltd. Ghana, batch no. DA1402A) dissolved in 100 ml of freshly boiled tap water. The mixture was stirred with appropriate vigour until it began to froth, indicating a uniform suspension. It was then cooled under running tap water to the ambient temperature of 28ºC. Fresh NCP suspension was prepared daily throughout the duration of the experiment, and G1 and G2 rats drank volitionally via water bottles for seven days before inoculation of parasitised RBC and 14 days post-inoculation. Administration of 0.5 mL – 0.8 mL NCP by oral gavage (per body weight of each rat) was done to equalise the stress to rats given AL by gavage and was given on the same days as AL administration.

Inoculation procedures and parasite counting

Cryopreserved parasites were taken through routine procedures to prepare an inoculum in a complete parasite medium (CPM) (Gibco, USA). After inoculation of the stock (donor) with parasites, a series of passages were run in subsequent donor rats in order to establish infection. The establishment of infection was confirmed by examination of thick and thin blood films prepared from the tail veins of

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the rats from which parasite density and percentage of parasite were calculated. Parasite density per µl of blood was determined by counting parasites against the total WBCs (~200 WBCs) counted in Giemsa-stained thick blood films, and the figure was multiplied by 8,000 (the standard WBC count per µl of blood). The calculation was done with the following equation: Parasite density = (number of parasites counted x 8000 WBCs) ÷ 200 WBCs counted. Parasitaemia was monitored every two days post-inoculation by Giemsa-stained thin blood films from tail veins, which were expressed as a percentage. Between 500 and 1000 RBCs were counted per slide with a mechanical hand tally counter (H-104, USA), and percentage parasitaemia was calculated as follows: Parasite (%) = (number of infected RBCs ÷ total number of RBCs counted) × 100.

After the desired parasite density and percentage of parasite was achieved (≥ 45%), a hypodermic needle containing 0.2 ml of trisodium citrate was used to draw blood directly from the rat by cardiac puncture using the xiphoid process as a guide. The blood was then put into Eppendorf tubes (Reagiergefab, Sarstedt Aktiengesellschaft and Co., Germany) containing 1.5 ml of normal saline. The diluted blood was transferred into a 15 ml falcon tube (Rohrchen Greiner bio-one, Germany) containing 2.0 ml of tri-sodium citrate to prevent clotting while inoculation was done. The rats were individually inoculated intraperitoneally (i.p.) with 0.2 ml of the diluted parasitised blood containing 1 x 10^5 *Plasmodium berghei* (NK65) parasites per µl of blood.

Artemether lumefantrine (AL) administration

A 0.6 mL of 20 mg/120 mg dispersible AL (Coartem, Novartis Pharma AG, Basel, Switzerland) purchased from a Licenced Chemist was administered via oral gavage once each morning to the rats in G2 and G3 groups on the 4th and 5th days after blood films have confirmed the presence parasites on day three post-inoculation.

Preparation of rats for liver harvesting and systematic uniform random sampling (SURS)

All animals were sacrificed on day 14 post-inoculation. Each animal was euthanised by diethyl ether (AVONCHEM, Wellington House, Waterloo St. West Macclesfield, Cheshire, UK) inhalation in an anaesthesia jar followed by perfusion fixation. Pain reflex tests (Rat Hands-on Laboratory, University of Washington) (Animal Use Training Sessions, 2021) were performed to assess the anaesthetic depth of each rat before the commencement of perfusion. Perfusion was performed intracardially via gravity by canulating the left ventricle with a hypodermic needle (23 gauge) attached to a blood-given set. The right atrium was punctured to allow effluent flow. Perfusion was started with normal saline until the liver turned pale, followed by a fresh fixative (2% formaldehyde and 2% glutaraldehyde buffered at pH 7.4 with 0.1M cacodylate). Adequacy of perfusion was determined if the liver was firm when touched with a pair of forceps. Following perfusion-fixation, the liver of each rat was excised in whole from the animal and separated into the right, left, median, and caudate lobes. Using a disposable microtome blade, each lobe was sliced at a thickness of 1.0 mm^3. Seven (7) liver slices were obtained from each of the right, left, and median lobes, whilst the caudate lobe yielded five (5) slices because it was the smallest of all the lobes. Representative samples were systematically selected for each rat liver by picking every 2nd, 4th, and 6th slice from the larger lobes and the 2nd and 4th slice from the smaller caudate lobe (Figure 1).

Processing for transmission electron microscopy

The systematically sampled slices of liver were taken through routine TEM tissue processing protocol of post-fixation in 3% cacodylate buffered glutaraldehyde for 3 hours, followed by washing in two 10-minute changes of 3% cacodylate buffer. Each liver slice was further cut into 10 – 15 mesh-like thin slices with a razor in a petri dish and further fixed in 2% glutaraldehyde for 1 hour. Three (3) out of each group of 10 – 15 mesh-like slices were randomly selected to represent each lobe. The slices were then dehydrated in graded alcohols, cleared with propylene oxide, and subsequently embedded in epoxy resin (Chiyoda Junyaku Inc.; Japan and LAAD Research Industries Inc.; USA). Ultrathin sections (70 nm) were cut with a Leica Ultramicrotome (Leica Company, Austria), mounted on a copper grid, stained with uranyl acetate, and observed in a TEM (JEOL JEM – 1010, JEOL LTD, Japan). A design-based stereological procedure was applied to transmission electron micrographs of the systematically sampled liver tissues. The photo tool of the electron microscope (JEOL JEM-1010, JEOL LTD, Japan) was used to systematically randomise the generation of micrographs of rat livers onto a negative film, which was developed using the COPINAL microfilm developer (Fuji Photo Film Company LTD, Tokyo, Japan). The developed films were later scanned with a special negative film scanner (Prime Film 7200, Pacific Image Electronics Co., LTD, Taiwan) together with another negative film scanner application (HELMUT film scanner, dk.codeunited.helmut.apk).

Hepatic ultra-structural variables (Kupffer cells, hepatic microvilli, sinusoidal diameter, and endothelial wall thickness) as indicators of liver injury

A design-based stereological system, i.e., the test system for grid-point counting (25) (Figure. 2), was used to estimate the volume density (Vv) of Kupffer cells, whilst the test frame for counting profiles (20) (Figure 3) was employed to count microvilli in the perisinusoidal spaces (of Disse). The 10 mm^2 counting frames with a test area of 58 mm x 80 mm and a total test point (Pt) of 4,640 were computer generated by Adobe Photoshop CS6 extended version and were superimposed on the micrographs. The number of points hitting the Kupffer cells was recorded as partial points (Pp). The ratio of Pp and Pt was expressed in percentage to give the volume density (Vv) of Kupffer cells. The mathematical expression of the volume density of Kupffer cells is described by the equation: Vv = Pp / Pt where: Vv = volume density, Pp = partial points and Pt = total test points.

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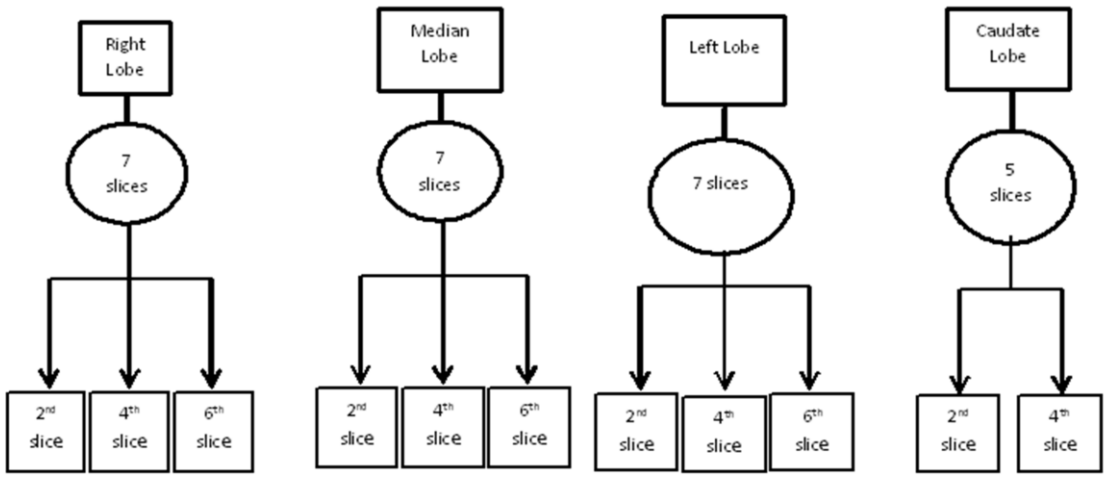

Figure 1. A chart illustrating how a rigorous systematic uniform random sampling (SURS) of liver lobes was performed in this study

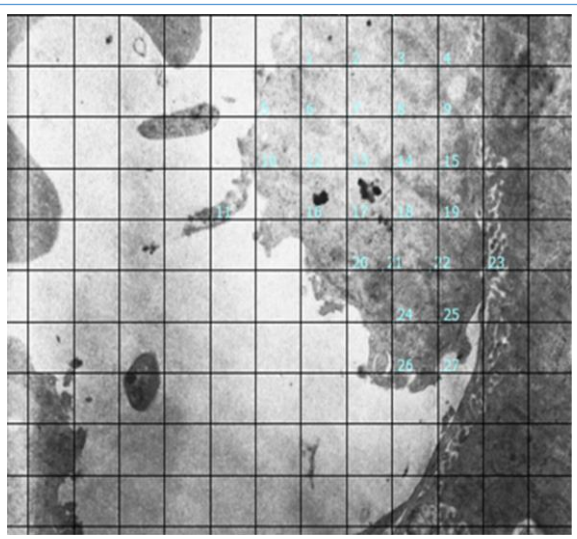

Figure 2. A transmission electron micrograph of rat liver superimposed with a lattice test grid. The numbered points (in light blue) are hitting a Kupffer cell. The stain is uranyl acetate. The bar represents 1µm.

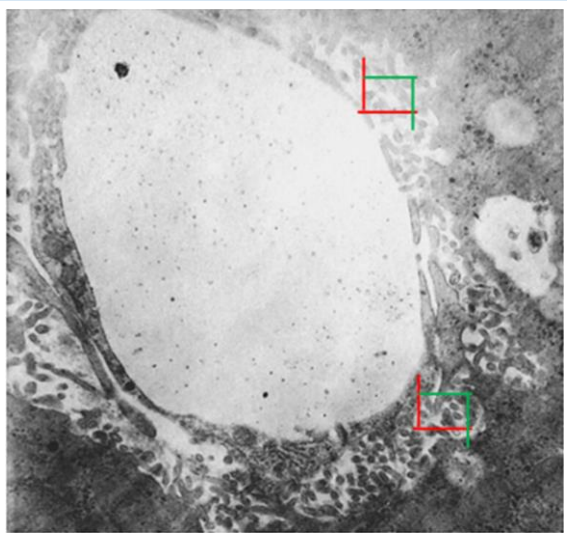

Figure 3. A transmission electron micrograph of a rat liver superimposed with a test frame for counting profiles. Hepatic microvilli are seen as extensions or profiles within the perisinusoidal spaces (of Disse) where two test frames are positioned. Stain: uranyl acetate. Bar represents 1µm.

The test frame for counting the peri-sinusoidal microvilli had an accepted line and a forbidden line. Microvilli, which hit the forbidden lines, were excluded from the counts. Counting was done by clicking the points of interest (i.e., microvilli) with the left button of the computer mouse, and the total counts were automatically calculated using the Photoshop counting tool. The Photoshop ruler tool was used to measure the short axes of sinusoidal diameters and endothelial wall thickness.

Biochemical assays

Between 10 ml to 15 ml of blood was collected transcardially from each rat using a 20 ml hypodermic syringe attached with a 21-gauge needle (BDH, England) after anaesthesia but before perfusion fixation. The blood

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was put in serum separator tubes and centrifuged at 10,000 rpm for 10 minutes. Activities of serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were assayed with an automated biochemical analyser (Flexor Lab E, VITA Scientific, Netherlands) at NMIMR, Legon Ghana.

Superoxide dismutase (SOD) and Glutathione (GSH)

SOD was measured using a diagnostic kit from Cayman Chemicals, USA. A volume of 10 ml of blood was collected by cardiac puncture with a 21-gauge hypodermic needle without any anticoagulants for the SOD and GSH analysis. The blood was allowed to clot for 30 minutes at 25 ºC and centrifuged at 2,000 rpm for 15 minutes at 4ºC. Serum was diluted at a ratio of 1:5 with sample buffer before assaying for SOD using the manufacturer's standard assay procedure (Cayman Chemicals, USA). GSH level was measured using a diagnostic kit from Cayman Chemicals, USA. Five (5) ml of the blood collected by cardiac puncture was deproteinated by dissolving in 5 g of metaphosphoric acid (MPA) (Sigma-Aldrich 239275) in 50 mL water. Subsequently, an equal volume (5 ml) of the MPA reagent was added to the sample and mixed by vortexing, and the mixture was allowed to stand at room temperature for 5 minutes and centrifuged at greater than 2,000 g for 2 minutes. The supernatant was carefully collected without disturbing the precipitate. A 4 M solution of triethanolamine (TEAM) was prepared in water by mixing 531 µl of triethanolamine with 469 µl of distilled water. Fifty (50) µl of TEAM was added per ml of the supernatant and vortexed immediately. All dilutions necessary at this stage were done with *N-morpholinoethanesulphonic* acid (MES) Buffer and assayed for total GSH by following the standard assay procedure provided by the manufacturer.

Statistical analysis

GraphPad Prism version 7.0 was used to analyse the data obtained from the study. After Bartlett's test for equal variance was done, a one-way analysis of variance (ANOVA) and Kruskal-Wallis tests were performed, followed by Bonferroni and Dunn's post hoc tests, respectively, to distinguish group(s) difference(s). All data were expressed as mean ± standard deviation (SD); P values < 0.05 were considered significant.

RESULTS

Two rats from G1 and a rat each from G2, G3 and G4 died during the study. The cause of death was not determined owing to the unavailability of a veterinary pathologist. Blood from the tail vein was used to prepare thin films for parasite counting. Parasitaemia was monitored from days 4 - 8 after animals were inoculated with parasites, as presented in Figure 4. ANOVA yielded a significant difference ($p < 0.0001$), and post hoc Bonferroni multiple comparison tests also showed significant differences between the groups (Table 1).

Hepatic sinusoidal diameters

A bar chart of the mean capillary sinusoidal diameters (µm) is presented in Figure 5(a). The values obtained were G1: 80.76 µm (SD 31.63), G2: 85.75 µm (SD 34.05), G3: 105.7 µm (SD 36.67), and G4: 118.7 µm (SD 76.19). Analysis of variance yielded a significant difference ($p < 0.0001$). Post hoc analysis confirmed the significant differences between the groups, except between G1 and G2 and G3 and G4. (Table 2).

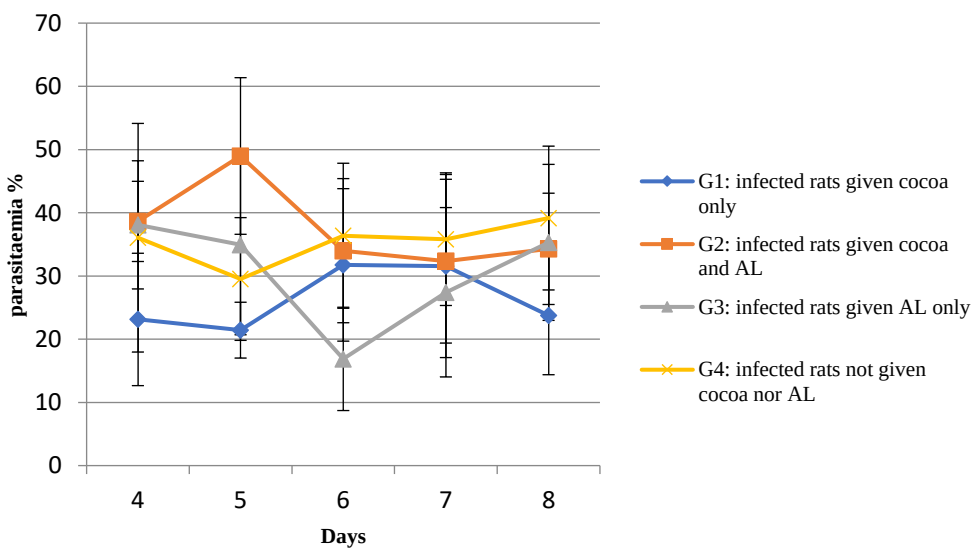

Figure 4. A line plot of trend of parasitaemia of parasitized RBCs in the infected rat groups for the from days 4 tofirst 8 days after parasite inoculation

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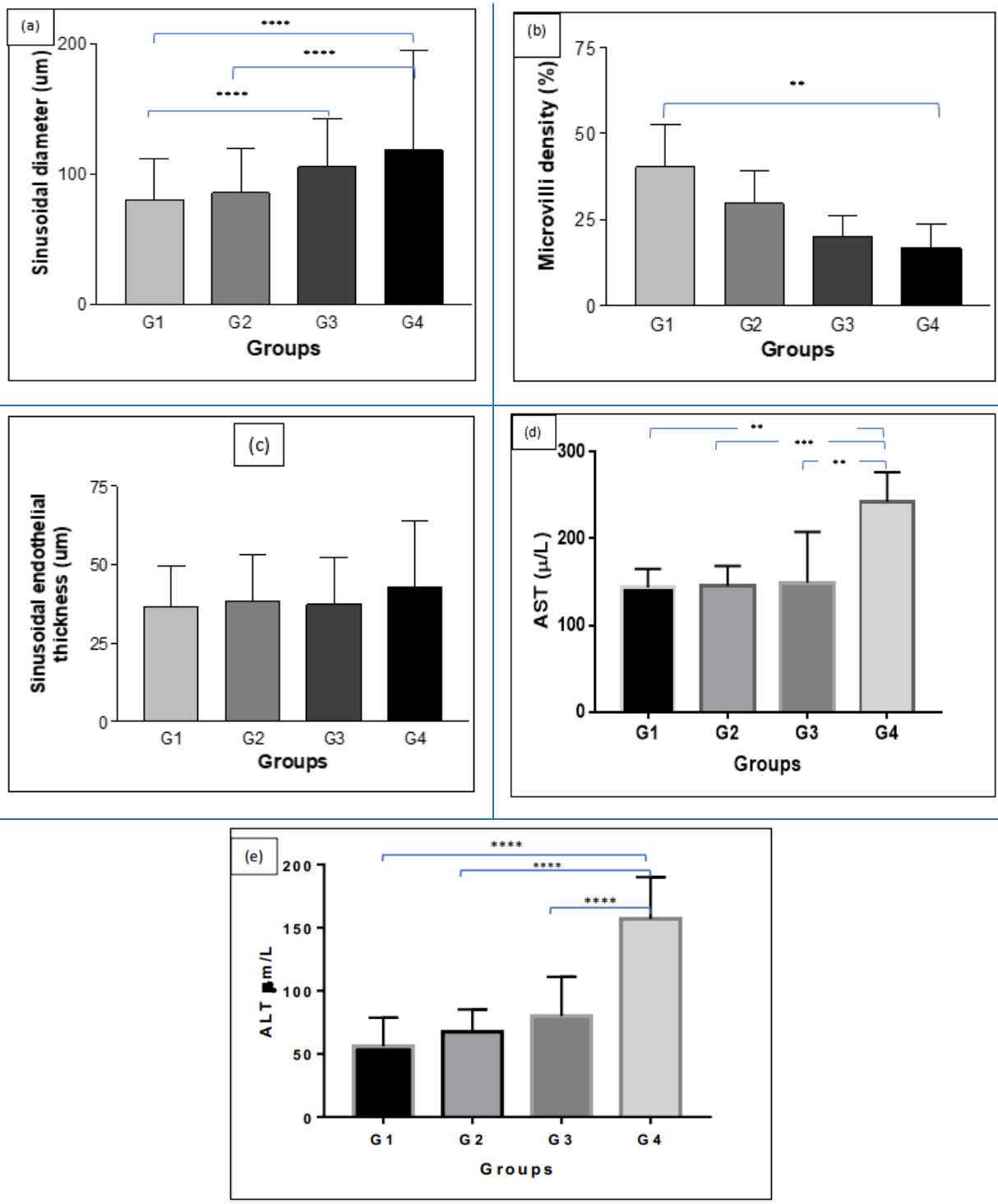

Figure 5 (a – e). Bar charts showing
(a) hepatic sinusoidal diameters (µm) in rats.
(b) Hepatic microvilli density (%) (within perisinusoidal spaces).
(c) Hepatic sinusoidal endothelial thickness (µm).
(d) Levels of AST (U/L) activity in rats after plasmodial inoculation.
(e) Levels of ALT (U/L) activity in rats after plasmodial inoculation.
Key: G1: infected rats given cocoa only, G2: infected rats given cocoa and AL, G3: infected rats given AL only, G4: infected rats neither given cocoa nor AL. Error bars represent standard deviation (SD). ** ($p < 0.005$); *** ($p < 0.001$); **** ($p < 0.0001$)

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Table 1. Bonferroni's multiple comparison tests of percentage parasitaemia among the rat groups

Groups	T	95% CI	P - value
G1 D4 vs. G3 D4*	3.703	-29.81 to -0.1059	0.0452
G1 D5 vs. G2 D5***	6.417	-43.33 to -11.76	<0.0001
G2 D5 vs. G4 D5***	5.892	7.314 to 31.59	<0.0001
G2 D5 vs. G3 D5**	4.167	1.651 to 26.45	0.007
G1 D6 vs. G3 D6**	4.519	2.771 to 26.97	0.0015
G2 D6 vs. G3 D6***	5.74	6.151 to 28.08	<0.0001
G4 D6 vs. G3 D6***	6.782	8.915 to 30.03	<0.0001

Table 2. Synopsis of Bonferroni's post hoc comparisons of sinusoidal diameters (µm) of the liver in the rat groups.

Groups	Mean Diff.	t	P - value	95% CI
G1 vs G2	-4.988	1.019	p > 0.05	-17.94 to 7.964
G1 vs G3	-24.93	5.435	p < 0.001	-37.08 to -12.79
G1 vs G4	-37.89	7.38	p < 0.001	-51.47 to -24.30
G2 vs G3	-19.95	3.364	p < 0.01	-35.64 to -4.253
G2 vs G4	-32.9	5.173	p < 0.001	-49.73 to -16.06
G3 vs G4	-12.95	2.114	p > 0.05	-29.17 to 3.267

Density of hepatic microvilli within perisinusoidal space

The mean volume density of apical hepatocyte projections into perisinusoidal spaces in the liver of rats was G1: 40.25 % (SD 12.55), G2: 29.67% (SD 9.61), G3: 20.00 % (SD 6.0) and G4: 16.57% (SD 6.95) Figure 5(b). A one-way ANOVA gave a significant value, but Bonferroni's post hoc tests showed a significant difference only between G1 and G4 (Mean difference 23.68, $t = 4.27$, $p < 0.01$, 95% CI = 6.44 to 40.92).

Thickness of sinusoidal endothelium

A Bartlett's test for equal variance indicated that data on sinusoidal endothelial thickness failed the normality test. Hence, a non-parametric test of ranks was used to compare values from the four groups of rats. Figure 5(c) shows a bar chart showing the mean thickness of the endothelium of sinusoids. The Kruskal-Wallis test for the medians of sinusoidal endothelial thickness (in µm) yielded a significant value ($p < 0.05$). Dunn's post hoc test produced only two significant differences. (i) Between G1 and G4 (difference in rank sum = -19.88; $p < 0.05$), and (ii) Between G3 and G4 (difference in rank sum = -20.89; $p < 0.05$).

Volume density of Kupffer cells

The respective median volume densities of Kupffer cells were 0.55%, 0.70%, 0.80%, and 1.00 % for G1, G2, G3, and G4 rats. The percentages of mean volume densities of Kupffer cells in the liver of the animals were G1 (0.55, SD 0.35), G2 (0.67, SD 0.58), G3 (0.83, SD 0.15), and G4 (0.97, SD 0.15). The values were not significantly different among the groups (F Statistic: 2.663; Kruskal Wallis value: 5.829; both with $p > 0.05$).

Serum GSH concentration and levels of SOD activity

The mean serum GSH concentrations (µM) measured were 1.83 (SD 0.28), 1.79 (SD 0.12), 1.73 (SD 0.16), and 1.64 (SD 0.24) for G1, G2, G3, and G4 rats, respectively. The mean serum SOD activity assayed in the rats was as follows. G1 (1.23, SD 0.42); G2 (1.26. SD 0.43); G3 (1.22, SD 0.41), and G4 (1.12, SD 0.41). One-way ANOVA on the mean serum GSH concentration (F value 0.7) and SOD activity (F value 0.09) did not yield significant differences ($p > 0.05$).

Biochemical markers of liver function

Serum AST values obtained from the rats at the termination of treatment are presented in Figure 5(d). A one-way ANOVA on serum AST showed significant differences between the four groups ($p < 0.05$). Bonferroni's post hoc tests confirmed statistical differences between the following treatment groups. G1 and G4 (mean difference = -99.03; $t = 3.94$; $p = 0.004$; 95% CI = -171.9 to -26.130, G2 and G4 (mean difference = -97.23; $t = 4.53$; $p = 0.001$; 95% CI = -159.4 to -35.06), as well as G3 and G4 (mean difference = -93.33; $t = 4.49$; $p = 0.001$; 95% CI = -153.5 to -33.13). However, no significant differences in serum AST existed between G1 and G2, G1 and G3, or G2 and G3. Serum ALT for the four rat groups is presented in Figure 5(e). A one-way ANOVA and Bonferroni's post hoc comparisons of serum ALT values in the rats produced statistics similar to those obtained for AST analysis. No significant differences were found between ALT values for rats in G1 vs. G2, G1 vs. G3, or G2 vs. G3. With a mean difference of 101.10, a t-value of 6.92 at $p < 0.0001$, and a 95% confidence interval of -142.7 to -59.53, the ALT was significantly lower in G1 compared to G4 rats. Comparably, ALT was lower in G2

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than in G4 rats (mean difference = -89.50; t = 6.92; p < 0.0001; 95% CI = -126.3 to -52.65). Group 3 rats had statistically depressed ALT levels compared to Group 4 rats (mean difference = -77.09; t = 5.67; p < 0.0001; 95% CI = -115.8 to -38.40).

DISCUSSION

In a previous study [19], we demonstrated at the light microscope level that hepatic damage was attenuated in mice infected with *P. berghei* when given free 24-hour access to 2% NCP. The present study extended the previous by comparing oral gavage administration of a therapeutic dose of AL with voluntary ingestion of 2% NCP and assessing morphological variables of hepatic damage at the ultrastructural level. Notably, NCP administration commenced seven days before plasmodium infection and continued throughout the 14 days of the experiment. The afforded hepatoprotection by NCP administration in the present study, therefore, reinforces its prophylactic efficacy [3]. To facilitate the explanation of liver tissue damage, percentage parasitemia was assessed daily from the 4th to the 8th day after animals were infected but was curtailed for the last six days of the study to forestall possible complications of results by blood loss through the daily sampling. The results of this study suggest that NCP conferred hepatoprotection not because of reduced parasitaemia since, by day 8, there was no difference in parasite load among the rat groups (Figure 4). It is apparent that whereas AL alone (G3 rats) significantly depressed parasitaemia on day 6, it increased steadily by day 8, possibly as a result of the waning of the efficacy of AL after the last shot on day five post-inoculation. AL combination with NCP did not show any significant difference since parasite load in G2 rats was not different from that of G1.

The parasite load in the untreated group (G4) was low on day five but then shot up steadily for the subsequent days up to day eight because there was no intervention (Figure 4). It is noteworthy, moreover, that in all other variables assessed in the present study, co-administration of AL/NCP (G2) consistently produced worse statistical values than NCP only (G1) but better results (albeit not always significant) than control (G4) and AL alone (G3). This finding is interpreted to mean that prophylactic use of NCP was better for preventing hepatic tissue damage in plasmodial infection. Given the mutually reinforcing roles of oxidative stress and inflammation in malaria pathophysiology [8], serum GSH and SOD, as well as AST and ALT, were assessed at the end of the experiment. AST and ALT are liver enzymes that reveal tissue damage and are elevated in various disease conditions, including severe/complicated [14] and uncomplicated malaria [26,27]. These liver enzymes also directly correlate with the inflammatory markers C-reactive protein (C-RP) [28,29]. The trends of serum antioxidants (GSH and SOD) measured in the present study were G1>G2>G3>G4 and G2>G1>G3>G4, respectively. It is apparent that the 14-day duration of this study could have been too short to elicit significant differences, as other studies recorded high antioxidant capacity when cocoa was added to the diet of humans and rats [30-35]. It is worth mentioning that other researchers in our laboratory who experimented over four weeks or longer registered significant serum antioxidant levels in animals given NCP [36,37].

It is interesting that in the present study, the trends of serum AST and ALT were G1<G2<G3<G4. The marginally higher levels of these enzymes in animals given AL-only may be explicable by the report that treatment with AL causes a harmless self-limiting rise in liver enzymes [38]. However, statistically lower AST and ALT in other rat groups compared with G4 rats suggest that treatment of rats with NCP only, NCP & AL, and AL-only did reduce inflammatory pressure exerted by plasmodium parasitaemia. Elevated AST levels reflect liver damage [39], and they are the most sensitive markers employed in the diagnosis of hepatic damage because they are cytoplasmic enzymes released into circulation after cellular damage [40]. Since plasma clearance of AST is modulated by the activity of sinusoidal liver cells, during progressive fibrosis and cirrhosis, the functions of these cells are progressively impaired, resulting in a relative increase in AST levels [41]. The present study focused on four ultrastructural indicators of liver tissue damage, namely, increased sinusoidal diameter, decreased density of microvilli in perisinusoidal space (of Disse), increased sinusoidal endothelial thickness, and increased volume density of Kupfer cells. According to these criteria, our findings were in order of mitigated hepatic damage: G1>G2>G3>G4. Animals given NCP only (G1) consistently had significantly less damaged liver than control (G4) and G3 rats according to sinusoidal diameter. Whereas rats given NCP & AL (G2) had statistically less damaged liver tissue than control (G4) and AL-only treated rats (G3) with respect to sinusoidal diameter, rats given only AL (G3) had less damaged liver than control (G4) based on sinusoidal endothelial thickness. Moreover, better attenuation of inflammation arising from the malaria infection by cocoa flavonoids [42,43], as evidenced by lower serum AST/ALT values discussed above, suggests better overall liver protection.

No differences were found in this study in terms of the volume density of Kupffer cells, which may have resulted from limitations of the stereological method used or the fact that parasitaemia did not reach the severe stage [24] that is characterised by increased numbers of these phagocytic cells. A noteworthy limitation of this study is our inability to determine the reason for the animal deaths recorded in all groups.

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Conclusion

Evidence has been produced to show that damage to the ultrastructural liver morphology in murine malaria was significantly mitigated by daily ingestion of NCP compared with AL treatment with respect to hepatic sinusoidal endothelial thickness and density of hepatic microvilli despite parasitaemia being comparable to untreated control rats. Correspondingly, the relatively minimised sinusoidal distension buttressed by lower serum AST/ALT but not GSH/SOD with NCP administration provokes the thesis that the anti-inflammatory activity of cocoa flavonoids may account for observed better hepatoprotection in this study.

DECLARATIONS

Ethical consideration

This study was independently approved by the Committee on Research, Publications and Ethics of the Kwame Nkrumah University of Science and Technology, School of Medical Sciences; and the Institutional Animal Care and Use Committee (IACUC) of the Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra.

Consent to publish

All authors agreed on the content of the final paper.

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Competing Interest

The authors declare no conflict of interest for this paper

Author contributions

EA conceived the idea which was his Ph.D project, designed the manuscript, analysed and interpreted the data, and approved it for publication. FKA contributed to the conception and design, reviewed the draft of this manuscript, and approved the final version for publication. He was the lead supervisor of the Ph.D. project. PA contributed to the design, advised on animal experimentation and statistical analysis, and approved the final version for publication.

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Availability of data

Data is available upon request to the corresponding author.

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Assessing gastric viability of probiotics: real testing in real human gastric fluid

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Abstract

Background: It is believed that the harsh conditions of the upper gastrointestinal tract, such as gastric fluid acidity, may affect the viability of ingested probiotics. Thus far, this notion has been verified in *vitro* by viability testing in simulated gastric fluid.

Objective: In this study, the survival of 8 probiotic bacteria was investigated in real human gastric fluid to determine the response of the bacteria in the actual biological medium.

Methods: Gastric tolerance of the different probiotic bacteria was determined by inoculation of the bacteria in human gastric fluid, sampling at 30 min, 60 min, 120 min, 180 min, serial dilution and spread plating. Tolerance was also determined in traditionally simulated fluids at pH of 2.2 ± 0.1 and 2.8 ± 0.1, mimicking the pH of the human gastric fluid.

Results: All the probiotic bacteria tested except for one strain, which showed less than 1 log CFU/mL loss in viability in the two fluids, were susceptible to the gastric fluids. The results showed significant ($p < 0.05$) strain-specific differences in the sensitivities of the bacteria in the gastric fluids. Some species were more sensitive to the real human gastric fluid than the simulated fluid. However, overall, the simulated gastric fluid did not significantly differ ($p > 0.05$) and hence provided a comparable environment to the actual human fluid at a similar pH.

Conclusion: More than 80% of the tested probiotic strains were susceptible to real human gastric fluids. The results demonstrated strain differences in the susceptibility of different probiotic bacteria to gastric fluid. Also noteworthy are the differences in the behaviour of some of the probiotic bacteria in the real fluid against the simulated fluid. The result highlights the importance of using biorelevant test systems in viability assays.

Keywords: Probiotic; lactic acid bacteria; human gastric fluid; gastric tolerance

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INTRODUCTION

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [1,2]. Probiotics are usually members of the lactic acid bacteria and bifidobacteria. Probiotics are obtained by isolation; some are available commercially for inclusion in food or other matrices and/or formulated into a dosage form mainly for oral administration or other applications. The amount of viable probiotics needed to obtain a clinical effect is generally quoted as 10^6 - 10^8 CFU/mL in the lower gastrointestinal tract [3,4]. Probiotic products are often required to contain greater than 10^8 CFU/mL due to likely viability losses that may occur after ingestion. For instance, Health Canada and the Italian Ministry of Health require a minimum of 10^9 CFU/mL viable cells per serving for probiotics [1]. The Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO) established a guideline for the evaluation of probiotics in food. The guidelines require that tests for establishing the health benefits of probiotics should first involve the use of an appropriate *in vitro* study before undertaking in vivo investigations [2,5]. The *in vitro* tests are to predict their

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ability to exert a function since the health benefits are derived from the growth and activity in the human body. *In vitro*, tests such as acid and bile tolerance, antimicrobial production and adherence ability to human intestinal cells are required to be performed depending on the anticipated health benefit. *In vitro* acid and bile tolerance studies of newly isolated or commercial lactic acid bacteria are consequently routinely performed when establishing the health benefits of nearly all potential probiotics [6-10].

These *in vitro* acid and bile tolerance tests are to mimic the harsh conditions of the upper gastrointestinal tract, such as the acid of the stomach, the presence of bile salts and digestive enzymes, as well as factors affecting residence or transit, which may affect the viability of ingested bacteria. The resistance of the probiotics during the *in vitro* gastrointestinal tolerance predicts that the ingested probiotics will be able to reach the lower gut in quantity sufficient to produce the intended health benefits of probiotics, such as improved gut health and enhancement of the immune system [1,2]. However, routinely, these *in vitro* gastrointestinal tolerance tests are conducted using either buffer or sodium chloride solutions adjusted to pH 1.2 – 4.0 and higher pH with or without bile salts [7,9,11,12] to simulate the gastric fluid and intestinal fluid. Pepsin is sometimes added to the saline solution to make it more biorelevant [7]. Also, growth media, adjusted to similar pH, have been used to simulate gastric fluid [13]. The probiotics are incubated in the simulated fluids, sampled and enumerated periodically over an average of 3 hours [3]. Cells that maintain viability during the tolerance study are often favoured. Techniques such as microencapsulation and coating mechanisms such as enteric coating, Phloral®, coating and many others are investigated to improve the chance of survival of those with poor tolerance but with other suitable properties [14-19].

For instance, a 6 log CFU/mL reduction in viable cells was obtained when *L. plantarum* was exposed for 120 min to simulated gastric fluid, whereas microencapsulated cells decreased by 2.9 CFU/mL in the simulated fluid under similar conditions. This indicated better tolerance and enhanced survival of the microencapsulated microorganism than free probiotic bacteria [19]. Gastric fluid is composed of swallowed saliva, hydrochloric acid, bicarbonate, bile salts, pepsin, phospholipids, lipids, lipase, potassium, sodium, chloride and calcium a with characteristic pH, buffer capacity, osmolality, surface tension and viscosity which could have a major influence on the survival capacity of probiotics [20,21]. The simulated fluids used routinely in the *in vitro* tests often lack this complexity. They only mimic the salt concentration of the gastric fluid through the addition of sodium chloride and the hydrogen ion concentration by the addition of HCl. This work aimed at exploring the survival of some lactic acid strains with established probiotic potential in real human gastric fluid. This was to determine the response of the cells in the real biological fluid and to ascertain if the routinely used simulated fluid was representative of the real fluid in terms of the sensitivities of the bacteria to the fluid. To the best of our knowledge, no prior study appears to have examined this topic.

MATERIALS AND METHODS

Microbiological media and chemicals

De man rogosa sharpe (MRS) broth (2276280) and agar (2465177) were from Oxoid Ltd, Basingstoke, UK. L-cysteine hydrochloride (22063/1a) was from Surechem Products Ltd., UK. HCl (37%) was purchased from Sigma-Aldrich, UK. NaCl and phosphate-buffered saline (PBS) tablets were from Fisher Scientific, USA. NaOH (10222/4) was obtained from Park Scientific, UK.

Probiotic strains

Eight (8) lactic acid strains were used for the study. Four (4) of the strains, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Enterococcus faecium*, which are component strains of the commercial product, Symprove™ with previously demonstrated probiotic properties were obtained from the manufacturer, Symprove™ Ltd, UK. Four local strains, which included *Lactobacillus fermentum* FSI3-D, *L. fermentum* FSI3-LBC, *L. plantarum* FSC3-LBC and *Lactobacillus salivarus* FSDI-D, were isolated from faecal samples in Ghana and previously demonstrated to have potential probiotic properties [9]. The strains were previously isolated on de Man Rogosa Sharpe (MRS) agar supplemented with 0.05% w/v L-cysteine hydrochloride and 0.002% w/v of bromophenol blue and incubated anaerobically at 37 ºC for 48 hours [9]. Pure colonies obtained were maintained in MRS broth or agar supplemented with 0.05% L-cysteine hydrochloride. Identification of isolates was done with Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) using the MALDI Biotyper® and validated with 16S rRNA gene sequence analysis [9].

Collection of human gastric fluids

Seven volunteers were recruited for the study. The volunteers were Ghanaians of normal body weight who were between the ages of 20 - 40 years. The volunteers had or were enrolled in tertiary-level education. Only samples from volunteers who did not have any upper gastrointestinal disease that was discovered during the examination were used for the study. Samples contaminated with intestinal content were also discarded. Gastric aspirates were obtained from the seven (7) healthy volunteers who had fasted for at least 12 hours prior to the procedure. The sample collection was carried out at the endoscopy unit of Korle Bu Teaching Hospital, Accra, using an upper gastrointestinal endoscopy. The inclusion criteria for the study were healthy adults who were 18 years and above without any chronic disease and who signed a consent form to participate after it had been explained to them. Persons with gastrointestinal disorders and on medications for acid blockade, as well as those on antibiotics, were excluded.

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On the day of the procedure, the volunteers were asked to sit and relax. They were briefed about the procedure, after which a 10% Xylocaine pump spray, a local anaesthetic, was sprayed into their throats, which they were asked to hold for a minute before swallowing. The volunteers were made to lie down on their left side and bite on a plastic mouthguard. An endoscopic tube was inserted through their mouth, down their throat, and into their stomach. Prior to insertion, 2% Xylocaine gel was applied to the tube for anaesthetic purposes. Gastric aspirates were collected by suction into a dry sterile trap inserted at the exhaust portion of the endoscopic tube during endoscopic examination. Collected aspirates, which measured between 15 mL and 40 mL, were stored in sterile 50 mL Falcon tubes, preserved with ice in a thermostat container, and transported over approximately 1 hour to the Microbiology laboratory at the School of Pharmacy, University of Ghana, Legon. They were examined, filtered with a 0.22 □m micropore filter, aliquoted into 1.0 mL portions into sterile cryovials, and stored at -80 ºC. Before use, they were thawed in a water bath with the temperature set at 40 ºC for 3 min and maintained at 37 ºC in an incubator.

Determination of pH and buffer capacity of gastric fluids

Simulated gastric fluid was prepared by dissolving 0.2 g of NaCl in 100 mL of purified water and adjusting pH to 2.2 ± 0.1 or 2.8 ± 0.1 with HCl. The pH and buffer capacity of the gastric fluids were determined using a calibrated pH meter (pHep®, HANNA, Instruments). The buffer capacities of the real and simulated gastric fluids were compared at the two different pHs [22]. The buffer capacities of the fluids were measured by adding NaOH standard solution (0.1 M) to 3 mL of each fluid and noting the volume.

Buffer capacities were determined using the equation:

$$\beta \text{ (mmol/l/}\Delta\text{pH)} = \Delta AB / \Delta pH$$

Where ΔAB is the small increment in mol/l of the amount of acid or base added to produce a pH change in the buffer. This equation can be rewritten as:

$$\beta \text{ (mmol/l/}\Delta\text{pH)} = \Delta AB/ \Delta pH = (M_a \times V_a)/ \Delta pH \text{ x } 1000/ V_b$$

Where M_a is the molarity of the acid, Va is the volume of acid in mL, V_b is the volume of buffer in mL, and ΔpH is the change in pH. The equation was multiplied by 1000 to express the volume in litres.

Gastric tolerances

Tolerance of the lactic acid strains to the gastric fluids was performed by inoculating 100 µL of a culture of each strain in 1 mL of respective gastric fluid. The cells were incubated at 37 ºC, and samples taken at 30 min, 60 min, 120 min, and 180 min serially diluted in PBS (pH 7.4) and spread-plated on MRS agar supplemented with 0.05% w/v L-cysteine hydrochloride for the determination of viable counts.

Statistical analysis

Experiments were performed in triplicates. Results of the gastric tolerance assay (viable cell count, log CFU/mL) were expressed as mean ± standard deviation. Statistical analysis was performed in Origin Pro Version 8.6 (Microcal Software Inc.). The significance of difference was evaluated with a t-test or analysis of variance (ANOVA). P values less than 0.05 were regarded as significant differences between means.

RESULTS

Two batches of gastric fluid from two of the volunteers were used for the study. The pH of the two batches of gastric fluid collected from the volunteers were 2.2 ± 0.1 and 2.8 ± 0.1. The buffer capacities of real human gastric fluid (HGF) and simulated gastric fluid (SGF) at pH 2.8 were 12 mmol/l/ΔpH and 8.08 mmol/l/ΔpH respectively. The buffer capacities of HGF and SGF at pH 2.2 were 4 mmol/l/ΔpH and 32.36 mmol/l/ΔpH, respectively. When the results for buffer capacities were compared to pH, a lower buffer capacity for increasing pH was noted for SGF and vice versa for HGF. The human gastric fluid (HGF) with pH 2.2 ± 0.1 was used for the tolerance assessment of the commercial strains, including *L. acidophilus, L. plantarum*, *L. rhamnosus* and *E. faecium*. The HGF with pH 2.8 ± 0.1 was used for the tolerance assessment of the locally isolated strains.

The results for the tolerance tests of the commercial strains are given in Figure 1. Significant differences in tolerance ($p < 0.05$) of the different strains were noted. Significant differences ($p < 0.05$) were observed between *L. plantarum* and *L. acidophilus; L. rhamnosus* and *L. acidophilus; E. faecium and L. rhamnosus* and *L. plantarum* and *E. faecium.* Both *L. acidophilus and E. faecium* did not show survival in human and simulated gastric fluids after 30 min. *Lactobacillus plantarum* maintained relative survival in both fluids for 120 min, although better survival was noted in SGF than in HGF, with reductions of 1.33 log CFU/mL and 2.28 log CFU/mL, respectively, after the test. *Lactobacillus rhamnosus* showed the strongest survival in the gastric fluids, relatively maintaining survival for the duration tested. It was, however, more sensitive in HGF than in SGF, showing about 2.31 log CFU/mL and 1.67 log CFU/mL reduction in viable cells, respectively, in the fluids after the test duration.

The results for the tolerance of the local strains in HGF are shown in Figure 2. Significant reductions in viable cells ($p < 0.05$) were observed. All strains lost viability by 60 min in HGF; only *L. salivarus* FSD1-D maintained survival in HGF with about 0.91 log CFU/mL reduction in viable cells, which was similarly noted in SGF. *Lactobacillus fermentum* FSI3-D and L. *plantarum* FSC3-LBC maintained some viable cells in SGF for the duration tested, showing less than 1 log CFU/mL and about 3 log CFU/mL reduction in viable cells, respectively, after 180 min. For all the studied strains, 50% maintained some viable cells in

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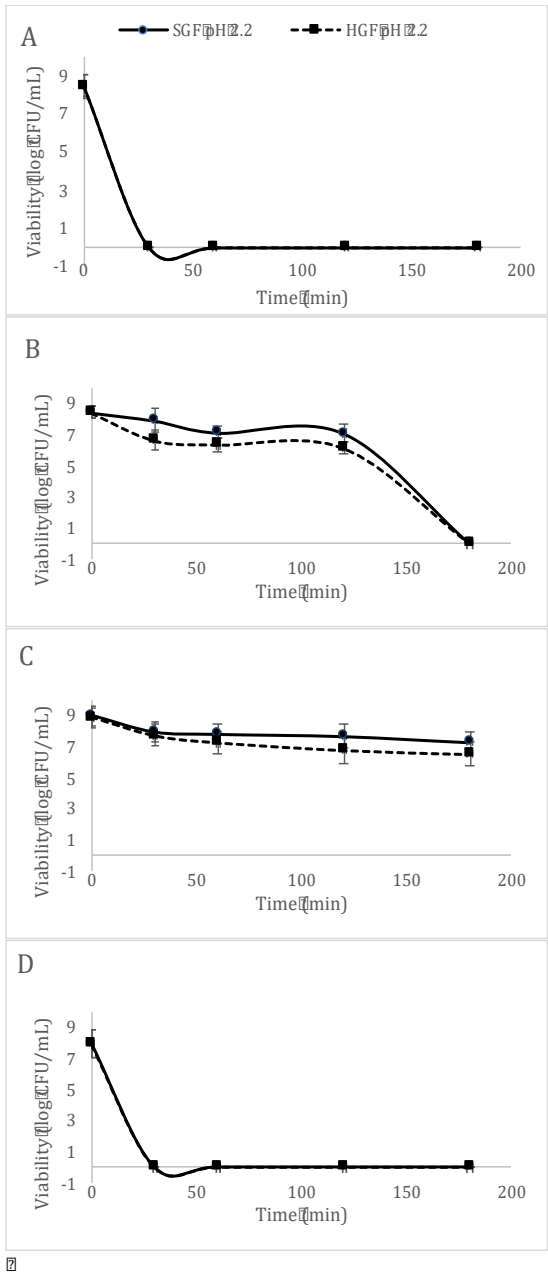

Figure 1. Viability (log CFU/mL) of commercial probiotic strains (A) *L. acidophilus*, (B) *L. plantarum*, (C) *L. rhamnosus*, (D) *E. faecium* in simulated gastric fluid (SGF) and human gastric aspirate (HGF) pH 2.2.

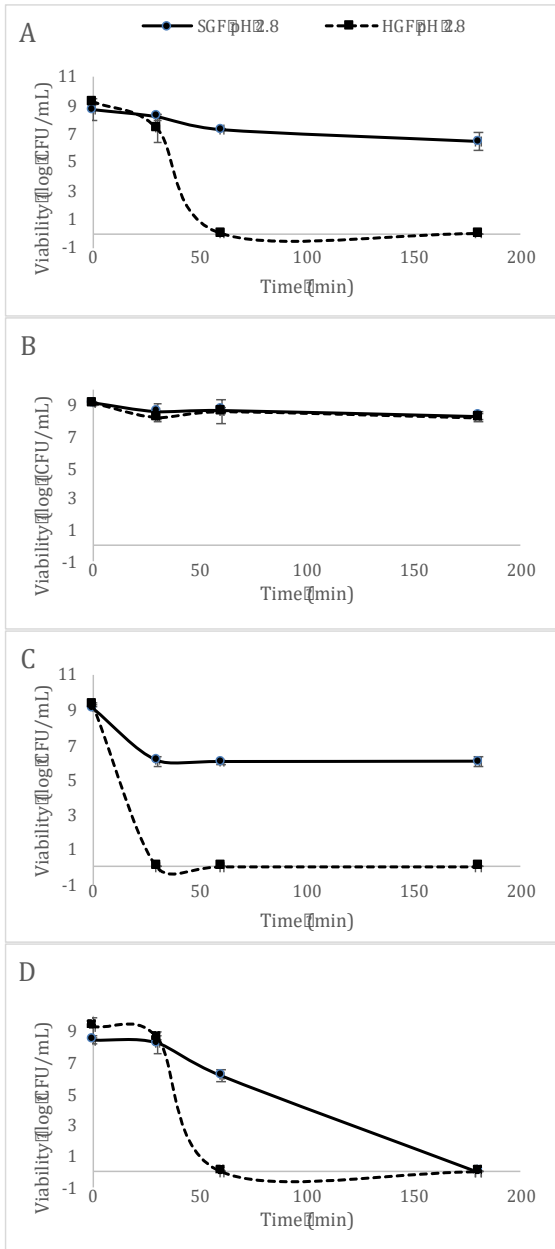

Figure 2. Viability (log CFU/mL) of isolated probiotic strains (A) *L. fermentum* FSI3-D, (B) *L. salivarus* FSD1-D, (C) *L. plantarum* FSC3-LBC, (D) *L. fermentum* FSI3-LBC in simulated gastric fluid (SGF) and human gastric aspirate (HGF) pH 2.8.

SGF, whereas 25% maintained relative viability in HGF for the duration tested.

DISCUSSION

The main objective of using simulated fluids is to mimic the *in vivo* behaviour of materials within the respective physiologic environment. The fluids' composition and their ability to replicate the physiological condition is, therefore, very critical. Considerable knowledge exists regarding the composition of the gastric fluid in both the fasted and fed states [20-24]. However, to date, the fluid is mostly simulated with an aqueous solution of sodium chloride, pH adjusted with hydrochloric acid. This study aimed to investigate the survival of four commercial lactic acid bacteria strains and four locally isolated strains in gastric

aspirate (from fasted volunteers) and simulated gastric fluid to determine if traditionally used fluid can accurately predict survival in the real fluid at a fasted state. The results indicate that overall, the viability of the tested probiotic strains in human aspirate was not significantly different ($p > 0.05$) from the viability in the simulated artificial fluid. Viability was, however, significantly different ($p < 0.05$) amongst strains. For the commercial strains, both *L. acidophilus* and *E. faecium* lost viability within the first 30 min of testing, whilst *L. plantarum* and *L. rhamnosus* maintained relative survival for more than 90 min.

Differences in survival among Lactobacilli species and strains in acidic conditions have been reported [25,26]. It is also known that probiotic species are generally less sensitive to pH above 3 [25] and have been demonstrated to show greater sensitivities at pH 2 [27]. *Lactobacillus rhamnosus* has demonstrated resistance to pH 2.5 for more than 4 hours [28] and has shown to be more resilient relative to *L. acidophilus* and *E. faecium* in a mixed environment [29]. For this study, both *L. rhamnosus* and *L. plantarum* demonstrated survival, although relatively lower survival was recorded in HGF than in SGF for 120 min. Whilst *L. rhamnosus* maintained some viable cells, *L. plantarum* lost viability after 120 min, showing that *L. rhamnosus* was more robust than the two. For the local isolates, except for *L. salivarus* FSD1-D, none of the tested strains survived beyond 60 min in HGF. The species demonstrated differences in viability in the fluids, as previously observed. A similar rate of loss in viability was noted for the two strains of *L. fermentum* in HGF. *Lactobacillus salivarus* FSD1-D was the more resistant strain. The buffer capacity, which is the resistance to change in pH, can be important to the survival of bacteria.

The buffer capacity of the gastric fluid is contributed by the physiological pH-regulating agents that are present in the stomach as well as any food and drink that has been ingested by a person. In the fasted state, the buffer capacity is mainly regulated by the concentration of hydrochloric acid, although a potential contribution of amylase, lipase, pepsin or other protein-based components to the buffer capacity of bulk gastric contents has been asserted [22]. A linear correlation between the buffer capacity and the hydrogen ion concentration of gastric aspirate has been reported [22]. Buffer capacity tends to decrease with increasing pH of gastric aspirates. This, however, does not concur with the aspirates in the present study, which can be explained by variability within the volunteers or sample treatment. The simulated fluid demonstrated a lower buffer capacity for increasing the pH of fluid, which is consistent with previous reports [8,22]. Although a significant difference in buffer capacity between the simulated fluid and human gastric aspirate at pH 2.2 was observed, this did not result in a greater sensitivity of the strains in the simulated fluid. The presence of other substances or properties of the human gastric fluid may have also contributed to the greater sensitivity of the cells. The results demonstrate that the rate of survival was species/strain dependent. The rate of killing was, however, not particularly dependent on pH.

Conclusion

Overall, the findings from the viability test suggest that the simulated gastric fluid provided an environment comparable to that of human gastric fluid at a similar pH. Whilst most strains showed similar trends in simulated and real fluid, this was strain-specific as significant differences were observed among some strains. This study demonstrates that the bactericidal action of gastric juice may be attributed to pH and possibly other components of the juice. It highlights the significance of using biorelevant fluids in viability assay and indicates the need to offer gastric protection to probiotics to maintain viable cells and obtain health benefits. This study has some limitations; it only considered eight (8) probiotic strains and one type of fluid used in simulating the gastric juice.

DECLARATIONS

Ethical consideration

Ethical approval was obtained from the institutional review board of Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Reference number NMIMR-IRB CPN 073/20-21. Informed consent was obtained from volunteers before participation.

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contribution

MFA participated in the conceptualisation, supervision, and analysis of the data for the manuscript. MFA and NAA-A participated in the Investigation and methodology. MFA, NAA-A and SG participated in the resources, writing, review and editing.

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Availability of data

Data for this work is available upon reasonable request from the corresponding author.

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Sustainable Development Goal 3.8 Universal Health Coverage from global perspectives: An analysis of the health insurance policies in Rwanda, Tanzania, South Africa, and Ghana

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Abstract

Background: The paper reviewed the policy strategies of four African states (Rwanda, Tanzania, South Africa, and Ghana) towards achieving Universal Health Coverage (UHC). It found that these four countries used national or community-based health insurance schemes as vehicles or the means to achieve UHC by 2030 in the context of the global agenda (the United Nations Sustainable Development Goals (UN SDGs).

Objective: The study aimed to specifically contribute to an interrogation of health insurance policy strategies in Africa.

Methods: It reviewed relevant literature on universal health coverage in selected regions like Europe, America, the Pacific, and Asia. It then added the materials to Sub-Saharan Africa. Data was obtained from secondary sources. Included criteria were the use of words such as United Nations Sustainable Development Goals SDGs), Universal Health Coverage (UHC), health, health insurance, health insurance scheme, and World Health Organization (WHO).

Results: The findings suggest that most African states have national or community-based health insurance schemes, and most of the health insurance schemes cover a good percentage of their population. However, most health insurance schemes cover less than half of the population. For the four cases, Rwanda and Ghana are excluded from the coverage of less than 50 percent of the population. Also, the four African states spent less than 10% of their GDP on health. These indicators suggest that the pathway towards achieving UHC in Africa by 2030 may still take some more years to be realised.

Conclusion: The study concludes that funding various health insurance schemes remains a challenge. Therefore, adequate funding by the African government for health and a positive attitude towards publicly funded health services are necessary to sustain African health insurance schemes or policies.

Keywords: SDG, universal health coverage, African states, Rwanda, Tanzania, South Africa, and Ghana.

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INTRODUCTION

This article addresses the question: Is Sub-Saharan Africa on the path to universal health coverage through national/community health insurance schemes? In response to this question, the paper engages in an extensive literature review in an attempt to answer the question. Also, there were document reviews of four Sub-Saharan African countries, namely Rwanda (Central), Tanzania (Eastern), South Africa (Southern) and Ghana (Western) were engaged. These countries were selected purposely to represent the four sub-regions of Africa, namely Central, Eastern, Southern, and Western Africa. These countries are also making efforts to improve universal health coverage (UHC). The article analyses the health insurance policies of the four Sub-Saharan African countries, their policy design factors, implementation structures (implementation process) and their implementation outputs or outcomes in the context of UHC. It specifically contributes to health

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insurance policy strategies in Africa. The United Nations Sustainable Development Goal (SDG) 3.8 focuses on good health and well-being (good health and wellness or well-being of the people of the world). The United Nations is interested in the health and well-being of all people in the world and urges member states to implement SDG 3 [1]. It is important to note that this study focuses on Goal 3, which is part of the broader goals and targets (17 goals and 169 targets) ratified by the United Nations (UN) member states in 2015 to be achieved by 2030. Member states of UN action are necessary, especially policymakers, to design their health policies and make their health systems robust to promote good health and well-being (Goal 3) through health education and health systems reforms, with the ultimate goal of making healthcare services accessible to all citizens or people [3].

The World Health Organization's (WHO) 1948 constitution defines health as the state of "complete physical, mental and social well-being and not merely the absence of disease or infirmity" [4]. Having the right state of mind is crucial, and it is not necessarily the absence of a disease/infirmity. This concept of health is used in this paper. Sustainable Development Goal 3 is one of the 17 goals set by UN member states to work with, and the hope is to achieve it by 2030. Goal 3 has nine targets for reducing the following: morbidity and mortality of vulnerable groups (target 1), preventable deaths of newborns and children under five years (target 2), to end communicable diseases-epidemics like AIDS, tuberculosis, malaria and others (target 3) and to reduce by one-third premature deaths from non-communicable diseases (target 4), risk factors like substance use, tobacco, harmful alcohol, (target 5), to reduce road traffic fatalities and injuries by half by 2020 (target 6), have access to universal sexual and reproductive health services (target 7), working towards achieving universal health coverage in terms of access to quality healthcare, affordability of healthcare, drugs/medicines and vaccines for all people by strengthening the health systems of countries across the globe (target 8), and finally, reduce the incidence of deaths and injuries from hazardous chemicals and forms of pollution - air, water, soil/environment and contamination (target 9) [5-6].

The timelines for all nine targets are very close. One of the targets (target 6) has passed (reducing road traffic fatalities and injuries by half by 2020). Time is not with UN member states to implement targets. The earlier, the better for states or countries, including those in Suh-Saharan Africa, to act right on goal 3 and the targets. The paper is interested in only one target: target 8 (universal health coverage), with emphasis on Sub-Saharan African states' efforts towards achieving target eight by 2030. In this regard, the paper engages in literature and document reviews of health insurance policy strategies of four countries, namely Rwanda, Tanzania, South Africa and Ghana, representing the four subregions of Africa (central, east, south and west respectively) on their pathways toward UHC.

MATERIALS AND METHODS

The study used largely secondary data sources from these diverse databases: Google Scholar, ResearchGate, Web of Science, and JSTOR. Also, other data used in the study were obtained from the National Health Insurance Authority website. In a few cases, the use of Google and other open search engines on universal health coverage, and states efforts towards UHC, searched and obtained various internet sources. Some of the keywords that the researcher used in the search for information or data gathering include: ‘universal health coverage’, ‘national health insurance scheme’, ‘community-based health insurance scheme’, ‘employee-based health insurance’, ‘SDG 3’, and ‘World Health Organization’. Moreover, other words including ‘challenges of UHC, ‘prospects of UHC’, and ‘politics of UHC were used to search for materials for the study. Based on these keywords, over 100 peer-reviewed journal materials, books, and a few internet sources of information were obtained. The next step was to search for those that related to the study’s objectives. Some 50 of the materials were indeed used. Some key themes emerged from the search which are presented in Table 1.

Table 1: Major themes from the secondary databases used in the study and their sources

Major themes	Key sources of information obtained from literature
Universal Health Coverage	• Ghebreyesus (2017) • World Bank (2022) • Craig et al (2022) • Kipo-Sunyehzi et al (2019) • Takura and Miura (2022) • Lagomarsino et (2012) • McKee et al (2012) • Kutzin (2013) • Michael et al (2020) • Ghanbari et al (2021)
Sustainable Development Goal 3 (SGD)	• Asi YM, Williams C. (2018) • Howden-Chapman et al (2017) • SDG (2022)
Prospects towards UHC	• Van Mah et al (2014) • Kimario, Muhanga, and Kayunze (2020)
Challenges towards UHC	• Unmeh CA. (2018) • Darrudi, Khoonsari Tajvar (2022)
Achieving UHC: a Political Choice (Politics of UHC)	• Ho et al (2022)
World Health Organization	• WHO (2005) • WHO (2022) • Kutzin et al (2017)
National Health Insurance Scheme	• Kipo-Sunyehai (2021) • Arhin (2013)
Community-based Health Insurance	• Melaka, Breen, and Binagwaho (2012) • Rwanda CBHI (2015) • Chinwa et al (2021) • Koch et al (2022)

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Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is widely accepted by scholars, and researchers in medicine and other scientific fields for several reasons including how the review of the literature was done, why it was done so and finally what was found in the review with specific reference to the PRISMA 2020 guidelines [52-54]. It is important to note this study approach did not fit exactly into the processes of systematic reviews, as the study had less adherence to PRISMA 2020 guidelines. Though some good efforts were made for strict adherence to the 27-item PRISMA checklist, with the four-phase flow diagram, admittedly the strict adherence was low in this study. As this study rather chose a synthesising approach targeting reports, journal articles, or studies which explicitly cover these headings and whose content covers UHC and SDG 3. Outside the two criteria constitute the excluded criteria used in the search for this study materials. Thus, these are the inclusive and exclusive criteria used. The selection was based on countries with national or community-based health Insurance schemes in each of the Sub-Regions of the African Union (AU) in Sub-Saharan Africa namely Southern Africa with South Africa, Central Africa with Rwanda, East African with Tanzania and West Africa with Ghana.

LITERATURE REVIEW

Historically, UHC was used in the German context as far back as 1883, when Germany introduced UHC to take care of its teeming young population. UHC became more popular when the World Health Assembly adopted it in 2005, and subsequently, the United Nations (UN) and its agencies like the World Health Organisation (WHO) pushed member states to embrace UHC and find innovative ways of financing it. Then, by 2010, the World Health Report emphasised health systems financing for states to build on toward achieving UHC. The WHO conceptualises UHC as access to healthcare services by all people of good quality when and where they need the healthcare services without financial barriers, limitations, or hardships. These healthcare services include access to health promotion, prevention, treatment, rehabilitation and palliative care, which are available to the people in their communities [7]. UHC is the means toward achieving the two core goals of the World Bank Group: to end extreme poverty in the world and increase equity and shared prosperity, a way to build on the human capital of countries [8]. UHC has moved away from being a health issue to be tackled by those in the health sector alone. It has gained global attention with a high level of political commitment by UN member states toward achieving UHC.

One of the political commitments is the member states' decision to carry out the Global Action Plan for Healthy Lives and Well-being (GAP) in September 2019 and the second international forum in January 2020. All these efforts aim to increase UHC's political commitment. Moreover, there have been efforts of G20 members to help developing countries achieve UHC since 2019, as well as many efforts across the globe on UHC. The World Bank and WHO urge all member states to increase spending on Primary Health Care (PHC) by at least 1% of their Gross Domestic Product (GDP) effectively from 2019 towards the 2030 period set for the global agenda for UN member states [8]. UHC is about free access to healthcare services or affordable healthcare services for all people in a country, irrespective of their socioeconomic status in society, which implies health financing to address extreme poverty. Some states or countries used general tax to provide UHC, other states used national health insurance schemes, and some states used community-based health insurance schemes as pathways to UHC. UHC is the best way out of out-of-pocket payments (cash payments) at the points of health service delivery, as UHC aims to reduce extreme poverty, reduce social inequity, and effectively improve good health and the general well-being of all the people/citizens [9-11].

UHC remains a highly contestable issue as there is no single definition or conceptualisation. In the Pacific regions, the Ministers of Health adopted a five-prone approach or areas/themes toward achieving UHC. These areas or themes include unifying action for UHC, integrated primary health care at the community level, building human resources for health, having access to reliable health information, and embracing digital health for the period 2015 - 2022. The Pacific region is very much concerned with the health of the 11.4 million population found in the hundreds of islands constituting the 22 Pacific Island Countries and Territories (PICTs) [12-14]. The Pacific countries and territories are working towards the realisation of UHC within United Nations Sustainable Goal 3.8 by 2030 to make healthcare services accessible to all people (as Healthy Islands' vision). Besides the conceptual issues or problematics, UHC politics exists across the globe. The political dimension seems to be overlooked or not factored much in the conceptualisation of UHC.

Universal Health Coverager is conceptualised as the strong political will or commitment at the highest level to implement a health reform or a health policy. Thus, UHC as a concept "involves the redistribution of resources across income groups, a political process that can rouse intense contestation between different groups" (15:2066). UHC entails states' commitment to the provision of physical infrastructure and human resource development, which all involve the political process and politicians' decisions. Where there is high political commitment, it is more likely to facilitate states' efforts toward UHC than in situations where there is little or no political will or commitment toward achieving UHC. The political choice may be influenced by the ideas, interests and political institutions of a state. These three factors can facilitate or inhibit governments' moves toward UHC. Thus, the pathway to achieving UHC is a political choice of governments or countries' political authorities to accept or reject UHC, as echoed by the Director General of WHO (Ghebreyesus) [16]. Even though moving toward UHC is a political choice, in reality, it goes beyond politics to include the

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roles/support of non-state actors and individuals as well as the availability of resources. This implies that for UHC to succeed, healthcare services must be publicly funded without these payments (out-of-pocket, cash and carry, extra medical billings for patients or service users). The recent global pandemic (COVID-19) showed that everyone, and not only the poor, is susceptible [15]. Universal Health Coverage (UHC) emphasises universalism over particularism, in which every member of society benefits from access to healthcare services without hardships or limitations. Also, not just access to healthcare services but good and quality healthcare services for all [17-19]. UHC is also conceptualised in the context of the number of healthcare services that are covered by the people in a country's health system. In this regard, some studies looked at UHC from the service coverage index (SCI) as a pathway toward achieving UHC. One such study focuses on 11 Asian countries from 2015 to 2017 and the relationship between SCI and some key socioeconomic indicators like gross domestic product (GDP), lack of jobs, health expenditure, and poverty.

The study found these major socioeconomic factors to affect the countries' progress toward UHC [20]. In Sub-Saharan Africa and Asia, a similar study analyses their progress toward UHC in the context of categories of healthcare services, cost and the population covered by the national/community health insurance schemes in nine countries -five in sub-Saharan Africa and four in Asia [21]. There are still no clear-cut indicators used to measure the level of progress made by countries or states towards UHC in both developed and developing countries. However, the more the people/citizens have free/low-cost access to healthcare services (good quality), the higher expenditure on health (to cover the cost of healthcare services, drugs/medicines/ vaccines), and closer toward the entire population coverage or more than 90% population coverage are clear indicators for UHC. Among the developed countries, UHC has been achieved through a publicly funded (government) general tax system or a mix of public-private schemes or employer-based insurance schemes. The United Kingdom (UK) has free healthcare through public facilities. Also, in Germany, UHC covers public and private healthcare facilities/medical doctors and is funded through government funds (pool) to pay for healthcare services. Many other developed countries, such as Canada, Australia, New Zealand, Austria, the Netherlands, and Italy, among others, are countries with UHC through public funding mechanisms. These public (government/state) funding mechanisms include the Nordic states like Norway, Denmark, Finland, and Sweden. However, such public funding mechanisms for UHC exclude the USA [22-24]. Achieving UHC in both developed and developing countries calls for the activation of these four core functions in the health systems of states: coherent and well-designed/aligned strategy for health financing, stewardship, creation of resources and delivery of healthcare services [24-27]. See Table 1 on the search for the conceptions of UHC across the globe.

Table 2. A summary of the main conceptions of UHC across the globe

Scholarly Works	Regions	Conceptualizations of Universal Health Coverage
[7], [17-19]	Global	UHC as access to quality healthcare services for all people
[15], [16]	Global	UHC as a political choice or commitment or will of leaders
[12-14]	Pacific	UHC as accessible and affordable healthcare services for all
[20-21]	Asia	UHC from service coverage index or socio-economic factors
[17, 21]	Africa	UHC from the services, the cost and the population coverage
[22-24]	Developed Countries	UHC from public-private provision (government) funded

RESULTS

Policy design and Implementation strategies/structures in some developed countries

In the United Kingdom, the government (public) is directly involved in the provision of healthcare services to the citizens and other people in the Kingdom through the National Health Service (NHS), but with some slight differences across the four nations (England, Wales, Scotland and Northern Ireland). The NHS relies on General practitioners (GPs) to provide primary healthcare services (PHCs) to the patients, and some prescriptions may be obtained from some accredited private pharmacies even though PHCs are generally provided by the public (state-funded or financed drug reimbursement). The UK spent some 10.2% of the GDP on healthcare in 2019. In Canada and France, for instance, some healthcare services are provided by the private sector; thus, there is a public-private mix in the provision of healthcare services to the citizens and people. In this arrangement, private healthcare providers are contracted to provide some healthcare services and are reimbursed by the public authorities in charge of the health insurance scheme.

In Canada, the public-funded health insurance is called Canadian Medicare, which is decentralised. It was passed in 1984 known as the Canada Health Act of 1984. The state or publicly funded is 70%, while private sector insurance takes care of the 30% of healthcare services that are not covered by Medicare. Some of these excluded services include eye, dental, and drug prescriptions. Other forms of health insurance exist in Canada, including employer-based schemes. As of 2017, Canada spent some 11.5% of its GDP on health. Historically, Germany has the world's oldest health insurance system, which started in 1883. Health insurance was imitated and implemented through Otto von Bismarck's social legislation, the Health Insurance Bill

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1883, by a liberal-conservative politician, leader, or statesman. In Germany, there is state support for persons who earn less than targeted salaries and private health insurance schemes, which are usually attended or visited by higher salary earners or the affluent who can pay more for a private health insurance scheme or policy. The German model of health insurance is termed a multiple-payer health insurance system or healthcare system (state vs private) blend of private--statutory-government/state health insurance scheme (Gesetzliche Krankenkasse or GKV) and private health insurance (Krankenversicherung or PKV). Employees pay 7.5% out of their salaries, and employers match this 7.5% on behalf of the employees. Thus, the health insurance scheme is co-financed in Germany between employers and employees. The employers pay such premiums into the national insurance pool on behalf of their employees. The German health insurance pool operates under the principle of "all for one and one for all" (solidarity). Equal healthcare for all people irrespective of their income differences. Some 11.2 of the Gross Domestic Product (GDP) is spent on healthcare in Germany. In terms of payment mechanisms, there has been a change from a fee for service to capitation and co-payment as a measure to contain the cost of medicines/drugs and healthcare.

In Japan, historically, employee health insurance kicked off in 1927, but Japan achieved UHC in 1961 when everyone was insured. Japan's health insurance covers all the people under universal healthcare and on the principle of equality for all people. However, the patients are required to have a form of insurance and to pay some 10 to 30% of the cost while the state or government pays the remaining cost of healthcare services. Also, all must have health insurance, but a few opted out of it and are not forced. The uninsured, in principle, pay 100% except for the very poor or poor households that receive government support or subsidy (for people in this category, medical fees are waived (paid by the state). As of 2018, the Japanese state spent 10.9% of the GDP on health care. The healthcare provision in Japan is public and private. There are multiple insurance schemes, largely the state National Health Insurance (Kokumin-Kenkō-Hoken), the employees' or workers' Health Insurance (Kenkō-Hoken) and the employers' -based health insurance schemes.

In Thailand, the first attempt at health insurance was the scheme to cater for the welfare of the poor and vulnerable Thais in 1975. Thailand has made a lot of progress towards achieving UHC since the adoption of the Universal Coverage Scheme (UCS) in 2002, in which the citizens have free access to healthcare services under the auspices of the Ministry of Public Health (MOPH). It is funded by the government (65%) and by private sources (35%). Healthcare access seems to be more prevalent in the urban areas and less prevalent in the rural parts of Thailand. The UCS, also known as the "gold card or 30-baht scheme", has a population coverage of 99.5%. It is funded by state-public revenues, with 4.3% of GDP on health. However, the UCS has funding challenges, including sustainable funding for the poor and the vulnerable across Thailand. Brazil is another developing country that has made a lot of progress on UHC, and all persons, including foreigners with legal residence, have free access to healthcare services in Brazil. Brazil's national health system is "Sistema Único de Saúde (SUS)"- Unified Health System (SUS). It started in 1988. Healthcare services are provided by both public and private health facilities or institutions. The federal Constitution of 1988 made healthcare a legal right of the people of Brazil. However, there are challenges in terms of access to healthcare services in many rural areas. Singapore, last but not least, is one of the countries in the developing world that have success stories on UHC for the people. The services are provided largely by the private sector (66%) and the state (public), which is co-funded by employers and employees and government subsidies. There is efficient service delivery as well as quality healthcare services. Singapore spent 3.4% of its GDP on healthcare. Singapore's healthcare system on UHC is making strides in Southeast Asia.

DISCUSSION

Rwanda formulated the health insurance policy to make healthcare services free and accessible to all its citizens, as well as persons with legal residence. The health insurance policy is known as Mutuelles de Sante (community-based health insurance (CBHI), which covers most of the Rwandan population. There are other government health insurance schemes, which include the Rwanda Social Security Board (RSSB) and the Military Medical Insurance (MMI). These schemes cover government workers or employees (formal sector who pay 15% of their Basic Salaries, which is shared equally between the employers and the employees) while the rest of the population (informal sector including the poor and the vulnerable ones in society) is covered by Mutuelles de Santé [29]. The Rwandan health insurance policy embraces a public-private mix (organisations). Community-based health insurance (CBHI) provides 80-90% of the population's coverage [30-33]. The policy makes healthcare services free for the poorest while the rich in society pay annual premiums (8256.41 Rwandan francs, an equivalent of $8 United States (US) Dollars) [28]. The financing mechanisms include contributions from members, government subsidies, foreign donors/external sources of funds and sources of funds from other health insurance schemes.

Since 2008, health insurance has been mandatory for all, and the Rwandan government spent 9.7% of its GDP on health. Also, Rwanda has a population of 12.9 million and a GDP of 837 USD as of 2019 [33]. The Rwandan CBHI has reduced out-of-pocket payments by patients at health facilities, which account for 10%, thus increasing healthcare service utilisation in Rwanda due to the CBHI policy [34]. Despite the strong political will toward UHC, the country is faced with financial and human resource shortages, especially among medical doctors or practitioners. The sustainability of the CBHI remains the

greatest challenge. This implies that for Rwanda to meet the ultimate goal of the global agenda of UHC by 2030, the Rwanda government has to diversify the sources of funding and increase the percentage of health spending. Such a move, if carried out more rigorously and effectively, should bring Rwanda very close to meeting or achieving the UHC by 2030. Historically, Tanzania's first attempt to provide healthcare services for all people was in 1987 through the Arusha Declaration ('Azimio la Arusha' in Swahili). Implementation was hampered due to rising costs of healthcare services, and it was only in the 1990s that a cost-sharing version of healthcare financing was adopted in the fashion of African Socialism, one people or oneness of the people or the 'Ujamaa', or brotherhood, everyone life is essential.

However, there were problems with the cost-sharing health reform, including inequity between the rich and the poor. Tanzania has multiple national health insurance schemes, some of which are private ones. Moving towards UHC, the Tanzania government adopted the Fourth (4th) Health Sector Strategic Plan (2015-2020) to address the challenges of access and to achieve healthcare for all [35-38]. The multiple schemes in Tanzania are a setback to the country's move towards achieving UHC due to variations in costs, population coverage, and healthcare services offered to patients. The National Health Insurance Fund (NHIF), established in 1999, is public and covers 13% of the population, and the Community Health Funds (CHF) covers 9% of the population. In all the categories of health insurance schemes, a total of 32% of Tanzanians are covered (insured), which is about one-third of the population as of 2019.

Tanzania spent 3.83 % of its GDP on health from 2000 - 2019 [42]. The other health insurance includes Private Health Insurance (PHI), which has a different benefits package and a fixed premium for members. The private micro-insurance sector covers only 1% of the population. There are other employer-employee schemes in Tanzania, a country with a population of 57.5 million [35-37]. The health sector or health insurance schemes are funded largely by government subsidies and external support such as the United States (US) government through the United States Agency for International Development (USAID) and the US Centers for Disease Control and Prevention (CDC), World Health Organization (WHO) expected and these sources are complemented by the private sources and the development plan 2022 - 2026 to make free health services accessible to all [35,36,38-39]. Tanzania's moves towards achieving UHC by 2023 imply the need for a possible merger of the multiple schemes with a central authority and the government to adopt policy measures to increase health budget/spending. South Africa has taken steps to make healthcare services accessible to all citizens and persons with legal residence. The pathway toward UHC started in 2011 when the health minister (Aaron Motsoaledi) initiated a National Health Insurance (NHI) for South Africa. It is important to note this scheme does not cover all the people of South Africa, as there are two parallel schemes in the country. One scheme is for the public, and the other is for the private healthcare system. The health insurance system in South Africa is complex in terms of access to healthcare services, the mode of payments, and other related issues, such as nationality and income level. The 2011 NHI policy targets a single fund, and access to free healthcare services is based on constitutional rights and not on membership in NHI. The NHI policy aims to decrease cash payments at the points of healthcare service delivery [40-41]. South Africa's move towards UHC spent 8.5% of GDP on healthcare (R332 billion), and about half of the money was spent on the private sector largely in favour of the socioeconomic and political elites, a minority group representing 16% while a majority of people of South Africa representing 84% healthcare services needs depend on the public sector which is under-resourced or underfunded [40,43].

South Africa relies on the pathways towards UHC, and these include NHI and Primary Health Care (PHC) reengineering [40]. It is based on public-private mix providers for 14 years to achieve UHC from 2012-2026 [44]. The healthcare system of South Africa is faced with many challenges, including bridging the wide gap between the rich and the poor and how to deal with the disease burden of TB, HIV/AIDS, violence, and trauma, among others. These are the financing mechanisms for the pathway toward UHC in South Africa, namely a unified health financing system, movement from voluntary contributions to a more mandatory prepayment system, improvement in cross-subsidisation, public-private providers mix and need for the broader tax base for more revenues [40, 44]. This implies that the biggest challenge for South Africa towards achieving UHC is how to effectively deal with the gap between the rich and the poor, where the majority of the people rely on the public-funded scheme, which is largely under-resourced. Thus, there is a need to support the majority scheme with more funds.

Ghana adopted the National Health Insurance Scheme (NHIS) in 2003, which was implemented in 2004 to increase access to basic health care services for Ghanaians and other persons with legal residence in Ghana. The NHIS is funded by the state/government and other sources, including private sources. It is a state-centred health insurance policy which has since been funded locally from diverse sources within Ghana [21,45-47]. Also, Ghana, as of 2019, spent 3.49% of its GDP on health [48], and the NHIS, as of 2019, covered 40% of the population of Ghana [49-50]. The biggest threat to Ghana's NHIS is funding with lots of delays in payments of health service providers/facilities, thus sustainable financing through increased budgetary allocations for NHIS since most of the beneficiaries are exempted from payments of the annual premium. All four Sub-Saharan African states have health insurance schemes that aim to achieve UN SDG3.8 on UHC within the timelines of the global agenda (by 2030). While Rwandan community-based health insurance has the highest population coverage, Ghana is the second Sub-

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Saharan African state to achieve slightly more than 40% population coverage ahead of Tanzania and South Africa as of 2019. In terms of budgetary allocation or public funding of 2019 for the health sector, the findings suggest that Rwanda has the highest health spending at 9.7% of the GDP [33], followed by South Africa with 8.5% of GDP on healthcare (R332 billion) [40,43] with Tanzania and Ghana at the bottom 3.83 % of its GDP on health for Tanzania from 2000- 2019 [42] and Ghana spent 3.49% of its GDP on health [48].

From our findings, it is obvious that Rwanda is on the pathway towards achieving the global agenda (UN SDG 3.8 on UHC) more than the other three countries per the two indicators used, namely population coverage and public spending for the health sector. These findings show that Sub-Saharan African states need to invest more in health and spend more money on healthcare services to achieve UHC for all people/citizens. The inability of this research to strictly comply with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines is a limitation. Also, the sole use of secondary data sources could not reflect the citizens' feelings, views and experiences of the four countries about their health insurance schemes and their health policy strategies towards achieving the global agenda (the United Nations SDG 3.8) by 2030. Primary data sources should be added to the same area to reflect the real perspectives of the people in the four countries. Efforts should be made to reflect on PRISMA guidelines in future research on this social phenomenon.

Conclusion

Funding various health insurance schemes in most developing countries, especially in Africa, remains a challenge. The pathway to achieving UHC by 2030 is most likely to be missed by the four African states studied. Rwanda has the best coverage for UHC. It is therefore recommended that adequate funding and a positive attitude towards publicly funded health services be addressed to sustain African health insurance schemes/policies. A change of attitude to embrace the various health insurance schemes or policies across Africa may protect schemes against abuses.

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High-Performance Liquid Chromatography applications and challenges in developing countries: A short communication

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Abstract

Over the years, developing countries have utilized High-Performance Liquid Chromatography (HPLC) to conduct extensive research on local and global health-related issues. However, challenges such as scarce service engineers, lengthy shipment procedures for spare parts, and other factors hinder HPLC from working at full capacity. This brief communication discussed HPLC research in developing countries, identified challenges, and proposed beneficial strategies for users and potential buyers.

Keywords: HPLC, developing countries, maintenance

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High-Performance Liquid Chromatography (HPLC) is a vital analytical technique used to separate, identify, purify, and quantify components from a heterogeneous mixture or solution. It is utilized in various fields, including disease diagnosis, drug discovery, drug monitoring, bioequivalence research, and forensic toxicology [1]. It is widely used in developing countries for pharmaceutical and research purposes, but its costly maintenance and servicing can lead to instrument malfunctions. The disparity between developed and developing countries in terms of the availability of consumables, operation, and engineering with respect to bioanalytical equipment, particularly HPLC, has not yet been published in any scientific journal. Therefore, this communication provides suggestions for both users and manufacturers, aiming to improve research in developing countries. Over the years, developing countries have utilized HPLC to conduct extensive research on local health issues (Figure 1). Africa's tropical location, harsh climate, and poor agriculture practices expose agricultural products to mold contamination, leading to the production of toxic chemicals by fungi [2,3]. HPLC is used for quantitative analysis of mycotoxins like aflatoxin, ochratoxin and other toxins due to its widespread use. In addition, it is used to analyze pollutants such as pesticide residues [4], polyaromatic hydrocarbons [5], and other toxic chemicals that are significant in developing countries. Drug discovery, primarily natural product research, has become an important area in developing countries, where most people rely on traditional medicine for their primary healthcare needs [6]. The HPLC is utilized in checking for adulterants in herbal preparations, drug monitoring, compound isolation, and pharmacokinetics [8,9]. Cation-exchange HPLC is used in some laboratories to diagnose sickle cell haemoglobin variants and glycated haemoglobin due to its sensitivity and specificity, especially in sub-Saharan Africa, where most of the global burden of sickle cell anaemia occurs [7]. Despite the advancement in the application of HPLC in developing countries, there are critical setbacks, which include scarce service engineers, the availability of spare parts and consumables, and other factors such as poor quality water for mobile phase preparation, erratic power supplies, and limited laboratory space (Figure 2). HPLCs are largely manufactured in the USA, Europe, and Japan. Few of these manufacturers have

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agents in developing countries. This usually requires trans-continental travel by engineers for both installation and servicing at the client's expense. In Ghana, the main HPLC service engineers are Wagtech Ghana Limited and MES Equipment Limited, whereas in Nigeria, the primary engineers are Synapse Technologies and Labstock Nigeria Limited. Due to their high workload and limited availability of spare parts, HPLCs can be abandoned or malfunction for years, causing costly maintenance. In addition, most developing countries lack service contracts with manufacturers or contractors due to the high cost of such contracts, thus presenting a huge burden on research. Therefore, it is important for institutions to train technicians or HPLC users to perform software and basic hardware servicing, which may not require an expert. HPLC users could be trained in replacing lamps, fixing columns, tubing, and fittings, as well as pump seals and general troubleshooting. It is also imperative for the institution to purchase supplementary parts while the equipment is functioning. Manufacturers of HPLC equipments should, therefore, critically assess this end-user need to expand their boundaries across developing countries and improve the quality of their products. Meanwhile, manufacturers such as Agilent, for instance, have a training university that renders both classroom training at Agilent facilities and online training for live e-learning, as well as on-site training at the customer's facility [8] that developing countries can access to build capacity in their staff to improve the performance of their equipment. Although developing countries' infrastructure has lately improved, further development is still required to enable equipment to operate at full capacity. Access to electricity has received little attention in developing countries [9]. Damage to equipment caused by powder fluctuation is common in developing

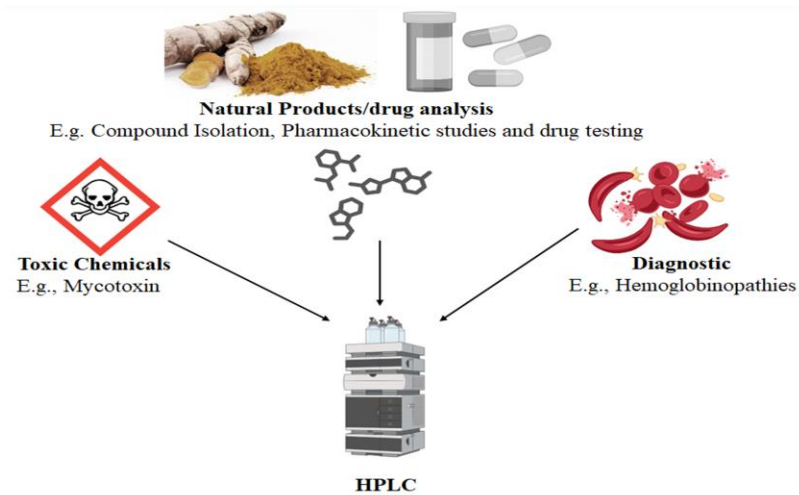

Figure 1. General application of HPLC in developing countries.

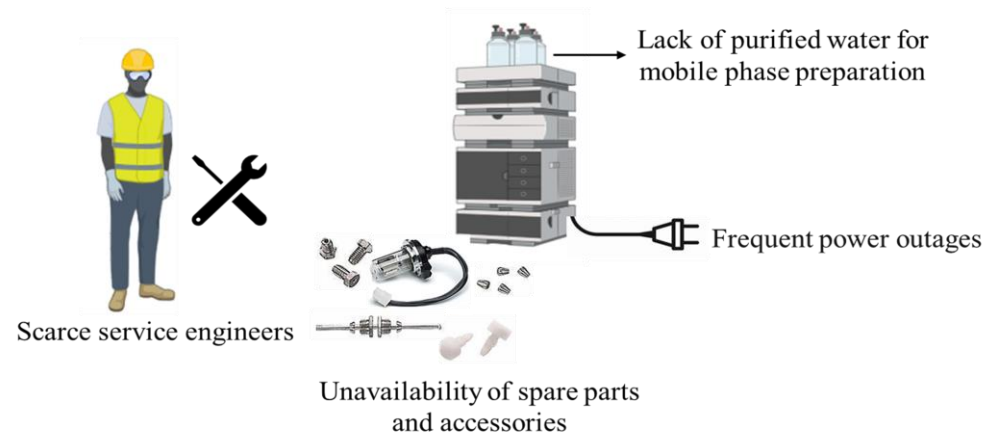

Figure 2. Challenges associated with the use of HPLC in developing countries

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countries. HPLCs, like any other laboratory equipment, need a stable power supply during operation. A sudden power outage during HPLC operation can cause electrical damage and retain molecules in the system, requiring thorough purging afterwards. Hence, a voltage-uninterruptible power supply (UPS) is recommended for temporary system operation. Some laboratories have reliable UPSs, while others have UPSs with weak batteries or rely on ordinary voltage regulators, which may not be sufficient for the system's operation. Institutions should invest in a stable power supply to prolong the lifespan of their HPLCs, as it is costly to acquire or repair. The hazard of dust in developing nations, particularly in Africa, is terrible because the Sahara Desert, the world's largest dust source, contributes more than half of worldwide dust emissions [14], and this can have a great negative impact on HPLC. A clean laboratory space, free of dust, can significantly improve the performance of the equipment. The average higher temperatures in most developing countries, coupled with global warming, pose outrageous impediments [15] and could play a major role in the performance of high-precision laboratory equipment such as HPLC. For instance, a warm temperature can affect the temperature of the mobile phase and the overall performance under warm conditions; in this case, good air conditioning is needed to enhance optimal performance. Water is one of the basic solvents that is widely used in the preparation of the mobile phase for reverse-phase RP-HPLC. Buffers and other solvents that are not HPLC-grade could be filtered through microfilters.

Due to the low quality of water supply in developing countries, the purification of water to meet HPLC requirements is cumbersome. In some cases, the water purification system in some laboratories gets clogged with dirt frequently, and this requires filter changes, which, in the end, incur additional costs. Some laboratories rely on other laboratories that have water purification systems for distilled water. Low-quality distilled water can clog HPLC tubing and columns, increasing system pressure, detecting elevated backgrounds, and reducing tubing and column lifespan. Ghost peaks, also known as artifacts or erroneous peaks, are caused by unknown impurities, such as impure water [10]. It is, therefore, crucial to consider a sustainable source of purified water supply when purchasing HPLC. In summary, developing countries have utilized HPLC to address a wide range of relevant areas despite the challenges associated with its usage. Institutions should, therefore, enhance staff maintenance capacity and consider quality water and a stable power supply when purchasing it. HPLC manufacturers should prioritize the needs of HPLC users in developing countries.

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Minimally invasive resin infiltration with DMG Icon for white spot lesions: A case report

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Abstract

White spot lesions (WSLs) which are frequent occurrences in dental orthopaedics and pediatric dental patients, often result from demineralisation of enamel due to poor oral hygiene and excessive sugar consumption. Patients with early-stage dental caries usually present with WSLs, which occur as a result of the deionisation of the outer covering of the teeth. The management of these early-stage carious lesions can be challenging, as the traditional approach involves invasive restorative procedures. Over the years, many treatment methods have been proposed, but none of them have shown good clinical results. However, the application of the DMG Icon, a minimally invasive treatment, resulted in the reversal and arrest of the progression of WSLs. This case report presents an 18-year-old female patient with the chief complaint of visible white spots on the upper anterior teeth for two months. Based on the clinical examination and history, the patient was diagnosed with WSLs on bilateral labial surfaces of the maxillary incisors and canines. The treatment plan involved using DMG Icon resin infiltration to prevent the WSLs and improve the structure of the anterior teeth. This case report presents a successful application of the DMG Icon in the treatment of WSLs in a young patient with WSLs on the anterior teeth. The report highlights the potential of DMG Icon as an effective alternative to conventional restorative methods.

Keywords: Resin infiltration, white spot lesion, hypo-mineralisation, enamel demineralisation, dental caries

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INTRODUCTION

Dental caries develop as a result of the deionisation of the outer covering of the teeth, known as enamel. Patients with this condition initially present as white spot lesions (WSL) on the tooth's enamel surface. Inadequate oral care, fluorosis, medicines, molar incisor hypo-mineralisation (MIH), and traumatic hypo-mineralisation are a few of the causes of dental caries [1]. The structural composition of enamel provides optical properties to this layer of teeth. Any developmental defects or external factors that can cause changes in this chemical composition of enamel can lead to WSLs. These WSLs typically appear as changes in colour and opacity of enamel. The changes in enamel composition give the characteristic opaque and discoloured appearance of the WSL [2]. These changes in enamel occur due to reduced ameloblastic activity during tooth development, which results in increased porosity on the hard surface of the tooth. WSLs that occur as a consequence of MIH may vary from mild opacities to complete breakdown of enamel after the eruption of teeth. Treating this condition poses a great challenge to dentists and patients. The dentin in the undersurface of the tooth is exposed following the breakdown of enamel. Exposure of dentin to the external environment increases the sensitivity, and thus, children fail to take proper oral hygiene. This makes teeth more prone to the rapid progression of caries [3]. The adherence of the filling material is weakened by increased enamel porosity.

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This makes teeth more sensitive to temperature changes and more prone to pulpitis. People who experience such changes frequently experience insecurity and avoid smiling because they are ashamed of their stained teeth. These factors may contribute to mental disorders, social disengagement, and dental fear in children due to inadequate anaesthesia [4].

The treatment method depends on the extent of cavitation of the lesion. The restorative approach is beneficial in treating cavitated lesions, and the preventive approach is suitable for the treatment of cavitated lesions [5]. Some of the treatment options that help in reducing the white spot lesions include remineralisation, abrasion of enamel, erosive infiltration, composite resin restoration, and bonding [6]. The resin infiltration technique has emerged as a highly appealing therapeutic approach in recent years since it may be employed without causing tooth tissue loss or requiring anaesthesia. The etched enamel surface can easily be penetrated by low-viscosity resin. Light curing of the teeth is done after the infiltration. The similarity between the refractive indices of resin and enamel helps explain why this procedure frequently yields favourable results [7]. To ensure adequate retention of resin and formation of strong bonds, deproteinisation of lesions using sodium hypochlorite should be carried out before the infiltration of resin. To improve the aesthetical appearance of anterior teeth, a minimally invasive color-masking procedure can be opted for. Procedures that help to stop enamel demineralisation and/or enhance tooth aesthetics include topical administration of remineralising chemicals and microabrasion [8]. Traditionally, invasive restorative procedures like drilling and filling have been employed to treat these lesions. However, advancements in dental materials have introduced minimally invasive options like DMG Icon (Icon® EDMG2204031; DMG Chemisch-Pharmazeutische Fabrik GmbH, approved by the food and drugs authority of India), a resin infiltration system designed to halt the progression of early-stage carious lesions. This case report highlights the application of the DMG Icon in treating WSLs.

CASE

An 18-year-old female patient came to the Department of Endodontics with the chief complaint of visible white spots on the upper anterior teeth for two months. She was already given oral prophylaxis. WSLs were discovered during the intraoral evaluation. Clinical examination revealed multiple WSLs on the labial surfaces of bilateral maxillary incisors and canines. The patient had a history of irregular brushing

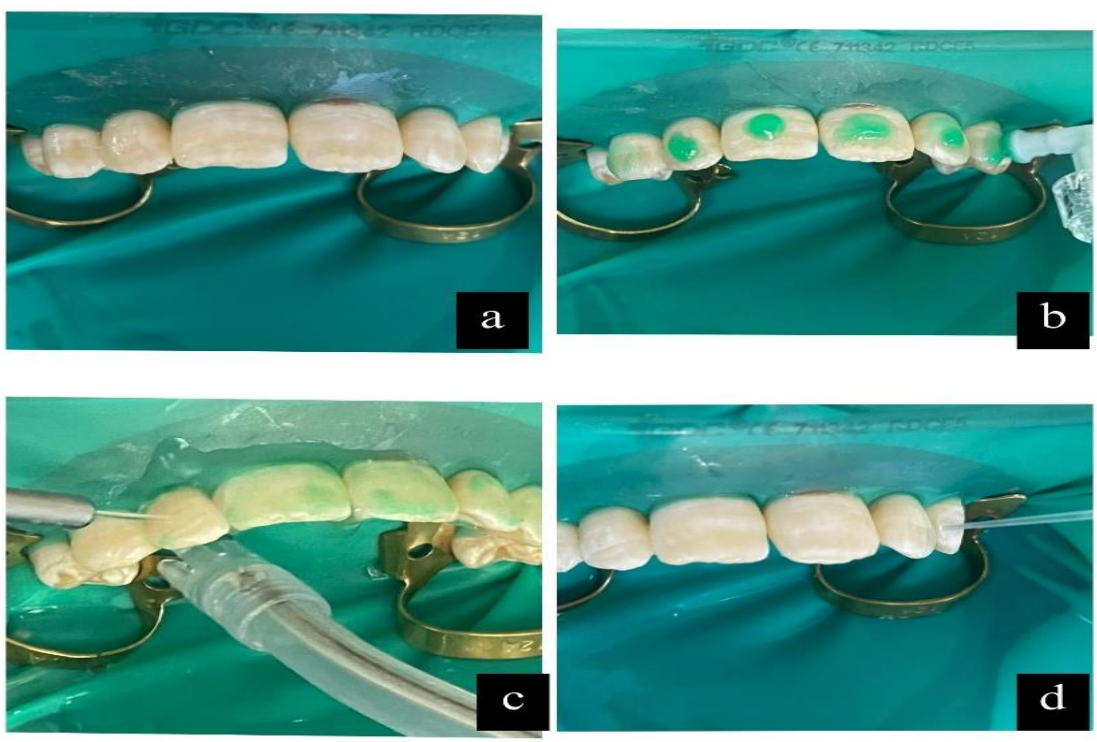

Figure 1. Isolation, cleaning, etching, acid rinsing, and drying.
a. Preparation and isolation of anterior teeth.
b. Etching of lesions using 15% HCL.
c. Rinsing of tooth surface with acid and drying with oil and water-free air.
d. Desiccation of teeth with Icon-dry.

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and high consumption of sugary snacks and beverages. The rest of her oral health was satisfactory, with no signs of cavities or gingival inflammation. Based on the clinical examination and history, the patient was diagnosed with WSLs on bilateral labial surfaces of the maxillary incisors and canines. The lesions seemed to be early-stage carious lesions, showing areas of enamel demineralisation. After being informed of all the treatment choices, the patient opted for the most conservative option, which was the resin infiltration procedure.

Treatment plan and procedure

The treatment plan aimed to halt the progression of the WSLs and improve the aesthetic appearance of the affected teeth. The treatment plan involved using DMG Icon resin infiltration to prevent the WSLs and improve the structure of the anterior teeth. Informed consent was obtained from the patient and her parents before the treatment. Firstly, the anterior teeth were isolated, rinsed, and cleaned, as given in Figure 1a. Cleaning the teeth using pumice and water slurry helps remove plaque and stains from the surface. The teeth were then isolated with cotton rolls to ensure a dry field. A 15% hydrochloric acid etchant gel was applied to the white spot lesions for two minutes, as shown in Figure 1b. This step created microporosities in the demineralised enamel, allowing better penetration of the resin infiltrant. The etched teeth were then rinsed for thirty seconds. This helped to remove acid from the surface. The teeth were gently dried using oil-free air to ensure the etched surfaces were moisture-free, as shown in Figure 1c. The lesions were desiccated with Icon-dry (99% ethanol) for 30 seconds. The white spots on the teeth were still visible, and thus, the etching and drying steps were repeated. On the third Visual inspection, good masking of white lesions was achieved. The DMG Icon resin infiltrant was applied to the demineralised areas using a micro brush, as shown in Figure 2a. The infiltrant penetrated the porous enamel and improved the refractive index of the enamel, reducing the visibility of the white spots. Then, interdental flossing was done, as shown in Figure 2b. After application, the resin infiltrant was light-cured for 40 seconds per tooth to polymerise the material, as shown in Figure 2c. An aesthetically convincing result was seen after infiltration treatment with Icon, as shown in Figure 2d. The treated

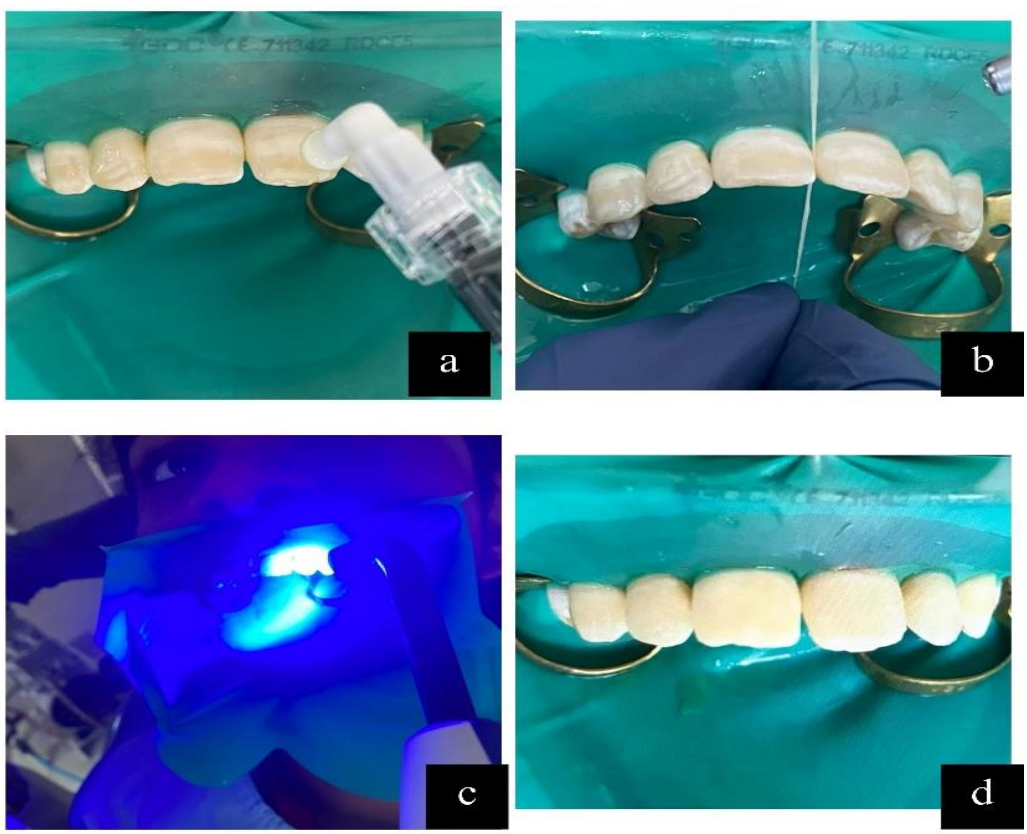

Figure 2. ICON resin application, interdental flossing, light curing, and final result.
a. ICON resin was applied for 3 minutes.
b. Interdental flossing after the application of resin.
c. Light curing of each tooth for 40 seconds.
d. Final result after infiltration.

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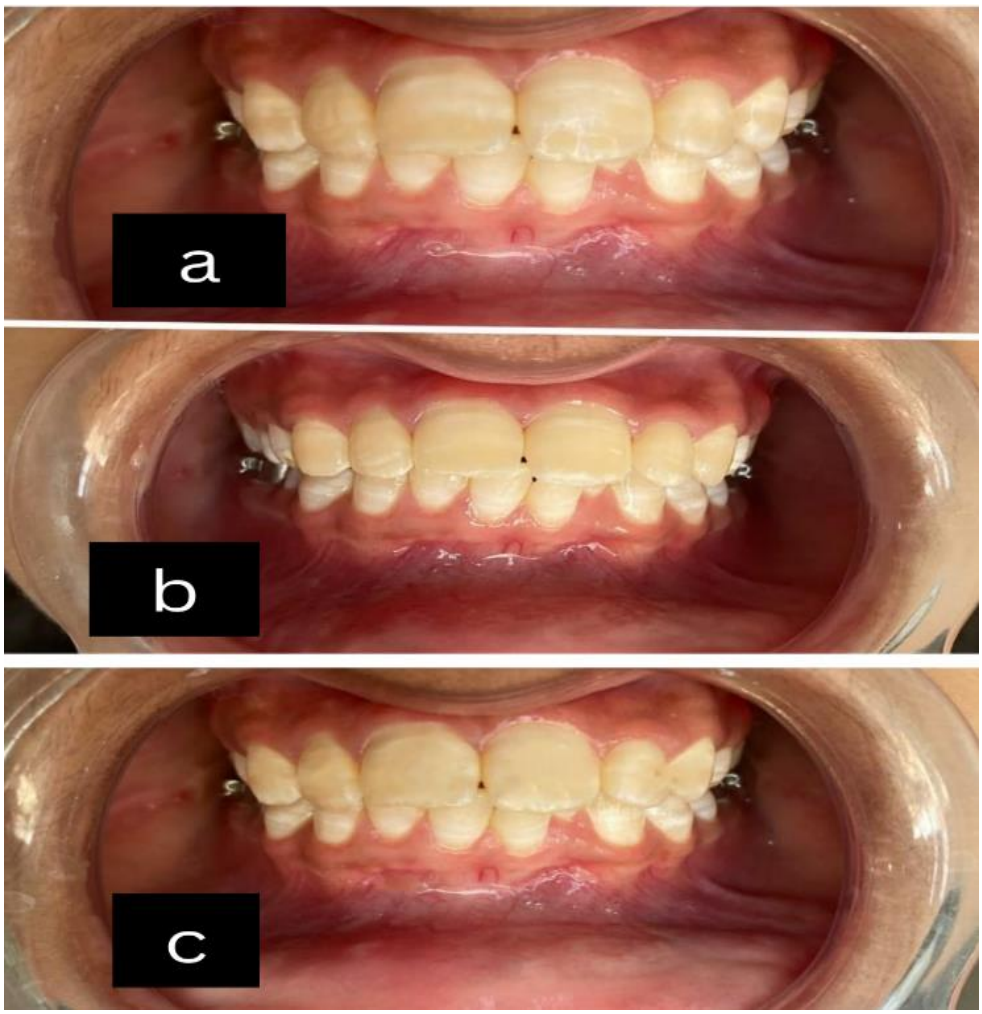

Figure 3. Follow up in the fourth week.
a. Appearance of anterior teeth before the treatment.
b. Appearance of anterior teeth immediately after the treatment.
c. Appearance of anterior teeth after the treatment.

surfaces were polished to achieve a smooth and natural appearance. The removal of water from the pores of the lesion is given in Figure 1d.

Follow-up and outcome

The patient was advised to follow good oral sanitation, reduce sugar intake, and avoid consumption of acidic and staining foods or beverages to prevent the formation of new white spot lesions. Regular follow-up appointments were scheduled to monitor the treated areas and overall oral health. Figure 3 shows pre-operative (Figure 3a), immediate postoperative (3b), and one-month follow-up findings (3c). The immediate postoperative examination revealed that the WSLs appeared less prominent, and the teeth exhibited a more uniform colouration, blending with the surrounding enamel, as shown in Figure 3b. At the one-month follow-up visit, the white spot lesions showed noticeable improvement in both size and appearance. The patient expressed satisfaction with the treatment outcome. Visual examination under daylight revealed stable results with no signs of lesion progression Figure 3c.

DISCUSSION

The application of DMG resin infiltration, in this case, offered a minimally invasive and aesthetically pleasing alternative to conventional restorative methods for treating WSLs. The procedure helped to halt the development of early-stage lesions and improved the aesthetics of the affected teeth, resulting in a more confident and satisfied patient. Furthermore, maintaining a healthy tooth composition is essential, especially in children undergoing orthodontic treatment, to maintain long-term dental health

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[9]. The resin infiltration technique was developed in the 1970s when resin materials with lower viscosity were used to improve enamel calcification. Further studies established the benefit of etching and the production of infiltrant with a higher penetration coefficient in the 2000s, broadening the concept of the infiltration method in clinical practice for masking and halting caries [10]. The resin infiltration method also helps in instant masking the WSLs in the deeper layer of the lesion [11]. Additionally, mechanical strength is increased for enamel as a result of this resin infiltration [12].

The Icon system is a pioneering method that has crossed the gap between caries restoration and prevention for one-third of dentine (D-1) [13]. It has many benefits, including maintaining the composition of teeth, instant results, mechanically stabilising the demineralised enamel, reducing the likelihood of filling leak from the pores, preventing secondary caries, having a better aesthetic result, and is widely accepted by patients [14]. The capillary reaction is what allows the resin to permeate porous enamel according to the resin infiltration rule. In this way, removing micropores, which enable acid diffusion pathways and melting materials, slows disease progression [15]. The use of the traditional Icon application method can result in partial etching without complete infiltration.

To overcome this problem, several recommendations were given, including longer etching duration, prior preparation of tooth structure, and a longer infiltration period. A prolonged application period leads to a deeper infiltration of the substance [16]. The application of etchant assists the resin penetration into the lesion through a hyper-mineralised enamel surface, and 15% HCl was thought to be a suitable etchant for adequate penetration. The water in the pores can be eliminated using 99% ethanol, and thus, capillary forces are created in the pores that aid the infiltration of infiltrant into them [17]. However, MIH teeth are frequently challenging to treat. The average bonding power of composites is decreased by resin infiltration on its own. The Icon system of resin infiltration prevents the acids and bacteria from reaching the structures located in the deeper layers of the teeth and thereby enhances the bonding strength of filling material with hypo-mineralised enamel. Thus, the use of the Icon system can be effective in treating even worse dental abnormalities [18].

Conclusion

The application of DMG Icon in treating white spot lesions is a promising minimally invasive approach that offers improved aesthetics and preservation of healthy tooth structure. This case report demonstrates the successful use of the DMG Icon in a young patient with WSLs, highlighting its potential as an effective treatment option for managing early-stage carious lesions. Further research and long-term studies are warranted to assess the long-term efficacy and stability of this treatment modality. The treatment process with the DMG Icon was straightforward and painless.

DECLARATIONS

Ethical considerations

The ethical clearance for this case report was obtained from the Departmental Review Committee, NIMS University, under the reference number NIMSDC&H/DOSP/42. Written and oral informed consent was obtained from the patient.

Consent to publish

All authors agreed on the content of the final paper.

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Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Author contributions

AM conceptualised the paper. AM and IJ participated in the study design, Literature review, and experimental and clinical studies. PK, SA, and RM participated in editing and reviewing the manuscript. AG supervised and approved the manuscript for publishing.

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Availability of data

Data is available upon request to the corresponding Author

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Catamenial Pneumothorax: A rare but important cause of chest pain in young adult females in the Ghanaian population

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Abstract

Catamenial Pneumothorax refers to a spontaneous pneumothorax due to thoracic endometriosis, often associated with menstruation in young women. It forms part of the spectrum of thoracic endometriosis syndrome, a term used to describe the occurrence of pneumothorax, hemothorax, haemoptysis, or chest pain associated with menstruation. We present three females between the ages of 30 and 40 years who sought medical attention at the Emergency Department of the University of Ghana Medical Centre with complaints of chest pain and difficulty breathing associated with their menstrual cycle. In all three cases, chest X-rays revealed right-sided pneumothoraxes. The diagnosis of Catamenial Pneumothorax was made in all three cases based on clinical findings. This write-up aims to underscore the importance of maintaining a high index of suspicion for diagnosing catamenial pneumothorax in young women presenting to the Emergency Department with cyclical chest pain associated with menstruation.

Keywords: Endometriosis, catamenial pneumothorax, female, menstrual cycle

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INTRODUCTION

Catamenial pneumothorax is defined as the spontaneous, often recurrent collection of air in the pleural cavity of women in the reproductive age group. It is closely associated with the menstrual cycle of these women [1]. Although catamenial pneumothorax was previously thought to be a rare clinical entity, recent studies suggest otherwise [2]. We present three cases of women in their reproductive age diagnosed with catamenial pneumothorax based on clinical findings identified at the Emergency Department (ED) of the University of Ghana Medical Centre (UGMC). In all three cases, chest tube thoracostomy was performed, and patients were subsequently discharged on Zoladex (GnRH agonist) implant 3.6 mg every 28 days for a duration of 6 months. These presentations underscore the importance of clinicians being mindful of this clinical picture in women of reproductive age to enable appropriate and early diagnosis and management.

CASE 1

A 38-year-old female living with endometriosis presented with difficulty breathing accompanied by dull right-sided chest pain, an occasional dry cough and fatigue on minimal exertion. The symptoms had a duration of two weeks and were noticed a week prior to her menstrual period but worsened during the menstrual period. She had a history of a similar episode about a year prior to the presentation, which resolved after taking over-the-counter medication she could not recall. No prior history of trauma was reported, and she was not known to be living with any chronic lung condition. Although her menstrual cycles were

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regular, she experienced secondary dysmenorrhea. Upon arrival, her vital signs were as follows: blood pressure was 121/72 mmHg, pulse was 89 beats/min, respiratory rate was 22 cycles/min, oxygen saturation (SpO2) was 100% on room air, and temperature was 36.3 ºC. However, she had decreased chest expansion hyperresonance on percussion with reduced breath sounds in the right middle and lower zones. The initial erect chest X-ray revealed a right lung collapse with loss of lung markings and increased radiolucency peripheral to the collapsed lung. Bedside ultrasound (USG) revealed absent lung sliding at the apical portion of the right hemithorax. The diagnosis made after clinical assessment was a right pneumothorax in a young woman with endometriosis. She underwent a chest tube thoracostomy using a size 24 Fr chest tube and continued to be managed in the cardiothoracic ward. The tube was removed after eight days of admission, and a repeat chest X-ray at the time of discharge showed complete re-expansion of the right lung without any complication. Subsequently, she was seen by the gynaecologist and started on Zoladex (GnRH agonist) implant 3.6 mg every 28 days for a six-month duration.

CASE 2

A 33-year-old female presented with difficulty breathing and associated right-sided pleuritic chest pain noticed three days after the onset of her regular menses. The presenting symptoms started a day prior to the presentation. She had no associated fever, cough, orthopnea, pedal oedema, hemoptysis, and no prior history of chest trauma. She was not known to have any chronic lung condition and did not smoke. However, her past medical history revealed cyclical episodes of chest pain and mild difficulty breathing during menses over the past six months leading up to the current presentation. Her vital signs on arrival were as follows: blood pressure - 108/79 mmHg; pulse - 93 beats/min; respirations - 20 cycles/min; SpO2 - 96% on room air; temperature - 36.3 ºC. However, she had decreased right chest expansion, hyperresonance on percussion, absent vocal fremitus, and absent breath sounds in the right hemithorax. An erect chest X-ray revealed a right lung collapse with loss of lung markings and increased radiolucency peripheral to the collapsed lung. Bedside ultrasound confirmed absent lung sliding on the apical portion of the right hemithorax. The clinical assessment led to a clinical suspicion of catamenial pneumothorax. A chest tube thoracotomy using a 24 Fr chest tube was inserted and connected to an underwater seal, and the patient was subsequently transferred to the cardiothoracic ward for continued management. A repeat chest X-ray on Day 11 showed lung expansion and resolution of the pneumothorax. She was also evaluated by the gynaecologist and started on Zoladex (GnRH agonist) implant 3.6 mg every 28 days for a six-month duration.

CASE 3

A 34-year-old female living with endometriosis presented with a week's history of right-sided chest pain, which progressively worsened and later became associated with shortness of breath. She noticed symptoms 72 hours after she had started her menses. She had no prior history of trauma and was not known to be living with any chronic lung condition but had a similar episode 2 years prior. On arrival, her blood pressure was 121/82 mmHg, pulse was 115 beats/min, respirations were 22 cycles/min, saturation was 95% on room air, and temperature was 37.2 ºC. However, there was decreased chest expansion, hyperresonant percussion note in the right hemithorax, and absent breath sounds. An erect chest X-ray showed a right total lung collapse with loss of lung markings and increased radiolucency peripheral to the collapsed lung. The diagnosis made after clinical assessment was a right pneumothorax in a young woman with endometriosis. She underwent a chest tube thoracostomy using a 24 Fr chest

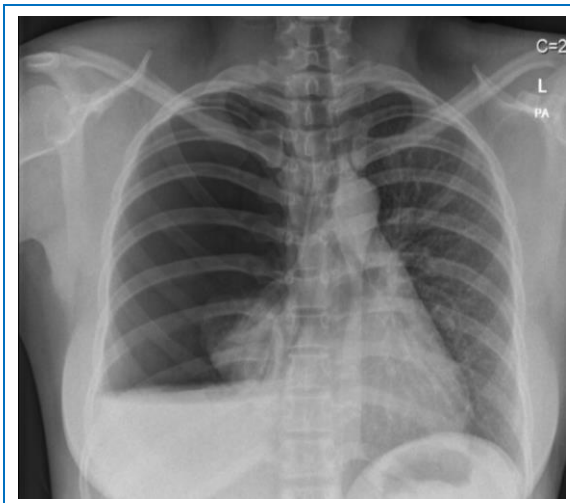

Figure 1. Case 2 Chest X ray on presentation prior to passage of chest tube

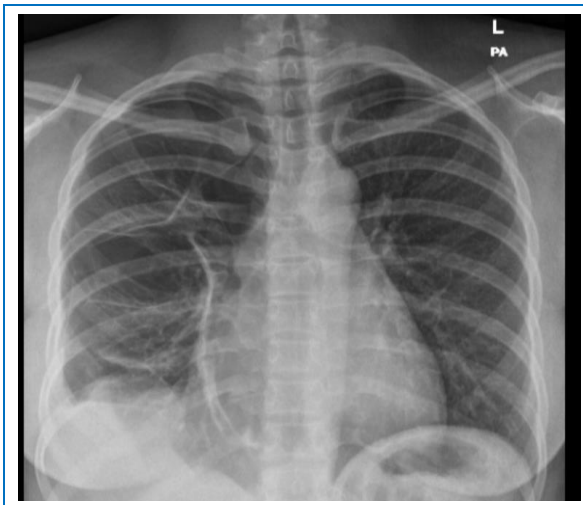

Figure 2. Case 2 - Chest X ray showing lung expansion after removal of chest tube

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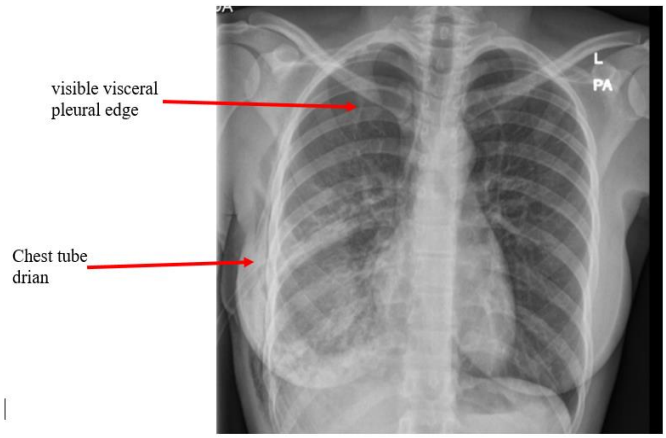

Figure 3. Case 3 Chest X ray showing pneumothorax and chest tube in situ

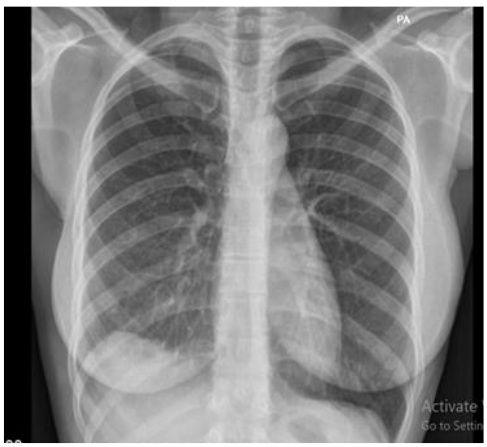

Figure 4. Case 3-Chest X ray showing resolution of pneumothorax.

tube as part of emergency management and was subsequently admitted to the Cardiothoracic ward. She experienced no complications and was discharged after a week of admission when a repeat chest X-ray confirmed full lung expansion. After an evaluation by the gynaecologist, she began a regimen of Zoladex (GnRH agonist) implant, with a dosage of 3.6 mg, to be administered every 28 days for a duration of 6 months.

DISCUSSION

Endometriosis is characterised by the implantation of endometrial tissue outside the uterine cavity [3]. While it typically occurs in the pelvis, it can rarely affect the labia, abdomen and thorax [4]. Tettey M et al. reported a total of 12 cases of thoracic endometriosis syndrome seen between 2004 and 2012 at their centre [3]. Catamenial Pneumothorax forms part of the spectrum of thoracic endometriosis syndrome and is defined as the recurrent spontaneous collection of air in the pleural cavity of women in the reproductive age group, usually occurring within 72 hours before or after the onset of menstrual periods [1]. It may also occur within 5-7 days of menses [5]. Clinical manifestations are predominantly right-sided, although left-sided or bilateral occurrences have been reported in rare cases [6]. The spectrum of thoracic endometriosis syndrome also includes catamenial haemothorax, haemoptysis and relatively uncommon endometrial lung nodules [4,7]. Catamenial Pneumothorax is a rare cause of spontaneous pneumothorax with an incidence of 2.6 - 5.8%, but recent clinical studies have reported a much higher incidence of about 30% [1]. Several theories have been proposed to explain the aetiopathogenesis of the thoracic endometrial syndrome and catamenial pneumothorax. The earliest theory involves retrograde menstruation with implantation in the peritoneum, followed by transdiaphragmatic migration through fenestrations produced by endometriosis [8]. The second theory describes the metastatic spread of endometrial tissue through the venous or lymphatic system [8]. The third involves coelomic metaplasia of undifferentiated stem cells into endometrial tissue, as both the abdominal and thoracic cavities are covered by the coelomic membrane [8]. Additionally, the pathogenesis of catamenial pneumothorax remains unclear. One explanation posits that ectopic endometrial tissue undergoes menstrual cycle phases, releasing air or blood into the pleural space, causing a recurrent spontaneous hemothorax or Pneumothorax [9]. Other explanations include spontaneous rupture of blebs, alveolar rupture due to prostaglandin-induced bronchiolar constriction, and the absence of a cervical mucous plug allowing the passage of air from the genital tract through diaphragmatic fenestrations [3,9]. A thorough history and clinical examination are crucial for the identification and diagnosis of catamenial pneumothorax [10]. Patients commonly present to the emergency department with complaints of chest pain (90%) and dyspnea (31%) associated with pneumothorax [11]. Additionally, pelvic endometriosis is frequently present, although it may be absent in some cases [3]. Other non-specific symptoms include cough and fatigue [12].

The presence of recurrent spontaneous pneumothorax associated with at least two menstrual periods suggests a catamenial pneumothorax [13]. The cases presented above detail young women of childbearing age who experienced right-sided pleuritic chest pain associated with difficulty breathing during menses. Chest X-rays performed in all the cases revealed a large right pneumothorax with partial or complete collapse of the right lung. Clinical findings in the described cases align with the features associated with thoracic endometriosis syndrome and catamenial pneumothorax. The diagnosis of catamenial pneumothorax is often delayed, hence, the need for clinicians to be mindful of this clinical presentation in women of reproductive age to enable appropriate and early diagnosis and management [14]. Chest X-ray, Computed Tomography (CT) scan, and Magnetic Resonance Imaging (MRI) are usually employed

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in the identification of pneumothorax. Chest CT and MRI may further reveal the presence of diaphragmatic and pleural endometrial lesions [7]. However, definitive diagnosis requires video-assisted thoracoscopy, allowing for direct visualisation and resection of endometrial lesions for histopathological analysis [15]. In the cases described, the diagnosis of catamenial pneumonia was based solely on a clinical picture and the presence of pneumonia on a chest X-ray. Emergency management of catamenial pneumothorax follows the same protocol as for other spontaneous and secondary pneumothoraces. Therapeutic options depend on the size and severity, which are crucial considerations. These options include observation, high-flow oxygen therapy, needle aspiration and tube thoracostomy [16]. The use of surgical and hormonal therapy to prevent recurrence is a definitive approach in catamenial pneumothorax management [14]. Surgical options include pleurectomy and resection of endometrial diaphragmatic implants with chemical or mechanical pleurodesis. Hormonal therapy, in most situations, involves the use of gonadotropin-releasing analogues [17].

Conclusion

It is imperative for clinicians to consider catamenial pneumothorax in all young women of childbearing age who present to the Emergency Department with recurrent chest pain and difficulty breathing associated with their menstruation. This consideration allows for prompt diagnosis and management. Long-term management includes surgical options and hormonal therapy using gonadotropin-releasing analogues such as Zoladex.

DECLARATIONS

Ethical consideration

Ethical clearance was granted under the ethical standards of the University of Ghana Medical Center Institutional Review Board. Informed consent was obtained from the patients for the publication of this article.

Consent to publish

All authors agreed on the content of the final paper.

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Competing Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author contributions

EN, GKM, AM-A and EMB participated in the management of the case, drafting of the case, summaries and researching for relevant literature review. PKB, AE, EA and SQ-P participated in drafting and editing the final manuscript. KE supervised the drafting of the report and reviewed the final work for submission. All the authors have read and approved the final version of the manuscript.

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Availability of data

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

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