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FOREWORD

On behalf of the Management and entire staff of the College of Health and Allied Sciences, University of Cape Coast (CoHAS, UCC), I am privileged to introduce you to the first edition of the CoHAS Journal – The Integrated Health Research Journal (IHRJ). UCC has the commitment to mobilize synergies by advocating for better health initiatives and information to meet the challenges in this dynamic world environment. This Journal, being the first scientific journal of UCC, will provide that unique platform where health-related research findings within the College, University, sub-region and beyond will be showcased to healthcare professionals, researchers and the industry community around the globe.

The IHRJ is a multidisciplinary journal which will cover all aspects of the health science disciplines and will be dedicated to publishing impactful healthcare (pre-clinical and clinical) research including original research articles, perspectives, short communications, case studies, case reports and reviews. It will thus provide an avenue for discussions, training and support as well as share our challenges and triumphs on health issues. We have made the Journal open-access so it will be available at no cost to all and it will be open to all researchers worldwide on other digital platforms. With time we hope to publish special issues that will deal with specific thematic health conditions. We firmly believe that this Journal has not only come to stay but will increase patronage of the latest research findings that will inspire changes in healthcare policies, move to greater heights and be the Journal of Ghana, West Africa and beyond speaking with one voice.

I will say a big thank you to the Vice-Chancellor, Professor Johnson Nyarko Boampong who first as Provost of the College pushed for the establishment of this Journal, and as Vice-Chancellor provided the needed devotion, passion and financial resources to make it happen. I will also acknowledge our committed team members of the Editorial Board, both local and international, who through their collective efforts have given us this very first edition. Special thanks also go to Professor Samuel Kyei and Dr. Akwasi Anyanful, Director and Deputy Director respectively of the newly established Biomedical and Clinical Research Centre (another vision of the Vice Chancellor), who provided all the needed support.

Congratulations team, you have made CoHAS and UCC proud.

Prof. Martins Ekor

Provost, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

SPECIAL ACKNOWLEDGEMENT

Prof. Johnson Nyarko Boampong

Vice Chancellor, University of Cape Coast

On the occasion of the launch of the very first issue of the Integrated Health Research Journal (IHRJ), it is appropriate and befitting to share a brief history of the journal in order to acknowledge and recognize the mind that first mooted the idea of establishing the journal and also put together actionable efforts leading to the establishment of IHRJ. Briefly, in 2013, the current Vice Chancellor of the University of Cape Coast, Prof. Johnson Nyarko Boampong, who was then the Head of the Human Biology Department, mooted the idea of establishing a journal that could provide: (1) Research and knowledge advancement in the sciences, (2) A platform for the dissemination of ideas and thoughts of scientists, (3) A forum for healthy scientific discourse on health issues, and (4) An impetus for institutional visibility and internationalization.

It was until 2019, when Prof. Johnson Nyarko Boampong became the Provost of the College of Health and Allied Sciences (CoHAS) that he had the opportunity to put in place actionable efforts that saw the formation of various committees tasked to consider modalities for restructuring and rebranding CoHAS including a sub-committee specially tasked to see to the formation of IHRJ. It must be stated that Prof. Johnson Nyarko Boampong has closely monitored and provided support in all forms to see to the establishment of IHRJ under the newly established Biomedical and Clinical Research Centre, headed by Prof. Samuel Kyei.

On the basis of this singular role in the establishment of IHRJ, the IHRJ management team wish to dedicate this very first edition of IHRJ (Vol. 1, Issue 1) to the Vice Chancellor, University of Cape Coast, Prof. Johnson Nyarko Boampong.

Editorial Team

Integrated Health Research Journal, Biomedical and Clinical Research Centre, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

ACKNOWLEDGEMENT

To all the chairpersons and members of committees, sub-committees, and sub-sub-committees, as well as the staff of the University of Cape Coast Online Journal System (OJS), the IHRJ management appreciates your time and sacrifices over the period with regards to the establishment of Integrated Health Research Journal.

We appreciate the role played by the underlisted in various boards and committees in the lead up to the establishment of Integrated Health Research Journal.

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EDITORIAL BOARD

Integrated Health
Research Journal (IHRJ)

The Official Journal of the
College of Health and Allied Sciences
University of Cape Coast, Ghana

EDITORIAL

Expanding Frontiers of Biomedical Research: The Contributions of Basic Scientific Research

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Introduction

As always, the scientific cause aims at improving human life at all levels through the use of time-tested scientific knowledge and practices. At the root of this lies scientific enquiry which fuels translational research efforts. Generation of scientific knowledge and its dissemination is critical to the scientific cause. In the light of this the Integrated Health Research Journal (IHRJ) was established to serve as the official research conduit of the College of Health and Allied Sciences (CoHAS), University of Cape Coast. Thus, the IHRJ provides a platform for researchers and scientists in the medical and biomedical fields to advance dissemination of quality research outcomes capable of stimulating translational research and generation of health policies to improve human health and wellbeing. In the first call for articles, a total of twenty-one submissions were received out of which seven were accepted for publication after rigorous editorial and review processes.

The focus of all the accepted articles were within that of the IHRJ. For instance, one of the articles highlighted the relationship between indiscriminate use of tetracaine hydrochloride ophthalmic solutions and development of multi-drug antibiotic resistance (Kyei, Boadi-Kusi, Effram-Menyah, Dua, 2023). Telehealth has emerged as one of the approaches to overcome barriers to healthcare coverage. Interestingly, one study assessed barriers to telehealth during the Covid-19 pandemic in rural North America and observed that few studies investigated barriers to telehealth in rural communities and those studies overly focused on short term health outcomes instead of long term health outcomes. Exposure to xenobiotics through matrices such as food, water, air and the soil put humans at high risk for certain diseases. Vowotor and colleagues determined hazard quotient of micronutrients in breastmilk and observed that manganese, sodium, magnesium, calcium, and copper were in levels above the upper limit of the WHO recommended dietary allowance possibly exposing breast feeding infants to risk of metal/metalloid-related toxicity (Vowotor, Sackey, Amuah, Huzortey, Aboh, and Druye, 2023).

Another study (Boachie, Piiga, Dadzie, Essuman, and Boye, 2023) reported transfusion-related adverse reactions from a district hospital in Kumasi detailing the incidence and the factors that contribute to transfusion-related adverse reactions. Non-communicable diseases such as chronic kidney disease (CKD) has been on the increase and it was refreshing to note from a study (Ewua-Gyan, Abdul-Wahab, Donkor, Awuku, Okyere, Botchway et al, 2023) which assessed predictability and accuracy of kidney failure risk equation. Finally, a study detailed the pattern of patient referrals in Northern Ghana.

Summarily, the first issue of IHRJ captured important topics in the core areas of medical and biomedical research and it is hoped that researchers will double their basic research efforts to inform translational research, policy and practice.

References

Boachie, J., Piiga, J. Y., Donkoh, I. E., Amponsah, S. B., Essuman, M. A., Dadzie, E., Boye, A. (2023) Transfusion-related Adverse Reactions: An update from a District Hospital in Ghana. Integrated Health Research Journal 1(1), 34-41.

Ewua-Gyan, M. L., Abdul-Wahab, O., Donkoh, I. E., Awuku, Y. A., Okyere, P., Botchway, F. A., et al. (2023) Assessment of Kidney Failure Risk Equation among Chronic Kidney Disease Patients: A cross-sectional study. Integrated Health Research Journal 1(1), 7-12.

Kyei, S., Boadi-Kusi, S. B., Effriam-Menyah, M. N., Duah, P. (2023) Effect of the Clinical Usage of Tetracaine Hydrochloride Ophthalmic Solution on Multiple Antibiotic Resistant *Staphylococcus Aureus*. Integrated Health Research Journal 1(1), 1-6.

Vowotor, M. K., Sackey, S. S., Amuah, C. L. Y., Huzortey, A., Aboh, I. K., Druye, A. A. (2023) Analysis of Seven Micronutrients in Breast Milk of Lactating Mothers from the Central Region of Ghana Using Epithermal Neutron Activation. Integrated Health Research Journal 1(1), 41-49.

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Integrated Health Research Journal (IHRJ)

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RESEARCH ARTICLE

Effect of the Clinical Usage of Tetracaine Hydrochloride Ophthalmic Solution on Multiple Antibiotic Resistant *Staphylococcus Aureus*

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Abstract

Background: The emergence of resistant strain bacteria is rendering many antibiotics ineffective in the management of infectious diseases.

Objective: The purpose was to investigate the effect of Tetracaine hydrochloride usage on the emergence of a resistant strain of bacteria.

Materials and methods: A total of fifteen New Zealand White rabbits of either sex weighing between 1400-2700 grams were used for this study. Infectious agents of Ophthalmia neonatorum were cultured, isolated and identified. Their susceptibility to various antibiotics was assessed. A resistant strain was picked up and inoculated on the conjunctiva of the rabbits to induce bacterial conjunctivitis. Rabbits were divided into three groups of five rabbits each. Group A received Tetracaine as treatment, group B received 0.3% Ciprofloxacin as treatment and group C received normal saline as a treatment for 14 days. Outcome measures included bacterial colony count, clinical signs and post-antibiotic susceptibility test.

Results: The tetracaine significantly reduced clinical signs (P=0.012) compared to normal saline (control) and 0.3% Ciprofloxacin reduced clinical signs (P =0.003) compared to normal saline (control). This means there was a significant decrease in clinical scores with the interventions as compared to the normal saline which showed minimal change in clinical score. Tetracaine hydrochloride significantly reduced bacteria load (P≤0.0001). It decreased multiple antibiotic-resistant index by 20%.

Conclusion: Tetracaine has a supplemental antibiotic effect on resistant strain of bacteria and does not worsen antibiotic resistance of bacteria. Clinical usage of tetracaine ophthalmic solution should therefore be applied after conjunctival swaps or corneal scrapping have been taken.

Keywords: Antibiotics, Antimicrobial, *Staphylococcus aureus*, Tetracaine hydrochloride.

Citation: Kyei, S., Boadi-Kusi, S. B., Effriam-Menyah, M. N., Duah, P. (2023) Effect of the Clinical Usage of Tetracaine Hydrochloride Ophthalmic Solution on Multiple Antibiotic Resistant *Staphylococcus Aureus*. Integrated Health Research Journal 1(1), 1-6. https://doi.org/10.47963/ihrj.v1i1.1173
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Introduction

Some studies have shown that anesthetics possess a supplemental antibiotic effect (Johnson, Saint John, & Dine, 2008). Several topical anesthetic agents have demonstrated distinct antimicrobial activity against specific bacterial strains and Candida (Pelosini, Treffene, & Hollick, 2009). Tetracaine hydrochloride ophthalmic solution is a local anesthetic indicated for processes requiring an ophthalmic anesthetic of fast and brief action. Its usage for diagnostic purposes could promote bacterial resistance to antibiotics since they are used momentarily and not formulated for treatment. The possibility of repurposing older drugs in an attempt to deal with unmet medical needs further reinforces the necessity for such evaluation to inform the decision in this line of thought (Takahashi et al., 2017).

Evidence from research indicates the intrinsic antimicrobial characteristics of local anesthetics against

a broad spectrum of human pathogens (Johnson et al., 2008). At levels typically used in the clinical environment, multiple local anesthetics inhibit the development of numerous bacteria and fungi under different circumstances. Early research noted that Tetracaine exerts its bactericidal effect on the bacterial cell by a mechanism of action. The writers discovered that tetracaine harmed *Pseudomonas aeruginosa* cell membrane through lysis, intracellular leakage, dehydrogenase activity, and enhanced permeability of the cell wall and greater temperature correlates with a rise in the inhibition of microbial development. Observation of cell lysis, intracellular leakage, dehydrogenase activity, and greater sensitivity of spheroplasts to Tetracaine than whole cells led to the conclusion that Tetracaine works by damaging the cell membrane(Leung & Rawal, 1977). This study aimed to investigate the antibiotic effect of Tetracaine hydrochloride on a resistant strain of bacteria to ascertain its contribution to the increasing spate of resistance to conventional antibiotics.

Materials and Methods

Drugs and Chemical Used: Tetracaine hydrochloride (Archdiosesan Health Pharmacy, Kumasi, Ghana) was used as an anesthetic and positive control drug was Ciprofloxacin eye drop (Ciron Drugs Ltd., India). Normal saline drops (MSB Pharma., India) was also used as a negative control drug in the study. Ketamine hydrochloride (Pfizer, USA) was used to anesthetize the rabbits before the induction of conjunctivitis.

Animal husbandry: A total of 15 New Zealand White rabbits of either sex weighing between 1.4kg-2.7kg were purchased from the School of Agriculture Rabbitry, University of Cape Coast and kept at the Animal House of the School of Biological Sciences of the University of Cape Coast, Ghana. The animals were housed singly in aluminum cages (34×47 ×18 -cm3) with softwood shavings as bedding, under ambient laboratory conditions (28 ± 2°C, relative humidity 60%-70%, and a normal light-dark cycle) and fed with normal commercial pellet diet (AGRICARE, Kumasi) and water ad libitum.

Culture and isolation of bacteria: Swabs from clinically diagnosed cases of Ophthalmia Neonatorum were cultured, isolated and identified in the microbiology laboratory using standard microbiological procedures in a previous study(Kang & Lee, 1989). The susceptibility to various antibiotics was assessed using an antibiotic disc (Abtek Biologicals. Liverpool, UK). Multiple antibiotic-resistant strains of *Staphylococcus aureus* (*S. aureus*) as determined in a previous study (Kang & Lee, 1989) were picked from the pure culture with a sterile standard loop was inoculated into 10 ml of nutrient broth. The isolated multiple resistant S. aureus was then incubated for 24 hours at 37°C.

Induction of infectious conjunctivitis: The method as described by Al-Waili (Al-Waili, 2004) and adopted by Ilechie (Ilechie, Kwapong, Mate-Kole, Kyei, & Darko-Takyi, 2012) was used. Each rabbit was anesthetized by an intramuscular injection of 10mg/kg Ketamine hydrochloride and a drop of 0.5% topical Tetracaine hydrochloride ophthalmic solution was instilled into the conjunctival cul-de-sac of the right eye of each animal. An abrasion was then made on the right bulbar conjunctiva of each rabbit. A 10uL of the bacterial suspension of multiple resistant *S. aureus* was inoculated into the abraded bulbar conjunctivae of the rabbits.

Experimental design: Rabbits were pre-examined for clinical signs of infectious conjunctivitis and those with no signs of conjunctivitis were selected and inoculated with the bacterial inoculum. Forty-eight hours after inoculation the rabbits that showed clinical signs of infectious conjunctivitis were selected and randomized for treatment (n=5). Swabs were taken to confirm positive cultures in the right eye of the animals before randomization.

They were randomized for treatment with 0.5% Tetracaine hydrochloride, 0.3% w/v of Ciprofloxacin (because it was the antibiotic that the strains were susceptible) and 0.9 w/v of Normal saline drop. All treatments were given twice daily (12 hourly intervals) for 14 days.

The right eyes of the animals were assessed for clinical signs of bacterial conjunctivitis using a handheld slit lamp every other day alongside swabbing for days of positive culture over the experimental period. The signs were scored and scale of 0-3 except for sensitivity to light. The scoring was as shown in **Table 1**.

The maximum possible score was 21. The swabs were used to assess for Bacteria Colony Count (BCC) and Antimicrobial Sensitivity Testing (AST). Plate count agar (PCA) was used for Bacteria Count Colony. The specimen swab was snapped off the swab shaft at the score line and cut shaft to fit into a test tube containing 9ml of sterilized freshly prepared peptone water. It was incubated at 37°C for 10h. Using a sterilized pipette, the culture was transferred into a sterilized tube containing 10ml of sterilized freshly prepared peptone water. Using a serial dilution method, 1ml of the aliquot was transferred into the sterilized tubes until least 10 x 5 dilution factor was reached. 1000µL of the serially diluted aliquot was transferred into an autoclaved media plate. Employing pour plate method, about 20ml of freshly prepared autoclaved media was then poured into the plates, swirled gently in both clockwise and anti-clockwise directions and allowed to solidify. The solidified plates were labelled and incubated (Panasonic MIR-154-PE Cooled incubator) at 37 ^{O}C for 24 hours. Samples in VRBA were allowed a maximum incubation time of 1 week. Two different dilution factors for each sample was used and the mean found. After incubation, single viable colonies were counted and recorded in CFU/ml, using the formula; CFU/mL= CFU x dilution factor x 1/aliquot.

Modified Kirby-Bauer disc diffusion was employed. 8ml of normal saline was inoculated with portions of at least 3 pure colonies with similar appearance using a hot-flamed sterilized straight wire. The turbidity of the suspension was then compared with 0.5 McFarland turbidity standard (1.5x108CFU/ml) and adjusted by either the addition of more colonies or normal saline. Freshly prepared Mueller Hinton (MH) agar in sterilized media plates were streaked with a sterilized swab stick soaked with the inoculums in

Table 1: Clinical and visual scoring of some features associated with bacterial conjunctivitis

Clinical features	0	1 (mild)	2 (moderate)	3 (severe)
Redness	Clear	Redness size of <2mm	Redness size of 2-5mm	Redness of >5mm
Discharge	None	Mild discharge	Moderate discharge	Severe discharge
Crusty eyelids	None	Mild crusty eyelids	Moderate crusty eyelids	Severe crusty eyelids
Wet eye	None	Mild wet eye	Moderate wet eye	Severe wet eyes
Edema	No edema present in the conjunctiva	Slight edema/general or localized conjunctival edema	General diffuse edema of the conjunctiva	Frank edema of chemosis of conjunctiva
Photophobia	No photophobia	slight difficulty with light causing occasional eye	Moderate difficulty with light requiring regular blinking	Severe difficulty with light cannot bear natural light
Blepharospasm	No spasm	Mild spasm at stimulation only	Visible spasm without impairment of daily life	Visible spasm with impairment of daily life
Photophobia	No photophobia	slight difficulty with light causing occasional eye	Moderate difficulty with light requiring regular blinking	Severe difficulty with light cannot bear natural light
Blepharospasm	No spasm	Mild spasm at stimulation only	Visible spasm without impairment of daily life	Visible spasm with impairment of daily life

the normal saline suspension. The whole surfaces of the media were streaked three (3) times at an angle 60^{O}. The plates were rotated each time, and allowed to air dry. Sterilized hot-flame forceps were used to place the gram appropriate antimicrobial-impregnated disk (Axiom) on the agar, and each disc was pressed gently to ensure complete contact with the surface of the agar. The plates were then incubated at 37 ^{O}C for 24 hours. After 24 hours, the diameters of inhibition zones were measured using a rule and recorded in millimeters (mm). Antimicrobial sensitivity pattern was determined as susceptible (S), intermediate (I) or resistant (R), with reference to AST interpretation chart.

Statistical analysis: Data obtained for control, test and reference drug effects were analyzed by one-way analysis of variance followed by Tukey's multiple comparisons test using GraphPad Prism (version 5.03; GraphPad, La Jolla, CA, USA). Values were expressed as the mean ±standard error of the mean. P≤0.05 was considered to be statistically significant.

Results

After 48 hours of inoculation of S. aureus, there were obvious signs of bacterial conjunctivitis in all treatment groups. The rabbits treated with normal saline showed significantly higher clinical signs of bacterial conjunctivitis as compared to the 0.3% Ciprofloxacin and Tetracaine hydrochloride treatment groups **(Table 2)**.

There was a significant reduction in redness, discharge, crusty eyelids and conjunctiva edema relative to the control; seen as significant decrements (P≤0.001-0.004) in clinical scores of bacteria conjunctivitis (Table 2, Figure 1). However, tetracaine significantly reduced clinical signs (P=0.012) compared to normal saline (control) and 0.3% Ciprofloxacin reduced clinical signs (P =0.003) compared to normal saline (control). This means there was a significant decrease in clinical scores with the interventions as compared to the normal saline which showed minimal change in clinical score **(Figure 1, Table 2)**.

Table 2: The effect of Normal saline (control), Ciprofloxacin and Tetracaine on clinical scores per treatment schedule

Day	Clinical Scores		
	Normal Saline	Ciprofloxacin	Tetracaine
1	13.00±2.12	10.8±0.530	11.4±2.088
3	9.200±2.332	4.4±1.030	6.8±0.860
5	8.400±0.812	5.4±0.40	7.8±0.735
7	8.400±0.510	4.8±0.374*	3.8±0.735*
9	7.600±0.872	4.8±0.374	3.8±0.490*
11	8.600±0.927	3.8±0.735***	4.8±0.970
13	8.400±0.600	4.6±0.675**	3.6±0.400***
15	6.800±0.860*	4.2±0.490***	2.8±0.490***

Significant clinical score on treatment days established using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. * P≤0.05;**P≤0.01, P≤0.001.

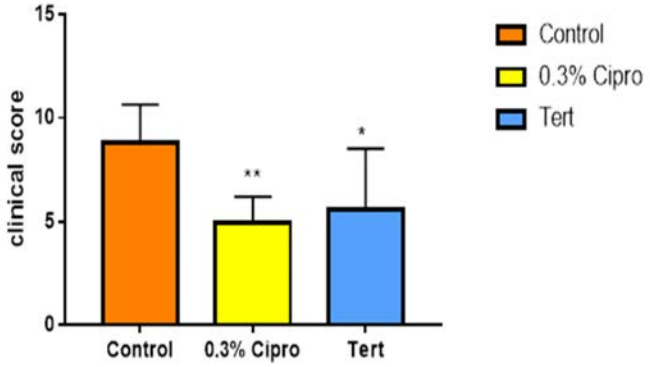

Figure 1: The effect of normal saline (control), 0.3% ciprofloxacin and tetracaine on clinical scores. Significant clinical score per treatment established using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. * P=0.012, **P=0.003.

Bacteria colony count: There was a significant decrease (P=0.002) in the bacteria colony count of rabbits treated with the Ciprofloxacin as compared to the control group (treated with normal saline) **(Figure 2)**. Tetracaine hydrochloride exhibited a significant (P=0.004) antibiotic effect on the multiple antibiotic-resistant S. aureus **(Table 3)**.

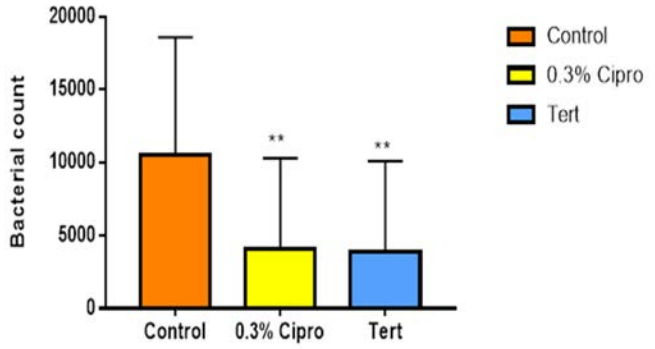

Figure 2: The effect of Normal saline (control), 0.3% Ciprofloxacin and Tetracaine on bacteria count after treatment. Significant reductions in bacteria count per treatment established using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. **implies P≤0.002-0.004.

Table 3: The effect of Normal saline (control), 0.3% Ciprofloxacin and Tetracaine on bacteria count per treatment schedule.

Day	Bacteria Count		
	Normal Saline	Ciprofloxacin	Tetracaine
1	22348±8749.50	19074.0±6279.15	18945.80±4411.30
3	23632±11391.15	4532.00±1441.023	3262.80±484.85
5	10406±3989.39	2766.00±688.56*	2718.00±199.16*
7	8944±3542.020	2542.20±694.61*	2177.00±302.29*
9	6694±29739.86	1415.00±950.65*	2087.20±387.39*
11	4828±100.10	1058.60±824.00*	1276.40±379.13*
13	4208±900.80	920.00±676.246*	617.40±283.05*
15	3168±463.41	337.80±173.10**	375.60±153.93**
Significant reductions in bacteria count per treatment days established using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. *P=0.002; **P=0.004			

Multiple antibiotic resistant index: The antibiotic susceptibility test did not show any significant multiple antibiotic-resistant in bacteria isolates treated with Tetracaine hydrochloride and normal saline (control). However, there was a significant increase in multiple antibiotic-resistance in isolates treated with Ciprofloxacin **(Figure3; Table 4)**.

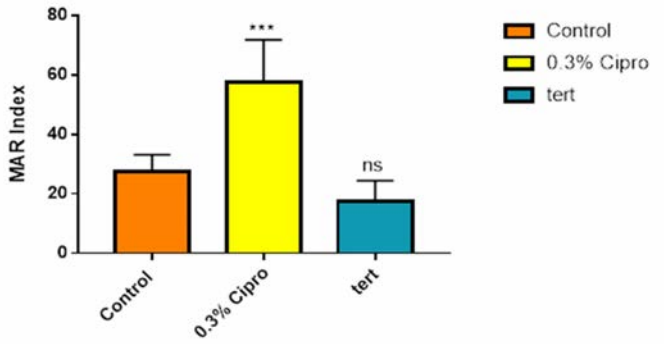

Figure 3: The MAR Indexes for all treatment groups. Significant reduction in MAR Index of treatment groups established using a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. **implies P≤0.01.

Table 4: The MAR Indexes for Normal saline, Ciprofloxacin, and Tetracaine treatment groups

Day	MAR Index values (%)		
	Normal Saline	Ciprofloxacin	Tetracaine
1	25	62.5	12.5
3	37.5	50	25
5	25	62.5	25
7	25	37.5	12.5
9	25	75	12.5
Average	27.500±2.500	57.500±6.374 **	17.500±3.062
One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. **implies P≤0.01. MAR, Multiple antibiotic resistance define MAR here			

Discussion

Bacterial conjunctivitis can be contracted directly from infected individuals or can result from abnormal proliferation of the native conjunctival flora (Azari & Barney, 2013). Contaminated fingers (Høvding, 2008), oculogenital spread (Varu et al., 2019) and contaminated fomites (Sattar, Dimock, Ansari, & Springthorpe, 1988) are common routes of transmission. Besides, certain conditions such as compromised tear production, disruption of the natural epithelial barrier, abnormality of adnexal structures, trauma, and immune-suppressed status predispose individuals to bacterial conjunctivitis (Varu et al., 2019). The most common pathogens for bacterial conjunctivitis in adults are Staphylococcal species, followed by *Streptococcus pneumoniae* and *Haemophilus Influenza* (Fishovitz, Hermoso, Chang, & Mobashery, n.d.). In children, the disease is often caused by Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis (Fishovitz et al., n.d.). The course of the disease usually lasts 7 to 10 days the reason for the decline in the clinical scores of conjunctivitis in the rabbits in the control group (Boadi-Kusi et al., 2021). Signs and symptoms include red eye, purulent or mucopurulent discharge, and chemosis (Fishovitz et al., n.d.). The period of incubation and communicability is estimated to be 1 to 7 days and 2 to 7 days, respectively (Leung & Rawal, 1977). Bilateral mattering of the eyelids and adherence of the eyelids, lack of itching, and no history of conjunctivitis were strong positive predictors of bacterial conjunctivitis (Sharma, 2011). This study aimed to investigate the antibiotic effect of Tetracaine hydrochloride on a resistant strain of bacteria to ascertain its contribution to the increasing spate of resistance to conventional antibiotics.

In this study resistant strain of Staphylococcus aureus was the causative agent of bacterial conjunctivitis. There was a significant reduction in clinical signs of bacterial conjunctivitis in the rabbits that were treated with Tetracaine hydrochloride ophthalmic solution. An earlier study (Reynolds, Greenwood-Quaintance, Patel, & Pulido, 2016) reported on the mechanism of action by which Tetracaine damaged the cell wall of bacteria through lysis, leakage of intracellular components, dehydrogenase activity and increased cell wall permeability.

A study on the effects of topical anesthetics on bacteria using a disk diffusion technique studied proparacaine and tetracaine at 0.5, 0.25, and 0.125% concentrations

found that tetracaine inhibited S. aureus growth at 5000 µg/mL and Pseudomonas aeruginosa at 2500 to 5000 µg/mL (Chiang, Penadés, & Chen, 2019). Also, a study using broth microdilution demonstrated that tetracaine inhibited strains of S. epidermidis at a concentration of 625µg/mL (Mullin & Rubinfeld, 1997). Tetracaine has demonstrated its antibiotic effects against coagulase-negative staphylococci and S. aureus (Johnson et al., 2008).

In the present study, bacterial colonies were counted from bacterial-induced conjunctivitis in rabbit eye treated with tetracaine and there was a significant reduction in bacteria count. This particular solution contained 0.5% Tetracaine hydrochloride as an active ingredient, a preservative which is Chlorobutanol and Boric Acid, Edetate Disodium, Potassium Chloride as inactive ingredients.

Bacteria may have innate resistance or acquired resistance to antimicrobial agents. This involves; mutations in cell genes (chromosomal mutation) leading to resistance, transformational gene transfer from one microorganism to another, conjugation which needs independent genetic elements including transposons (Tns) and plasmids and also transduction which involves independently replicating bacterial viruses, known as bacteriophages (Kaewjiaranai, Srisatjaluk, Sakdajeyont, Pairuchvej, & Wongsirichat, 2018). The current class of antimicrobial agents targets certain major bacterial processes such as metabolic pathways, cell wall, functions of the cell membrane, protein and nucleic acid synthesis (Rietveld, van Weert, ter Riet, & Bindels, 2003). After the bacteria have gained resistance genes against various antimicrobial agents, the bacteria can, therefore, use several biochemical types of resistance mechanisms including drug inactivation or enzymatic degradation involving enzymes that destroy the antibiotic. These enzymes include B-lactamases that cleave the amide bond in the B-lactam ring (Loka, Sumadja, & Resmi, 2017). Another mechanism is by extrusion of the antibiotic before it reaches the target by the efflux pump. This mechanism extrudes the antibiotic before it reaches the bacteria target-site (Kaewjiaranai et al., 2018). Target modification occurs by an altered bacteria cell wall which modifies the target region. An example is MRSA which involves a modified Penicillin Binding Protein (PBP2a) that confers methicillin resistance to certain S. aureus strains (Loka et al., 2017).

In this study, the antibiotic susceptibility test on bacteria isolates treated with 0.3% Ciprofloxacin showed multiple antibiotic-resistant which was not the case before treatment was commenced. There was no increase in multiple antibiotics resistant in groups treated with Tetracaine hydrochloride and normal saline. A study by Sharma (Sharma, 2011), among 2408 eyes in South Florida revealed an increase resistance of gram-positive by 2-fold and 3-fold to erythromycin and ciprofloxacin respectively. The primary disadvantage associated with treatment with an antibiotic is the probable future resistance. The findings of this study support the assertion that there exists a link between the consumption of antibiotics and subsequent resistance (Leibovici et al., 2001). This was not the case Tetracaine hydrochloride treatment given credence to the fact that the short to medium term diagnostic does not contribute to the growing spate of antibiotic resistance. The limitation to this study was that there were no genetic studies to confirm this finding.

Conclusion

Despite Tetracaine possessing supplemental antibiotic effect as a diagnostic agent, it has no consequential effect on the development of antibiotic-resistant strains of bacteria. It is recommended that further studies involving molecular methods be adopted to confirm this finding.

Declarations

Ethical considerations: The study protocol was approved by the University of Cape Coast Institutional Review Board, UCCIRB, Ghana with reference number: UCCIRB/CHAS/2018/57. All activities performed during the studies conformed to the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. Biosafety guidelines for the protection of personnel in the laboratory were observed.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Authors' contributions: Author SK conceived the idea, designed the study and wrote the protocol. SBB-K managed the analyses and the interpretation of data. ME-M managed the literature searches, collected data, and wrote the first draft of the manuscript. All authors read, critically revised the content and approved the final manuscript.

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RESEARCH ARTICLE

Assessment of Kidney Failure Risk Equation among Chronic Kidney Disease Patients: A cross-sectional study

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Abstract

Background: Patients with chronic kidney disease (CKD) are at increased risk for kidney failure, cardiovascular events, and both cause mortality. Accurate models are needed to predict and identify patients at risk of progression to kidney failure which may facilitate more optimal nephrology care.

Objective: This study assessed the kidney failure risk equation among CKD patients to predict when they need to start renal replacement therapy.

Materials and methods: Total of 38 CKD patients were recruited from the renal unit of the Cape Coast Teaching Hospital. Estimated glomerular filtration rate (eGFR) and albumin creatinine ratio were measured from each patient. Information on age, gender, body mass index, blood pressure, cause of CKD and type of medication on were obtained from the patient's medical records. The 4-variable Kidney Failure Risk Equation (KFRE) was used to assess the risk of progression to kidney failure among the participants. The KFRE uses 4-variables (age, sex, estimated GFR, albumin).

Results: For both 2-year and 5-year risk of progression, serum creatinine and ACR increases as the risk increases from participants with low risk of progression through to high risk. Mean eGFR decreased from participants with low risk of progression through to those with high risk. Serum creatinine and ACR positively correlated with both 2-year and 5-year risk progression. eGFR, on the other hand, negatively correlated with both 2-year and 5-year risk progression. Participants with high 2- year and 5-year risk were on Linopril/Calcitonin/Atorvostatin drug combination, while participants with low and intermediate risks for both 2-year and 5-year were on lisinopril only.

Conclusion: KFRE was able to discriminate which participants have low, intermediate and high 2-year and 5-year risk of progression to end stage kidney failure and which participants need to start renal replacement therapy.

Keywords: Chronic kidney disease, End stage kidney disease, Kidney failure risk equation, Renal replacement therapy, Risk of progression.

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Introduction

Chronic kidney disease (CKD) is a global health concern and adequate preparation is required to provide education, consider renal replacement therapy, mortality option and plan for the initiation of renal therapy. CKD describes the persistent urine and structural abnormalities or impaired excretory renal function which is suggestive of a loss of functional nephrons. CKD is an adverse clinical condition because renal impairment may prelude to the development of end-stage renal disease (ESRD) requiring the need for dialysis and transplantation (Zoccali, Kramer, & Jager, 2009). Also, CKD amplifies the risk for cardiovascular complication (Chobanian et al., 2003). It is reported that patients with stage 4–5 CKD, independent of other risk factors have a death risk for cardiovascular complications which is 2–4 times higher than that of the coeval general population, whilst patients with ESRD have absolute higher risk (Baigent, Burbury, & Wheeler, 2000). Worldwide, people have predicted when CKD patients would start

dialysis or transplantation in order to put in measures to reduce the rate of progression of CKD to kidney failure (Tangri, 2017).

Cases of CKD across sub Saharan Africa keeps increasing due to the ever-increasing number of predisposing factors (Nahas, 2005). Instigated by CKD and ESRD in both adults (Kimmel & Patel, 2006) and children (McKenna et al., 2006) are increased mortality, morbidity and poor quality of life which further imposes high direct and indirect costs to society. The situation is further compounded by the low number of primary health care facilities and the lack of specialist personnel to manage cases of CKD. The kidney function risk equation developed by Tangri and colleagues (Tangri, 2017) is useful in predicting when one will need to start renal replacement therapy. This will enable nephrologists to put in measures to reduce the rate of progression of CKD which will help reduce some of the financial stress associated with renal replacement therapy. However, data on the use of such a tool especially across Sub-Saharan Africa is scanty. Thus, we assessed the kidney function risk equation among CKD patients to predict when they need to start dialysis or transplantation.

Materials and Methods

Study design: A cross-sectional study design was used to recruit CKD patients from the renal unit of Cape Coast Teaching Hospital. Currently, the hospital is a 400-bed capacity referral hospital in the region. The hospital also serves as the teaching and practical biomedical site for several nursing and allied health programmes. Presently, the hospital has accreditation for the training of resident physicians in Internal Medicine and Surgery.

Study Population: Thirty-eight (38) CKD patients were recruited from the renal unit of the Cape Coast Teaching Hospital. Both gender (male and female) with stages 1, 2, 3 CKD patients and are undergoing treatment were recruited as participants for the study.

Eligibility criteria/Ethical clearance: Participants with CKD stage 1, 2 and 3 with written informed consent were enrolled in the study. Patients with CKD stage 4, end stage kidney failure, on dialysis and those who have been transplanted were excluded from this study. Ethical clearance was sought from the Ethical Review Committee of Cape Coast Teaching Hospital, Cape Coast and written consent was also obtained from all the participants.

Experimental Protocols

Blood Sample Collection: After 8 – 12 hours overnight fast, two (2) ml of venous blood was drawn from each participant and a drop was used for fasting blood glucose (FBG) was estimated with ACCU-CHEK glucometer (manufactured by Roche Diabetes Care GmbH, Germany). The blood was put in a gel tube and allowed to clot and serum separated and stored. Serum creatinine was measured using the automated Selectra Pro S Clinical Chemistry analyzer (manufactured by ELITechGroup, Puteaux France). eGFR was calculated from the creatinine values for each participant using Chronic Kidney Disease Epidemiological Collaboration Equation, CKD-EPI.

Measurement of Albumin– Creatinine ratio (ACR): About 10 ml of a clean-catch urine sample was collected from each participant into a dry, sterile wide-mouth container. Semi quantitative CLINITEK albumin and creatinine strips (SIEMENS Healthineers, Germany) was used to detect the amount of albumin and creatinine in the urine sample to get the albumin creatinine ratio.

Using the Kidney Failure Risk Equation: The Kidney Failure Risk equation (KFRE) models are based on laboratory data and demographic variables that predicts the risk of developing kidney failure. (Tangri et al., 2011). The sex, age, eGFR and albumin creatinine ratio of each participant was input into the KFRE to calculate for Kidney failure in over 2 years and over 5 years (renal). The formulas are:

$P = 1\text{-}S_{ave}$ (t=1,826)

$a = -0.55418x[(eGFR/5)\text{-}7.22] + 0.26940 \times (male\text{-}0.56) + 0.45608 \times [in\ (ACR) - 5.2774]\text{-}0.21670 \times [(age/10) - 7.04]$

Where:

- p = Five-year risk of kidney failure
- S_{ave} (t=1826) = Five-year survival rate for an individual with the average value of covariates in the risk equation and was 0.929 in the development dataset
- eGFR = Estimated Glomerular Filtration Rate (eGFR) (ml/min/1.73m2)
- ln (ACR) = Natural logarithm of Albumin-Creatinine Ratio (ACR) (mg/g)
- male = Indicator for sex (male = 1, female = 0)
- age = Age (years) at test date

Data Analysis: Independent t-test was used to compare mean scores between two groups. One-way ANOVA was also employed to compare the mean scores of more than two groups. Correlation (Pearson correlation) and linear regression analyses were also performed. P<0.05 was considered statistically significant. Data was analyzed with SPSS version 16 (SPSS Inc. Chicago).

Results

Table 1 shows the general characteristics of study participants stratified by gender. Most (77.8%) of the males had normal weight, while most of the females (45%) were obese. All the males and most (60.0%) of the females had significantly high creatinine concentration (P<0.005).

Table 1: General characteristics of the study participants.

Parameter	Total (N=38)	Male (N=18)	Female (N=20)	P-value
Age (years)	58.50±13.60	60.72±12.55	56.50±14.50	0.346
Age group (years)				
21-35	3 (7.9)	1 (5.6)	2 (10.0)	0.644
36-50	7 (18.4)	3 (16.7)	4 (20.0)	
51-65	15 (39.5)	6 (33.3)	9 (45.0)	
>65	13 (34.2)	8 (44.4)	5 (25.0)	
Diagnosis				
Diabetes	6 (15.8)	3 (16.7)	3 (15.0)	0.888
Hypertension	32 (84.2)	15 (83.3)	17 (85.0)	
BP (mmHg)				
Optimal	3 (7.9)	1 (5.6)	2 (10.0)	0.688
Normal	2 (5.3)	1 (5.6)	1 (5.0)	

Prehypertension	2 (5.3)	1 (5.6)	1 (5.0)	
Hypertension	21 (55.3)	12 (66.7)	9 (45.0)	
ISH	10 (26.3)	3 (16.7)	7 (35.0)	
BMI (Kg/m2)				
Normal	17 (44.7)	14 (77.8)	3 (15.0)	<0.001
Overweight	12 (31.6)	4 (22.2)	8 (40.0)	
Obese	9 (23.7)	0 (0.0)	9 (45.0)	
FBG (mmol/l)				
Normal	31 (81.6)	15 (83.3)	16 (80.0)	0.791
High	7 (18.4)	3 (16.7)	4 (20.0)	
Creatinine (µmol/l)				
Normal	8 (21.1)	0 (0.0)	8 (40.0)	0.003
High	30 (78.9)	18 (100.0)	12 (60.0)	
eGFR (ml/min/1.72m2)				
≥90	1 (2.6)	0 (0.0)	1 (5.0)	0.352
60-89	2 (5.3)	0 (0.0)	2 (10.0)	
45-59	13 (34.2)	7 (38.9)	6 (30.0)	
30-44	14 (36.8)	8 (44.4)	6 (30.0)	
15-29	7 (18.4)	2 (11.1)	5 (25.0)	
<15	1 (2.6)	1 (5.6)	0 (0.0)	
ACR (mg/g)				
<30	8 (21.1)	5 (27.8)	3 (15.0)	0.335
30-300	30 (78.9)	13 (72.2)	17 (85.0)	
FBG = Fasting Blood Glucose ACR = Albumin Creatinine ratio ISH = Isolated Systolic Hypertension BMI = Body Mass Index				

Figure 1 illustrates the distribution of 2-year and 5-year progression to kidney failure risk among participants. For the 2-year risk of progression, 94.7%, 2.6%, 2.6% of the participants had low risk, intermediate risk and high risk of progression respectively whereas for the 5-year risk of progression, 68.4%, 18.4%, 13.2% of the participants had low risk, intermediate risk and high risk of progression respectively and were statistically significant (P=0.012).

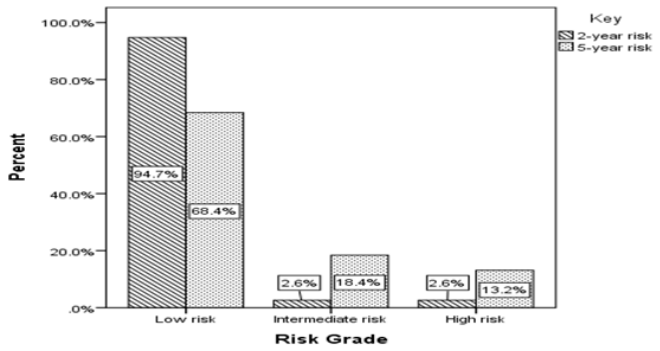

Figure 1: Distribution of 2-year and 5-year progression to kidney failure among participants (P=0.012).

Figure 2 presents the 2-year risk progression of participants in relation to gender. For the males, 94.4%, 0%, 5.6% of the participants had low, intermediate, and high risk of progression respectively whereas for females, 95.0%, 5.0%, 0.0% of the participants had low, intermediate, and high risk of progression respectively and were statistically insignificant (P=0.366).

Figure 3 presents 5-year risk progression of participants in relation to gender. For the males, 66.7%, 16.7%, 16.7% of the participants had low, intermediate, and high risk of progression respectively whereas for females, 70.7%, 20.0%, 10.0% of the participants had low, intermediate, and high risk of progression respectively and were statistically insignificant (P=0.822).

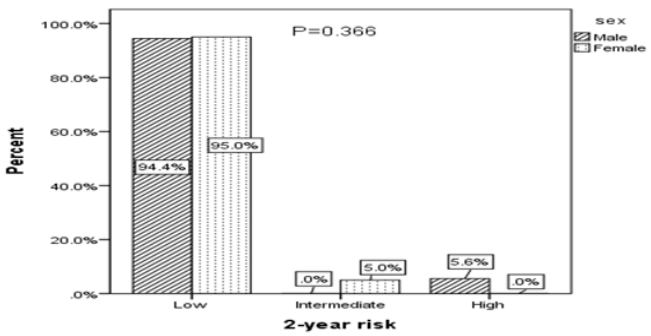

Figure 2: Two-year risk progression of participants in relation to gender.

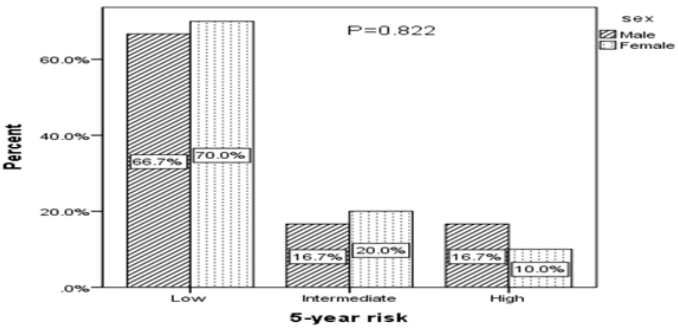

Figure 3 Five-year risk progression of participants in relation to gender.

Table 2 presents the socio-demographic, and distribution of clinical and biochemical characteristics of participants stratified by 2-year kidney failure risk. Most (41.7%) of the participants with low 2-year risk progression were within 51-65 years. The participants with intermediate risk and high risk were within 21-35 years and 36-50 years respectively.

Table 2 Socio-demographic, and distribution of clinical and biochemical characteristics of participants stratified by 2-year kidney failure risk.

Parameter	Low (N=36)	Inter mediate (N=1)	High (N=1)	P-value
Age (years)	59.47±13.20	34.00±0.00	48.00±0.00	0.133
Age group (years)				
21-35	2 (5.6)	1 (100.0)	0 (0.0)	0.011
36-50	6 (16.7)	0 (0.0)	1 (100.0)	
51-65	15 (41.7)	0 (0.0)	0 (0.0)	
>65	13 (36.1)	0 (0.0)	0 (0.0)	
Diagnosis				
Diabetes	6 (16.7)	0 (0.0)	0 (0.0)	0.820
Hypertension	30 (83.3)	1 (100.0)	1 (100.0)	
BP (mmHg)				
Optimal	3 (8.3)	0 (0.0)	0 (0.0)	0.989
Normal	2 (5.6)	0 (0.0)	0 (0.0)	
Prehypertension	2 (5.6)	0 (0.0)	0 (0.0)	
Hypertension	19 (52.8)	1 (100.0)	1 (100.0)	
ISH	10 (27.8)	0 (0.0)	0 (0.0)	
BMI (Kg/m2)				
Normal	16 (44.4)	0 (0.0)	1 (100.0)	0.487
Overweight	11 (30.6)	1 (100.0)	0 (0.0)	
Obese	9 (25.0)	0 (0.0)	0 (0.0)	
FBS (mmol/l)				
Normal	29 (80.6)	1 (100.0)	1 (100.0)	0.788

High	7 (19.4)	0 (0.0)	0 (0.0)	
Creatinine (µmol/l)				
Normal	8 (22.2)	0 (0.0)	0 (0.0)	0.755
High	28 (77.8)	1 (100.0)	1 (100.0)	
eGFR (ml/min/1.72m2)				
≥90	1 (2.8)	0 (0.0)	0 (0.0)	0.001
60-89	2 (5.6)	0 (0.0)	0 (0.0)	
45-59	13 (36.1)	0 (0.0)	0 (0.0)	
30-44	14 (38.9)	0 (0.0)	0 (0.0)	
15-29	6 (16.7)	1 (100.0)	0 (0.0)	
<15	0 (0.0)	0 (0.0)	1 (100.0)	
ACR (mg/g)				
<30	8 (22.2)	0 (0.0)	0 (0.0)	0.755
30-300	28 (77.8)	1 (100.0)	1 (100.0)	
Drug used				
Lisinopril	26 (72.2)	0 (0.0)	0 (0.0)	0.006
Lisinopril+Metformin	6 (16.7)	0 (0.0)	0 (0.0)	
Lisinopril+Atorvostatin	2 (5.6)	0 (0.0)	0 (0.0)	
Linopril+Calciton+Atorvostatin	2 (5.6)	1 (100.0)	1 (100.0)	

Majority (91.7%) with low risk had eGFR ranging from 30 to 60. Participants with intermediate risk and high risk had eGFR of 15-29 and less than 15 respectively. Both participants with intermediate and high 2-year risk were on Lisinopril/Calciton/Atorvostatin drug combination while majority (72.2%) of low risk participants were on lisinopril.

Mean clinical and biochemical characteristics of participants in relation to 2-year risk of kidney failure is shown in **Table 3**. Mean serum creatinine [140.48±37.72; 242.50±0.00; 838.00±0.00, P<0.05] and ACR [35.56±15.53; 60.00±0.00; 150.00±0.00, P<0.0001] significantly increased as the risk increased from participants with low risk through to high risk. Mean eGFR significantly decreased from participants with low risk through to high risk [44.36±15.77; 21.70±0.00; 5.80±0.00, P=0.031].

Table 3: Mean clinical and biochemical characteristics of participants in relation to 2-year risk of kidney failure.

Parameter	Low (N=36)	Inter mediate (N=1)	High (N=1)	P-value
SBP (mmHg)	158.08±24.49	140.00±0.00	180.00±0.00	0.517
DBP (mmHg)	89.97±11.76	90.00±0.00	110.00±0.00	0.257
Weight (Kg)	68.19±9.29	72.00±0.00	69.00±0.00	0.919
BMI (Kg/m2)	26.87±5.58	28.48±0.00	23.20±0.00	0.786
FBG (mmol/l)	6.19±1.46	5.90±0.00	6.00±0.00	0.973
Creatinine (µmol/l)	140.48±37.72	242.50±0.00	838.00±0.00	<0.001
eGFR (ml/min/1.72m2)	44.36±15.77	21.70±0.00	5.80±0.00	0.031
ACR (mg/g)	35.56±15.53	60.00±0.00	150.00±0.00	<0.001

Table 4 presents the socio-demographic, and distribution of clinical and biochemical characteristics of participants stratified by 5-year kidney failure risk. Majority (88.4%) with low risk had eGFR less than 60. All participants with intermediate risk and high risk had eGFR ranging from 30 to 60. Majority (80%) with high 5-year risk were on Linopril/Calciton/Atorvostatin drug combination. Most of the participants with low (84.6%) and intermediate (57.1%) risks were on lisinopril.

Mean clinical and biochemical characteristics of participants in relation to the 5-year risk of kidney failure is presented in **Table 5**. Mean serum creatinine [125.09±28.10; 166.14±18.53; 344.60±276.83, P<0.001] and ACR [29.80±10.44; 41.43±9.00; 85.00±37.42, P<0.001] significantly increased from participants with low 5-year risk progression through to high risk. Mean eGFR significantly decreased in participants with intermediate and high 5-year risk progression compared to those with low risk [49.62±15.41; 32.67±3.69; 21.16±8.84, P<0.001].

Table 6 shows the correlation of age, clinical and biochemical characteristics of participants with 2- and 5-year kidney failure risk. Serum creatinine and ACR positively correlated with both 2-year and 5-year risk progression (P<0.05). eGFR on the other hand, negatively correlated with both 2-year and 5-year risk progression (P<0.05).

Table 4: Socio-demographic, and distribution of clinical and biochemical characteristics of participants stratified by 5-year kidney failure risk.

Parameter	Low (N=26)	Inter mediate (N=7)	High (N=5)	P-value
Age (years)	61.23±12.43	54.00±16.94	50.60±12.36	0.176
Age group (years)				
21-35	1 (3.8)	1 (14.3)	1 (20.0)	0.327
36-50	3 (11.5)	2 (28.6)	2 (40.0)	
51-65	13 (50.0)	1 (14.3)	1 (20.0)	
>65	9 (34.6)	3 (42.9)	1 (20.0)	
Diagnosis				
Diabetes	5 (19.2)	1 (14.3)	0 (0.0)	0.554
Hypertension	21 (80.8)	6 (85.7)	5 (100.0)	
BP (mmHg)				
Optimal	2 (7.7)	1 (14.3)	0 (0.0)	0.859
Normal	1 (3.8)	1 (14.3)	0 (0.0)	
Prehypertension	2 (7.7)	0 (0.0)	0 (0.0)	
Hypertension	14 (53.8)	3 (42.9)	4 (80.0)	
ISH	7 (26.9)	2 (28.6)	1 (20.0)	
BMI (Kg/m2)				
Normal	11 (42.3)	3 (42.9)	3 (60.0)	0.771
Overweight	8 (30.8)	2 (28.6)	2 (40.0)	
Obese	7 (26.9)	2 (28.6)	0 (0.0)	
FBS (mmol/l)				
Normal	19 (73.1)	7 (100.0)	5 (100.0)	0.138
High	7 (26.9)	0 (0.0)	0 (0.0)	
Creatinine (µmol/l)				
Normal	8 (30.8)	0 (0.0)	0 (0.0)	0.096
High	18 (69.2)	7 (100.0)	5 (100.0)	
eGFR (ml/min/1.72m2)				
≥90	1 (3.8)	0 (0.0)	0 (0.0)	<0.001
60-89	2 (7.7)	0 (0.0)	0 (0.0)	
45-59	13 (50.0)	0 (0.0)	0 (0.0)	
30-44	9 (34.6)	5 (71.4)	0 (0.0)	
15-29	1 (3.8)	2 (28.6)	4 (80.0)	
<15	0 (0.0)	0 (0.0)	1 (20.0)	
ACR (mg/g)				
<30	8 (30.8)	0 (0.0)	0 (0.0)	0.096
30-300	18 (69.2)	7 (100.0)	5 (100.0)	
Drug used				
Lisinopril	22 (84.6)	4 (57.1)	0 (0.0)	<0.001
Lisinopril+Metformin	4 (15.4)	1 (14.3)	1 (20.0)	
Lisinopril+Atorvostatin	0 (0.0)	2 (28.6)	0 (0.0)	
Linopril+Calciton+Atorvostatin	0 (0.0)	0 (0.0)	4 (80.0)	

Table 6: Correlation of age, clinical and biochemical characteristics of participants with 2- and 5-year kidney failure risk

Parameters		Age	SBP	DBP	BMI	FBS	CR	GFR	ACR
2-year risk	R	-0.191	0.144	0.281	-0.093	-0.054	0.977	-0.47	0.845
	P	0.252	0.389	0.087	0.579	0.746	<0.001	0.003	<0.001
5-year risk	R	-0.238	0.135	0.278	-0.076	-0.086	0.971	-0.556	0.890
	P	0.149	0.421	0.091	0.652	0.608	<0.001	<0.001	<0.001

Table 5 Mean clinical and biochemical characteristics of participants in relation to 5-year risk of kidney failure.

Parameter	Low (N=36)	Inter mediate (N=1)	High (N=1)	P-value
SBP (mmHg)	157.73±23.13	151.57±31.42	169.80±19.45	0.445
DBP (mmHg)	90.00±11.26	87.29±15.33	97.60±8.99	0.319
Weight (Kg)	69.15±9.14	67.57±9.98	65.00±8.22	0.637
BMI (Kg/m2)	26.76±5.23	28.01±7.89	25.42±2.54	0.727
FBG (mmol/l)	6.44±1.64	5.53±0.52	5.72±0.36	0.242
Creatinine (µmol/l)	125.09±28.10	166.14±18.53	344.60±276.83#	<0.001
eGFR (ml/ min/1.72m2)	49.62±15.41	32.67±3.69*	21.16±8.84*	<0.001
ACR (mg/g)	29.80±10.44	41.43±9.00	85.00±37.42*#	<0.001

*: statistically significant from low risk group; #: statistically significant from intermediate risk group

Discussion

This study seeks to assess the kidney failure risk equation among CKD patients and to predict when they need to start dialysis or transplantation. The study showed that the KFRE was able determine which proportion of patients are likely to progress to low, intermediate and high kidney failure over the next 2 to 5 years. For both 2-year and 5-year risk of progression, serum creatinine and ACR increased as the risk increased from participants with low risk of progression through to high risk. Mean eGFR on the other hand decreases from participants with low risk of progression through to high risk. Serum creatinine and ACR positively correlated with both 2-year and 5-year risk progression. eGFR, on the other hand, negatively correlated with both 2-year and 5-year risk progression. Participants with high 2-year and 5-year risk were on Linopril/Calciton/Atorvostatin drug combination, while participants with low and intermediate risks for both 2-year and 5-year were on lisinopril only. For 2-year risk of progression, 94.7%, 2.6%, 2.6% of the participants had low risk, intermediate risk and high risk of progression respectively. For 5-year risk of progression, 68.4%, 18.4%, 13.2% of the participants had low risk, intermediate risk and high risk of progression respectively.

The abbreviated 4-variable KFRE was able to discriminate which patients need to start dialysis or transplantation. For the 5-year risk of progression, the KFRE showed that 5 of the participants need to start dialysis and for the 2-year risk of progression, it showed 1 of the participants need to start dialysis.

With respect to the 2-year risk progression in relation to gender, 94.4%, 0%, 5.6% of the male participants had low, intermediate, and high risk of progression respectively. For the females, 95.0%, 5.0%, 0.0% of the participants had low, intermediate, and high risk of progression respectively and were statistically insignificant (P=0.366). With regards to the 5-year risk progression, 66.7%, 16.7%, 16.7% of the male participants had low, intermediate, and high risk of progression respectively whereas for females, 70.7%, 20.0%, 10.0% of the participants had low, intermediate, and high risk of progression respectively and were statistically insignificant (P=0.822). In this study, the risk of progression of CKD to end stage kidney failure is higher in males than in females which is similar to findings by Kaiser Permanente and the RENAAL study group (Nephrology A clinical trial–Reduction in Endpoints in patients with Non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan), who reported that a lower estimated eGFR, higher albuminuria, younger age, and male sex predict faster progression to kidney failure (Acedillo, Tangri, & Garg, 2013).

Most (41.7%) of the participants with low 2-year risk progression were within 51-65 years. The participants with intermediate risk and high risk were within 21-35 years and 36-50 years respectively. Majority (91.7%) with low risk had eGFR between 30 to 60. Participants with intermediate risk and high risk had eGFR of 15-29 and less than 15 respectively. CKD in younger age in the midst of Lower estimated eGFR, higher albuminuria and male sex has a faster progression rate to kidney failure than old age (Acedillo et al., 2013).

Both participants with intermediate and high 2-year and 5-year risk were on Lisinopril/Calciton/Atorvostatin drug combination while participants with low risk were on only lisinopril. Most CKD patients with high risk progression to kidney failure have symptoms like high blood pressure, hypocalcemia, and high cholesterol level (Crook, 2012) whereas low risk patients are mostly hypertensives and asymptomatic (Sanguankeo, Upala, Cheungpasitporn, Ungprasert, & Knight, 2015) as such, medications and symptoms are to be carefully monitored in these state.

Mean serum creatinine and ACR significantly increased as the risk increased from participants with low 2-year risk progression through to high risk whereas mean eGFR significantly decreased from participants with low 2-year risk progression through to high risk. Mean serum creatinine and ACR significantly increased from participants with low 5-year risk progression through to high risk whereas mean eGFR significantly decreased in participants with intermediate and high 5-year risk progression compared to those with low risk. In a study by (Tangri, 2017), the mean serum creatinine level and eGFR were 2.30 mg/dl (203.32 umol/l) and 31 ml/min/1.72m2 respectively. The serum creatinine was very high in the CKD patients and the eGFR was very low in the same population. This was consistent with our study as majority (78.94%) of the participants had high mean serum creatinine (161.53 umol/l) and most (92.10%) had low mean eGFR (42.75 ml/min/1.72m2). The difference in the mean eGFR and serum creatinine may be due to the low sample size used in this study. A eGFR level less than 60 mL/min per 1.73 m2 represents loss of half or more of the adult level of normal kidney function. Below this level, the prevalence of complications of chronic

kidney disease increases (Levey, 2000).

Serum creatinine and ACR positively correlated significantly with both 2-year and 5-year risk progression. eGFR, on the other hand, significantly correlated negatively with both 2-year and 5-year risk progression.

This study, though the first in the sub-Saharan region had a few limitations. First, the small sample size used which was due to the small number of CKD patients at the renal unit of Cape Coast Teaching Hospital. Second, ACR was estimated using semi quantitative albumin dipstick instead of High Performance Liquid Chromatography which is relatively more sensitive. Third, though the KFRE should be used in patients with CKD stage 1 to stage 3, this study had participants with stages 4 and 5 which could be due to some participants who could not afford renal replacement therapy.

Conclusion

The KFRE was able to identify participants with both two- and five-year risk of progression to end stage renal failure. The KFRE showed an association with gender, serum creatinine, ACR eGFR, and medication used. Prospective studies are needed to verify whether its implementation can result in better resource allocation of nephrology team CKD care.

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RESEARCH ARTICLE

Patient Referral Pattern in Northern Ghana: A Retrospective Study of the Tamale Teaching Hospital

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Abstract

Background: Patient referral involves transferring the responsibility for the care of a patient from one level of care to the other. Several factors account for the referral of patients, and all categories of patients are referred.

Objective: This study sought to assess the most common medical and surgical conditions that are referred, as well as the demographics of patients mostly involved in medical referrals.

Materials and Methods: A retrospective cross-sectional study was conducted to examine the patterns of patient referral from peripheral facilities into the Tamale Teaching Hospital in 2021. Data on patient referral into the facility, including medical condition, age, gender, and other demographic information within a period of one year was retrieved from the nursing department under the consent of the nurse manager and analyzed using SPSS version 23.

Results: A total of 1565 referrals were made into the facility within the period under review. The main reasons for referral were to perform diagnostic investigations and for further management. Majority of the patients (53%) referred to facility were males within the ages of 20-49years. The commonest conditions that were referred were head injuries (19.9%) followed by fractures (12.8) most of which were sustained through road traffic accidents. Eighty two percent (82%) of the attempted referrals were successful whilst 12% was rejected for various reasons including improper referral procedures, whilst the remaining 6% were to call back after some issues have been resolved.

Conclusion: There is a high rate of patient referral in northern Ghana, most of whom are as a result of head injuries and fractures resulting from road traffic accidents.

Keywords: Conditions, Ghana, Hospital, Patients, Referral pattern.

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Introduction

Patient referral has been defined as the process of transferring the responsibility for the overall or partial care of a patient, temporarily or permanently to another health provider (At, Allied, & Emergency, n.d.). Most cases of patient referrals are sent to the next level in the health care system. Some few instances however warrant patients be sent to a lower-level facility either for specialist care or on patient request. The ministry of health in their referral policy document alluded to the fact that, the Ghanaian referral system is bedeviled with various challenges such as inadequate primary health care facilities, lack of confidence in first level facilities, lack of standard procedures for referrals, delays in referrals, not using referral forms, negative perceptions on referrals and lack of feedback by receiving facilities (Referral-Policy-Guidelines.Pdf, n.d.). Efficient referral system is an indication of robust health sector and specifically an indicator of the effectiveness of the primary health care in the country (Steinmann, Baimatova, & Wyss, 2012). A successful referral demands an inter-facility collaboration, cooperation and information transfer. It indicates the health worker's ability to identify signs of a severe or worsening condition at an early stage (Referral-Policy-Guidelines. Pdf, n.d.). The World Health Organization developed the Integrated Management of Childhood Infections (IMCI) as an effective guideline for the management of childhood infections for children between the ages of 2 months to 59 months. An effective referral is an integral part of the IMCI guideline (Beyene, Kassa, Tadele, & Persson, 2021).

Various forms of patient referral exists in health care, including internal referral, which is referral within the same health facility, external referral which involves two health facilities and external referral which sends the patient overseas (Referral-Policy-Guidelines.Pdf, n.d.). Other forms such as the emergency or unplanned referral

describes the transfer of an acutely ill patient, while planned or routine referral transfers a patient for expert opinion, for admission or investigative purposes on a planned basis (Khoja, Shehri, Abdul-Aziz, & Aziz, n.d.).

Excessive or over referral tend to burden and overwhelm receiving facilities such as teaching hospitals. Again, it results in unwarranted medical investigation and procedures, increasing cost. Under referral of the other hand mostly result in medical complications that could have been avoided with specialty care. This variability in over and under referral stems from the lack of certainty on the appropriate referral practice for certain medical conditions (13). The ministry of health policy on referral however states that, all emergency referrals must be received and at least first aid treatment administered. No health facility is allowed to turn away a patient on emergency referral (Referral-Policy-Guidelines.Pdf, n.d.). However, management of medical emergencies are poorly organized, and the essential resources are mostly not available at the receiving facilities. Several factors account for referral of patients from one health facility to the other. Major medical factors sighted in available literature include to get expert opinion about a medical therapy, to obtain assistance in diagnosing and to confirm a diagnosis, patient's condition been too complicated for a general practitioner to manage, to perform a therapeutic procedure and to perform a diagnostic procedure. Non-medical factors that may warrant a referral includes to meet the stipulated guideline in patient care, to fulfill patient's request, to motivate patient to adhere to medical advice and to benefit medical trainees working with a specialist (Donohoe et al., n.d.).

This study sought to identify the most common medical and surgical conditions that are referred into the accident and emergency unit of the Tamale Teaching Hospital as well as the demographics of patients mostly involved in these referrals.

Materials and Methods

Study Design and Setting: This is a retrospective study record review that was conducted at the Accident and Emergency unit of the Tamale Teaching Hospital. The Tamale Teaching Hospital is the third largest teaching hospital in Ghana, and the only tertiary health facility in the northern part of Ghana. The facility receives referral cases from the five regions of northern Ghana as well as from parts of the middle belt. The hospital was established in 1974 to serve as a regional hospital for the then northern region. The hospital was however upgraded to the status of a teaching hospital in 2005 with over 480 bed capacity (Dosoo & Adongo, 2020).

As the third largest tertiary health facility in the country, the hospital has specialized medical, surgical, and gynecological departments. As a teaching hospital, the facility engages in the training of all calibers of health personnel as well as a center for medical research.

The accident and emergency ward is an international standard emergency ward with approximately 50 bed capacity. The ward is divided into four units, often referred to as zones which includes triage, red, orange, and yellow zone. Patients are admitted into the various zones according to the severity of their conditions which is decided by their triage scores. The ward manages both adult and pediatric medical and surgical conditions. The accident and emergency unit abides by the ministry of health's policy on referral for health facilities. The unit has a 24-hour communication system to which referral facilities are expected to call and present cases prior to referrals. The unit receives over a thousand patients annually, both referrals and walk-in-patients.

Data Collection and Analysis: The accident and emergency ward has a robust communication system through which all peripheral health facilities call in to inform and present cases before referrals are made. Information on any call received is documented by the receiving nurse, which is compiled and made available at the nurse manager's office. This information was retrieved and analyzed. All telephone calls received concerning patient referrals to the ward were included in the study. Data collected during this telephone conversation includes patient's name, age, sex, condition and vital signs of patient, referral diagnosis, name of referral hospital and location of referral hospital. Information on patients that were referred into the ward from October 2020 to October 2021, with a total 1565 patients was retrieved and analyzed with the permission of the ward management. Statistical Package for Social Sciences (SPSS) version 23 was used to analyze the data. Data was analyzed using frequencies and charts.

Ethical consideration: Permission to use the data was requested from the nursing department of the facility as the data was readily available. Permission was subsequently granted before the data was released and analyzed for publication. Consent from participants was not possible as it was a retrospective study and participants could not be reached.

Results

A total of 1565 patients including males, females, children, adults and the aged were referred into the ward within the period under review. The referral of 273 cases were unsuccessful for various reasons. Various conditions including medical, surgical as well as cases of road traffic accidents (RTA) were referred to the unit. Majority (21%) of the patients referred to the ward were between the ages of 20-29 years. This was closely followed by those aged 30-39 years which amounted to 18%, then 14% of the patients were within the age bracket of 40-49 years. The aged constituted the least age group with those above 90 years being only 3 patients whilst 4% were within 80-89 years group **(Figure 1)**.

Majority of the patients that were referred into the ward within the period under review were males. With a population of 1064 patients, the male gender constituted 69% of patients referred into the emergency ward of the Tamale Teaching Hospital, whilst the female gender made up the remaining 31% **(Figure 2)**.

Most of the cases referred into the ward within the period of

review were surgical cases. This was made up 941 patients which constituted 62% whilst medical cases were 583, constituting 62%. Patients that were referred as a result of road traffic accidents 594, constituting 39%.

With regard to referral diagnosis from referring or peripheral facilities, patients that were referred in with a referral diagnosis of head injuries were 311, which constituted 19.9%. Patients referred in with fractures were 200, whilst those with other surgical conditions such as acute abdomen, lacerations, burns, hernias, intestinal obstructions, gastric outlet obstruction were 449 which constituted 28.7%. cardiovascular conditions such as hypertension, cardiovascular accident (CVA) and heart failures constituted 16.7% **(Table 1)**.

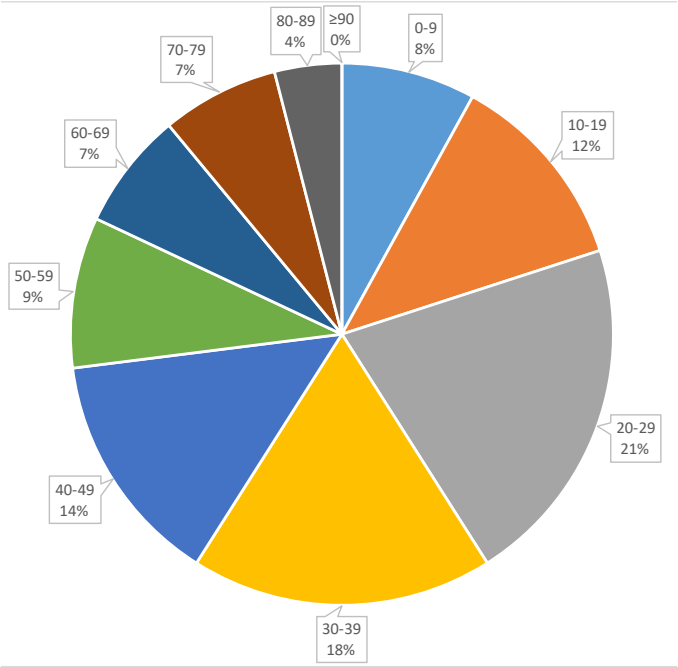

Figure 1: Age distribution of study participants.

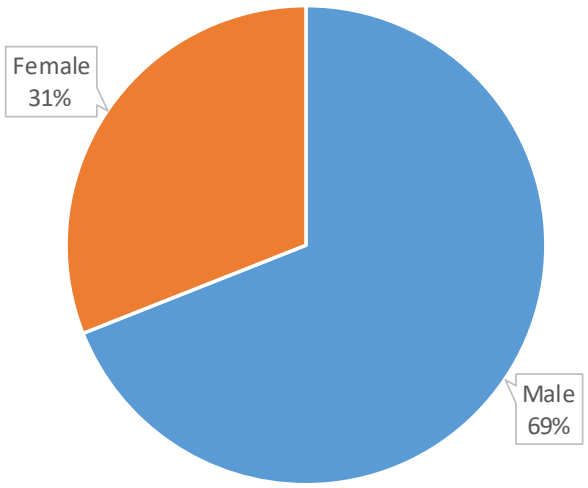

Figure 2: Sex distribution of study participants.

Table 1. Referral diagnosis of patients referred into the emergency ward.

Condition	Frequency	Percentage
Head Injuries	311	19.9%
Fractures	200	12.8%
Cardiovascular conditions	261	16.7%
Other surgical conditions	449	28.7
Other medical conditions	322	20.6
Missing	22	1.5
Total	1565	100

Table 2. Gender distributions of the various conditions.

Condition	Male (%)	Female (%)	Total
Head Injuries	213 (68.5)	98 (31.5)	311
Fractures	135 (67.5)	65 (32.5)	200
Cardiovascular conditions	159 (60.9)	102 (39.1)	261
Other surgical conditions	293 (64.3)	156 (35.6)	449
Other medical conditions	253 (78.6)	69 (21.4)	322

With regards to the number of patients that were permitted to be referred to the facility during the telephone conversation, 82% of patients were permitted whilst 12% were not permitted **(Figure 3)**.

The northern region emerged as the region with the most frequently referred cases with a total number of 716, constituting 48%. This was followed by the upper east region with 24% and the Savannah region with 10% of the total referred cases. The Ahafo region, Western north and Central regions did not refer any case to the facility during the period under review **(Table 3)**.

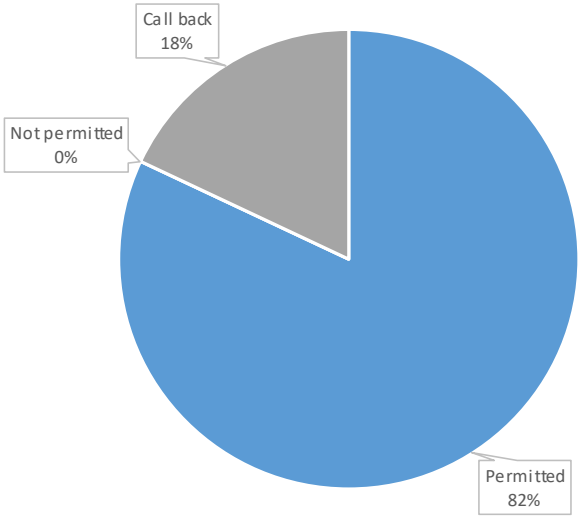

Figure 3: Patients that were permitted to be referred to the facility during the telephone conversation

Discussion

Patient referral has been an integral part of the health care system. This study sought to identify the most common medical and surgical conditions that are referred into the accident and emergency unit of the Tamale Teaching Hospital as well as the demographics of patients mostly involved in these referrals. The age distribution of patients referred to the facility reveals that, more than 60% of patients are below 50 years old. This can be attributed to

various factors. To begin with, the population of Ghana is generally described as a youthful population and as such, young people are always expected to dominate in all aspects of the population (Kpessa-whyte, 2018). Furthermore, the major medium of transportation in northern Ghana is through motorcycles, which are mostly used by the youth. This comes with a high prevalence of road traffic accidents. It is therefore not unexpected that, RTA cases constitutes a chunk of referrals to the facility. This further explains the finding that, majority of the patients referred are males. The male gender as a result of their masculine activities are daring and more likely to engage in risky behaviors, leading to RTAs (Dinye & Ahmed, n.d.). Finally, the accident and emergency unit is the first point of contact for all surgical and trauma cases into the Tamale Teaching Hospital.

Table 3. Distribution of regions and number of patients referred to the facility.

Region	Number of referrals	Percentage
Northern	716	45.7
Upper East	359	22.9
Savannah	151	9.6
Bono East	92	5.8
North East	84	5.3
Oti	58	3.7
Upper West	28	1.7
Volta	6	0.38
Bono	4	0.25
Ashanti	2	0.13
Western	1	0.06
Eastern	1	0.06
Grater Accra	1	0.06
Ahafo	0	0
Western North	0	0
Central	0	0
Missing	62	3.9
TOTAL	1,503	100

According to the nurses at the accident and emergency unit, most of the patients have been referred to the facility due to the severity of the condition, whilst others such as those with head injuries were referred for diagnostic investigation such as computerized tomography scan (CT scan). These are the reasons stated by the referral facilities during telephone conversations regarding referrals. In the whole of northern Ghana, only Tamale has a functional CT scan. This has been sighted as major causes of referral in other jurisdictions as well (Berkeley & Med, 1976). Fractures, which are mostly sustained from RTAs was observed as the second most referred conditions after head injuries. This is similar to other studies conducted in Australia where fractures were the number one condition referred to a tertiary health facility (At et al., n.d.).

In the health sector, some attempts to refer patients to the next level may be unsuccessful for various reasons. Some factors that impede patient referrals include inadequate bed space at the receiving facility, inadequate essential materials such as oxygen, diagnostic equipment and specialist health personnel, missing and inadequate information, challenges with communicating systems (Michael Weiner, M.D., M.P.H. Anthony J. Perkins, M.S.1, and Christopher M. Callahan, 2015). Majority of the calls that were received regarding referrals were successful and the patients were brought in. However, various challenges such as inadequate bedspace, incomplete information, and inadequate essentials such as oxygen impeded the referral of about 12% of cases.

The majority of patients referred into the facility were surgical cases. This includes patients with acute surgical conditions and RTAs. Road traffic accidents are a major cause of patient referral in Ghana and specifically in northern Ghana. Most RTAs results in head injuries which in most cases leads to a referral to the facility. The facility is the only health facility in the entire northern Ghana with a functional CT scan equipment as well as a highly recognized neurosurgeon. The Tamale Teaching Hospital has a robust surgical department which attend to general surgical and neuro-surgical condition as well as other specialized surgical cases. This results in a huge referral of surgical cases besides those of trauma and RTAs. This is expected as referrals to neurosurgeons has been documented as a major referral point in Australia (At et al., n.d.).

Cardiovascular conditions such as hypertension, heart failures and cerebrovascular accidents were the third major referral diagnosis. Cardiovascular conditions such as hypertension has ranked in the top five out-patient and emergency ward visits for the past 15yrs in Ghana (12). These conditions are part of a broader group of conditions described as chronic non-communicable diseases. These conditions account for about 60% of the estimated 58million deaths each year globally (Health, 2012). In other jurisdictions, cardiovascular conditions were reported as the third major most common conditions referred to a specialist (At et al., n.d.).

This study highlights the common and major conditions which accounts for patient referral in northern Ghana. It also highlights the gender and age distributions of patients most commonly referred.

The study is limited by the fact that it is a retrospective study and thus researchers lacked the opportunity to ascertain from participants the challenges encountered during referral. Also, incomplete missing data was a challenge as the authors could not correct or complete any incomplete data.

Conclusion

The accident and emergency unit of the Tamale Teaching Hospital receives a huge number of referred cases from peripheral facilities across both northern and middles belts of Ghana on annual basis. Most of the patients referred to the facility are males within their youthful ages. The major conditions referred to the facility are surgical conditions, including head injuries, fractures, and other surgical emergencies such as acute abdomen. Medical conditions commonly referred to the facility includes cardiovascular conditions such as hypertension, heart failures and stroke. Adequate and complete record keeping should be practiced

among nurses. This will reduce the occurrence of missing information and provide a complete database for future care of patients.

Declarations

Conflict of Interest: The authors have no competing interest to declare. The study was self-funded with no external support.

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RESEARCH ARTICLE

Identification of High-Risk Groups of Falciparum Malaria in Western Region of Ghana: the predictive value of ABO Blood Group Typology

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Abstract

Background: Several studies have linked malaria to ABO blood groups with still others reporting insignificant association between ABO blood group system and malaria. Blood group 'O' has been shown to confer protection against severe malaria by studies in various populations but indecisive reports have been given about non-O blood groups in relation to their protection or vulnerability to severe malaria.

Objective: The present study sought to investigate the ABO blood group typology and the risk of developing severe malaria.

Materials and Methods: A total of 280 participants (140) with P. Falciparum malaria patients and 140 healthy controls) screened for ABO blood groups by Tile method were enrolled into the study. Thick and thin blood films stained with 10 % Giemsa were prepared for falciparum – infected individuals and their full blood counts obtained from the haematology analyzer (Cell Dyn 1800, Abbot Diagnostic Division, USA). Parasite counts were categorized into severe and uncomplicated malaria and further grouped into varying degrees of parasitaemia. The effects of parasite densities on some haematological parameters were then studied. Severe malaria was defined as hyperparasitaemia, malarial anaemia and thrombocytopenia.

Results: The frequency of blood group 'A' was significantly higher in patients with severe malaria compared to other blood groups ($p = 0.042$). Blood groups 'A' and 'B' showed higher parasite densities while 'A' and 'AB' blood groups revealed low platelet counts. Anaemia was severe in blood groups 'A' and 'O' ($p \leq 0.05$). Previous studies including this current one highlighted the protected nature of blood group 'O' to severe malaria ($p \leq 0.05$).

Conclusion: The present study provides the evidences that, individuals of blood group 'A' are highly susceptible to malaria infection and inferably with an increased risk of developing severe malaria than the other blood groups.

Keywords: Anemia, Blood group, Ghana, Malaria, *Plasmodium falciparum*.

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Introduction

Malaria is a vector-borne disease caused by Plasmodium parasites which is transmitted through the bite of the infected female Anopheles mosquito or from mother to child or via blood transfusion (Ashley et al., 2014). It is a major cause of illness and death particularly among children and pregnant women. The disease is endemic in Ghana, accounting for 40% of all outpatient visits to hospital (Awine, Malm, Bart-Plange, & Silal, 2017) and it has been reported that households spent between US$5.70 on uncomplicated malaria and US$48.73 on severe in Ghana (Nonvignon et al., 2016). Statistics shows that between 3.1 and 3.5 million cases of clinical malaria are reported in public health facilities each year, of which 900,000 cases are in children under five years. On the whole children under five years and pregnant women constitute 20 % and 4 %, respectively, of the general

population affected by malaria (Kayentao et al., 2018), making malaria an infection of great worry.

Case management has been and continues to be one of the main strategies of controlling malaria in the country. There have been many large-scale initiatives undertaken during the last few decades with the goal of reducing or eradicating the burden of malaria in the developing world. These ambitious goals set by these programmes for reducing malaria burden in the near future however, appear unlikely to be met (Attaran et al., 2004). This is because mortality from malaria continues to be the major burden in developing countries, especially in children under five years of age. Effective treatment for malaria exists, but it must be administered promptly and timely by trained personnel in order to be effective as delay in treatment may be detrimental to the infected individuals (Alonso et al., 2011; Smith, Jones, Meek, & Webster, 2009)

It is generally accepted then, that most malaria deaths can be prevented when clinical cases are promptly diagnosed and effectively treated. In young children, malaria can progress from mild to severe cases within 24 hours after the onset of symptoms. Illness progression to the severe stages of malaria and mortality can be reduced if there is prompt diagnosis and timely malaria treatment within 24 hours after onset of first symptoms (Luz et al., 2013; Turuse, Gelaye, & Beyen, 2014).

Pathophysiologically, the response to malaria infection with P. falciparum is highly variable, depending on several different biological factors such as previously acquired immunity and blood polymorphisms (Modiano et al., 1996; Perkins et al., 2011). In light of this, researchers are investigating especially in developed countries on how best malaria infection and its progression can be curtailed through destruction of human cell-parasite interaction, most importantly, the destruction of the erythrocytic phase of the plasmodium cycle. One of such researches is the study of the interaction between ABO blood group antigens and plasmodium proteins, most especially, the Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) antigens, as it is believed to influence disease progression of malaria in different individuals (Cserti & Dzik, 2007). Blood group antigens A and B have been reported as co-receptors for P. falciparum rosetting whereas blood group 'O', lacking these antigens may offer some protection against severity of malaria by reduced rosetting (Barragan, Kremsner, Wahlgren, & Carlson, 2000). There are also increasing evidences that both the risk of acquiring P. falciparum malaria and the risk of developing severe complications are determined by host genetic factors (De Mendonça, Goncalves, & Barral-Netto, 2012; Fortin, Stevenson, & Gros, 2002). However, there are scarce studies and inconsistent data particularly in Ghana, relating a particular ABO blood group phenotype to the risk of developing severe malaria.

This study is therefore aimed at identifying the specific blood type(s) that is/are at a greater risk of developing severe malaria by determining the association between ABO blood groups and P. falciparum malaria.

Materials and Methods

Study site: This was a randomized cross-sectional study carried out from January to March 2013. The study took place at the medical laboratories of four (4) hospitals in the Sekondi-Takoradi Metropolis (STM); Effia-Nkwanta Regional Hospital (ENRH), Takoradi Hospital (TH), Kwesimintsim Hospital (KH) and Essikadu Hospital (ESKH).

Sekondi-Takoradi Metropolis, the administrative capital of the Western Region of the Republic of Ghana covers a land area of 385 km2 with Sekondi as the administrative headquarters. The Metro is bordered to the West by Ahanta West District, to the North by Mpohor Wassa East, to the East by Komenda-Edina Eguafo-Abrem and to the South by the Gulf of Guinea. The Metropolis is strategically located on the south-western coast of Ghana, about 200 km west of Accra and 130 km East of La Cote D'Ivoire (Sekondi – Takoradi Metropolitan Assembly, 2013). STM is a densely populated district having the largest share (23.5 %) of the region's total population of 2,376,021. The current population of the Metropolis stands at 559,548 with 273,436 males and 286,112 females (Fiave, 2017).

The central part of the Metropolis is low lying and occupied by muddy lagoons. It also has an equatorial type of climate and bi-modal type of rainfall. Temperatures are high with an average of 22 °C. With a mean annual rainfall of 1,380 mm, the metropolis experience heavy rainfall in March and July with minor rains occurring between August and November (Ministry of Food and Agriculture, 2013).

Study population: The study was cross-sectional and recruited two hundred and eighty (280) consenting individuals from patients who were referred to the laboratory for malaria test. One hundred and forty (140) malaria positive patients and another one hundred and forty (140) healthy individuals as control were enrolled into the study using a selection criterion described below. Malaria positive participants were confirmed Plasmodium falciparum infected patients of all age groups at the medical laboratories of the four hospitals. Healthy controls were non-malaria infected healthy individuals attending the outpatient department for routine check-up and the blood bank of ENRH for blood donation. Personal data included name, age and sex.

Ethical consideration: Ethical approval was given by the Department of Biomedical Sciences research committee students' research. Permission to undertake this study was obtained from the administration of all the four hospitals. The potential risk involved in the study was explained to the participants and finally, written consent from older study participants and permission from care givers of all younger participants were obtained prior to enrollment into the study. Records were kept strictly confidential hence there was no conflict of interest.

Inclusion criteria

- Patients who tested positive for malaria infection (irrespective of age and sex), in the medical laboratory of the hospitals and have not taken anti-malarial drugs before the study.

- Healthy non-malaria infected individuals at ENRH for routine check-up and for blood donation during the time of study.

Exclusion criteria

- Patients who took anti-malarial drugs before visiting the laboratory and those who were sickle cell positive were excluded.

Sample collection and processing

Sickle cell slide test: Sickle cell test was performed on each blood sample of malaria patients to exclude patients who were positive. One drop of blood was mixed on a slide with a drop of sodium metabisulphite, (di-sodium disulphite, Merck, Darmstadt, Germany) 0.2g in 10ml of distilled water covered with a cover glass and incubated at room temperature for up to 1 hour. This was observed under the microscope (Olympus; model CHA, USA) with 40x objective lens. A positive control blood from a known sickle cell trait person was set up.

Preparation, staining and examination of blood films for malaria parasite: Blood samples of both malaria patients and healthy individuals were taken into dipotassium Ethylenediamintetra- acetic acid (K2 EDTA) blood collection tubes (Cangzhou Yongkang, China). The blood samples were first confirmed by the trained microscopists at the laboratories to be either positive or negative for malaria. Confirmed malaria positive and negative blood samples for malaria infected patients and non- malarial healthy study participants respectively were then obtained from the laboratories for processing. Thick and Thin blood films were prepared on the same slide for malaria parasite quantification and identification respectively.

Thick film: Thick film was used to concentrate the parasite and to determine the degree of parasitaemia. The smear was prepared by placing 6 µl of blood on a lower half of a grease-free slide. The edge of another slide was used to spread the blood in a circular form of about 2 cm in diameter.

Thin film: Thin film consists of a single layer of red cell, which was prepared using 2 µl of blood on the lower part of the second half (about 1 cm apart from the thick smear) of the grease-free slide. A second slide with smooth but chopped edge was used as a 'spreader'. The 'spreader' was placed just before the 2 µl of blood and allowed to run along its edge. The spreader was then pushed along the slide at an angle of 30˚ to make the smear. The films were labeled correctly with the corresponding patient number and air dried.

Staining: Thin film was fixed with absolute (100 %) methanol after air drying both thick and thin films. The slides were placed on the staining rack, making sure that all thick films were at one end of the rack. A 1:10 or 10 % Giemsa stain solution (1 ml of Giemsa stock solution added to 9 ml phosphate buffered water of pH 7.2) was prepared and then filtered before it was used to avoid the introduction of dirt particles. The slides were covered with the stain for 10 minutes after which the stains were washed with excess buffered water. The back of the slides was blotted and air dried in a draining rack.

Examination of thick and thin smears: Plasmodium falciparum parasites were counted per 200 or 500 leucocytes, which were used to estimate the parasite density per microlitre of blood. The entire smear was first screened at a low magnification (40x objective lens) to examine first for stain reaction and in the case of thin film to select an area where the cells were evenly distributed and the parasites well seen. Smears were then examined using 100x oil immersion. White cells were symmetrically counted against the number of parasites in each field covered on the thick film using hand tally counters. Thin films were examined to confirm the species and stage identification on the thick film. A second opinion was sought when in doubt.

Estimation of parasite density: Parasite counts were done in the thin film (against 2,000 red blood cells) as a result of heavy parasitemia (greater or equal to 100 parasites on thick film per high power field), parasites counted were recalculated with 200 white blood cells (WBCs). The parasite density was estimated using the formula below (WHO, 1991):

Number of parasites per µl of blood (n)

n = (Number of parasites counted x Total white cell count)/200 WBCs counted

ABO blood grouping: Two spots of blood from each subject were made on the white plain tile and a drop of each antiserum A and B (Span Diagnostics Limited, Gujarat) was applied to each spot respectively. The mixture was further stirred with a plastic stirrer and rocked for some time. Signs of agglutination were observed.

Haematological analysis: The full blood counts (FBC) analysis for each malarial participant's sample was obtained from the automated haematology analyzer (Cell-Dyn 1800, Abbot Diagnostic Division. USA). Daily internal quality controls and the scheduled external quality assessment programme were adhered to as a quality measure.

Severity of infection: Development of severity of the malaria disease was determined based on the parasite density (parasitaemia) calculated as above, anaemia and thrombocytopenia (WHO, 1991). Malaria cases were further grouped into severe malaria (SM) and uncomplicated malaria (UCM).

Parasitaemia

- High parasitaemia was defined as parasitaemia> 10,000 parasites / µl.
- Moderate parasitaemia was defined as parasitaemia between 1000-9999 parasite /µl.
- Low parasitaemia was defined as parasitaemia between 1- 999 parasites /µl.

Anaemia

- Severe anaemia was defined as haemoglobin concentration of < 5g / dL or hematocrit < 15 % and RBC count <3.0 M / µL)

- Moderate anaemia was defined as haemoglobin concentration between 5 – 8 g / dL, hematocrit between 15 – 24 % and RBC count 3.0 - 4.20 M / µL).
- Mild anaemia was defined as haemoglobin concentration > 8 g / dL or hematocrit >24 % and RBC count >4.20 M / µL).

Thrombocytopenia

- Severe thrombocytopenia was defined as platelet count < 20,000 / µl.
- Moderate thrombocytopenia was defined as platelet count < 50,000 / µl but > 20,000 / µl.
- Mild thrombocytopenia was defined as platelet count < 150,000 / µl but > 50,000 / µl.

Severe malaria: Based on World Health Organization established criteria for severe malaria (WHO, 2000), severe malaria was defined as hyperparasitaemia> 250,000 parasites / µl of blood and / or hematocrit < 15 % or haemoglobin< 5 g / dl in the presence of parasite count > 10,000 / µl of blood.

Uncomplicated malaria: Uncomplicated malaria was defined as parasitaemia> 10,000 parasites / µl of blood and / or hematocrit >24 % or haemoglobin concentration > 8 g / dl in the presence of parasitaemia.

Severity was based on the presence of high parasitaemia, severe anaemia and severe thrombocytopenia. Normal reference ranges of the haematological parameters are shown in Appendix.

Statistical analysis: Data was entered in Microsoft Excel, checked for its correctness, and exported to and analyzed using SPSS version 16 (SPSS Inc. Chicago). Chi-square test was used to assess the difference between frequencies. One-way ANOVA was used to compare the mean scores of more than two groups. A probability value less than 0.05 ($p<0.05$) was considered statistically significant. The statistical evaluation of the data was done by mean, standard error and Chi square value (X2).

Results

The study enrolled 140 P. falciparum malaria patients representing the cases and 140 non- malaria infected individuals representing healthy controls. In general, the control group was significantly older, a mean age of 38.3 ± 1.17, than the malaria infected individuals with mean age of 18.0 ± 1.53 ($p = 0.001$). Of the malaria patients, 59 (42.1 %) were males and 81 (57.9 %) were females in contrast to 63 (45.0%) males and 77 (55.0%) females of the healthy controls. The ABO blood groups of both falciparum-infected patients and healthy controls were also determined. Correspondingly, 50 %, 23 %, 20 % and 6.4 % among the cases were found to be of blood types 'O', 'B', 'A' and 'AB' while 51 %, 22.9 %, 17.9 % and 7.9 % also of 'O', 'B', 'A' and 'AB' were found among the controls. Furthermore, among the patients with parasites, those below 5 years of age, 48 (34.3 %), represented the largest proportion whilst age group 37 – 51, 5(3.6 %), represented the smallest with 41 (29.3 %), 37 (26.4 %) and 9 (6.4 %) falling within age groups 5 – 20, 21 – 36 and greater than or equal to 52 years respectively **(Table 1)**.

Table 1: General characteristics of study participants.

Variables	Control (N = 140)	Case (N = 140)	p-value	(X2)
Age mean ± (SE)	38.3 ± (1.17)	18.0 ± (1.53)	0.001	
Gender				
Males	63(45.0)	59 (42.1)	0.63	0.232
Female	77(55.0)	81 (57.9)		
ABO Groupings				
A	25(17.9)	28 (20.0)	0.937	0.413
B	32(22.9)	33 (23.6)		
AB	11(7.9)	9 (6.4)		
O	72 (51.4)	70 (50.7)		
Age range (years)				
< 5	0 (0.0)	48 (34.3)	0.001	12.152
5 to 20	4 (2.9)	41 (29.3)		
21 – 36	76 (54.3)	37 (26.4)		
37 – 51	33 (23.6)	5 (3.6)		
≥ 52	27 (19.3)	9 (6.4)		
SE (Standard error); N (%): frequency (percentage). Differences between values are statistically significant at $p<0.05$				

In relating gender of malaria patients to their haematological parameters, males were found to be more infected with malaria than females, showing a higher mean white blood cell count of 8.4 ± 0.57 and mean parasite density of 30502 parasites / µl of blood though between them these differences were not statistically significant ($p>0.05$). Females however, recorded relatively lower mean values in the red cell indices; haemoglobin level, red blood cell count and hematocrit, compared to males. With the range of clinically normal platelet count to be 150 – 450 K / µl, it can be seen that males showed a near significant ($p=0.8$) lower platelet count (143.2 ± 13.5) than females (173 ±10.8) **(Table 2)**.

Table 2: Hematological characteristics of malarial subjects in relation to gender

Variables	Male	Female	p-value
	N = 59	N = 81	
Parasite density	30502 ± (581)	30084 ± (591)	0.97
Hb (g / dl)	10.6 ± (0.33)	10.2 ± (0.25)	0.32
RBC(M / µl)	4.02 ± (0.11)	3.9 ± (0.09)	0.34
HCT(%)	31.5 ± (1.01)	30.6 ± (0.74)	0.47
Platelet (K / µl)	143.2 ± (13.5)	173 ± (10.8)	0.08
WBC(109 / l)	8.4 ± (0.57)	7.9 ± (0.44)	0.51
Hb: Haemoglobin; RBC: Red blood cell; WBC: White blood cell; HCT: Hematocrit; N: Frequency. Values are in mean ± SE (Standard error). Differences between Mean values are statistically significant at $p<0.05$.			

Looking at the ABO distribution among malaria-infected participants, infection was more prevalent in blood group 'O' and less prevalent in blood group 'AB' in both males and females. The trend of high infection was then followed by blood group 'B' (15.00 %) before A (10.71 %) in females but took a reverse trend in males **(Figure1)**.

Degree of parasitaemia showed association with age as reported in most studies. From this study the highest prevalence of high parasite density was among children

under-five years of age (20.0%) as compared with older age groups but this difference was not statistically significant (p>0.05). Noticeably patients within the age group 32 – 57 years recorded the lowest incidence of parasitaemia (1.4 %) of the total population **(Table 3)**.

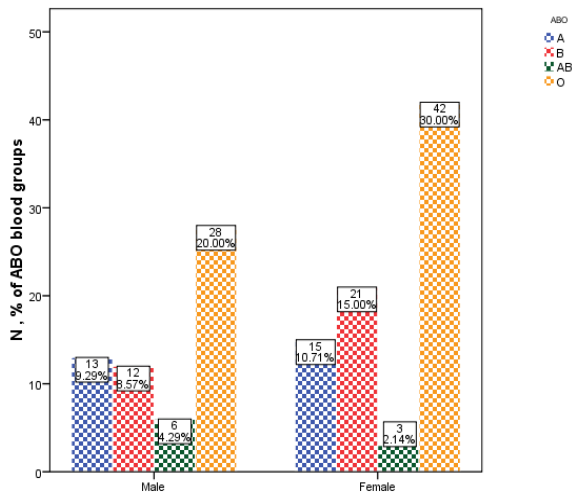

Figure 1: Frequency of ABO blood groups among malaria subjects per gender

Table 3: Relationship between age groups and the degree of parasitaemia

Age (years)	**LP**	**MP**	**HP**	**Total**
	N = 12 (8.6 %)	N = 63 (45.0 %)	N = 65 (46.4 %)	N = 140
< 5	3 (2.1)	17 (12.1)	28 (20.0)	48 (34.3)
5 – 20	3 (2.1)	22 (15.7)	16 (11.4)	41 (29.3)
21 – 36	6 (4.3)	17 (12.1)	14 (10.0)	37 (26.4)
37 – 51	0 (0.0)	3 (2.1)	2 (1.4)	5 (3.6)
≥ 52	0 (0.0)	4 (2.9)	5 (3.6)	9 (6.4)
Values in parentheses indicate percent values. N: Frequency; LP: Low parasitaemia; MP: Moderate parasitaemia; HP: High parasitaemia. Differences in percentages were analyzed using Chi-square statistical tool. Chi-square value (X2) = 8.764, df=6, p – value=0.363.				

To establish the possible association between ABO blood group system and severe P. falciparum malaria, the distributions of each ABO blood group in severe malaria and uncomplicated malaria patients were compared. Of the nine (9) patients with severe malaria, a significantly higher percentage (3.6 %) were blood group 'A' patients (X2 = 8.190, p = 0.042). Additionally, blood group 'O' patients were significantly associated with uncomplicated malaria than with severe malaria indicating its likely protective role **(Table 4).**

Parasite densities and their corresponding haematological parameters among each blood group were compared **(Table 5)**. In general, malaria infection displayed a significant association with blood groups (F = 5.573, p = 0.009), with the highest level of parasitemia observed among blood group 'A' patients (52318 parasites / µl of blood) and the lowest among blood group 'AB' (12086 parasites / µl of blood). Concerning the Mean platelet count, only a trend was observed between blood group 'AB' and the other blood groups (p = 0.06).

Table 4: Distribution of ABO blood group in severe and uncomplicated malaria

ABO blood group	SM	UCM	Total
	N = 9 (6.4%)	N = 131 (93.6%)	N = 140 (100.0 %)
A	5 (3.6)*	23 (16.4)	28 (20.0)
B	2 (1.4)	31 (22.1)	33 (23.6)
AB	0 (0.0)	9 (6.4)	9 (6.4)
O	2 (1.4)	68 (48.6)*	70 (50.0)
Values in parentheses denote percentages. N: Frequency; SM: Severe malaria; UCM: Uncomplicated malaria. Differences in percentages were analyzed using Chi – square statistical tool. Chi – square value (X2) = 8.190, df = 3, p – value = 0.042. ABO blood group frequency of healthy controls (N = 140): 'A' = 17.9 %, 'B' = 22.9 %, 'AB' = 7.9 % and 'O' = 51.4 %. * Shows the highest frequency in SM and UCM.			

On the whole, 4.3%, 2.1%, 1.4% and 0.7% of the 140 P. falciparum infected participants belonging to blood group 'B', 'O', 'A' and 'AB' respectively had low parasitemia (< 1000 parasites / µl of blood). Conversely 25.0 %, 9.3 %, 7.9 % and 2.9 % of them with blood type 'O', 'A', 'B' and 'AB' respectively had moderate parasitemia (1000-9999 parasites / µl of blood). High parasitemia (>10,000 parasites / µl of blood) was also most prevalent (22.9%) among blood group 'O' patients, followed by blood group 'B' (11.4 %), 'A' (9.3 %) and 'AB' (2.9 %). This difference was statistically insignificant (p = 0.356, X2 = 6.636) **(Table 6)**.

Severe malarial anaemia was defined as haemoglobin (Hb) value of <5 g / dl or hematocrit (HCT) < 15 % in the presence of parasitaemia. Severe anaemia was prevalent among blood group 'A' and 'O' patients; both recording 1.43 %. Moderate anaemia (Hb: 5 – 8 g / dl and / or HCT: 15 – 24 %) and mild anaemia (Hb: ≥ 8 g / dl or HCT: ≥ 24 %) were both prevalent among blood group 'O' malarial subjects (5.71 % and 42.86 % respectively) compared to non-O subjects. This high prevalence was followed by blood group 'A' (3.57 %) in moderate anaemia and blood group 'B' (21.43%) in mild anaemia **(Figure 2)**.

Thrombocytopenia is common in malaria. In finding the association between thrombocytopenia and ABO blood groups, their distribution in relation to platelet counts was studied. Platelet counts among the patients were stratified into low (< 150,000 / µl) and normal (> 150,000 / µl) platelet counts. Low platelet count (Thrombocytopenia) was further classified as severely (<20,000 / µl), moderately (< 50,000 / µl but > 20,000 / µl) and mildly (< 150,000 / µl but > 50,000 / µl) low. Among these categories just a patient each for blood group 'A' and 'O' reported with severe thrombocytopenia representing 0.7 % of the total population of malaria patients. Furthermore, blood group 'O' patients were associated with moderate and mild thrombocytopenia with still the highest percentage of them observed among patients with normal platelet counts. Among the non-O blood groups, blood group 'A' recorded the highest prevalence of severe (0.7 %) and moderate (2.1 %) thrombocytopenia whilst blood group B showed the

Table 5: Haematological characteristics of malarial subjects stratified by ABO blood groups

Variables	A (N = 28)	B (N = 33)	AB (N = 9)	O (N = 70)	Total (N = 140)
Parasite density	52318 ± (514)a	36890 ± (462)b	12086 ± (169)c	20647 ± (405)d	30260±(413)
Hb (g/dl)	9.5 ± (0.49)a	11.3 ± (0.43)b	10.4 ± (0.63)a	10.3 ± (0.23)a	10.4±(0.19)
RBC (M/µl)	3.6 ± (0.19)a	4.3 ± (0.14)b	4.1 ± (0.25)a	3.9 ± (0.09)a	3.9±(0.07)
HCT (%)	28.5 ± (1.51)a	33.9 ± (1.31)b	31.6 ± (2.01)a	30.6 ± (0.7) a	31.0±(0.61)
Platelet(K/µl)	131.8 ± (18.03)c	171.1 ± (17.6)a	99.7 ± (13.12)b	174.0 ± (12.4)a	160.3±(8.49)
WBC (109/l)	9.65 ± (0.83)a	7.83 ± (0.73)a	6.64 ± (0.91)a	7.78 ± (0.48) a	8.104±(0.34)

Differences between mean values of the various groups were analyzed with one-way ANOVA. Mean values in row with different superscripts are statistically significant ($p < 0.05$). Parasite density, ($F = 5.573$, $p = 0.009$); RBC ($F = 3.349$, $p = 0.021$); Hb ($F = 3.132$, $p = 0.028$); HCT ($F = 3.114$, $p = 0.028$); Platelet ($F = 2.811$, $p = 0.061$); WBC ($F = 1.893$, $p = 0.134$). Parasite density is in parasites / µl of blood.

highest prevalence in both mild thrombocytopenia (12.1 %) and normal platelet count (11.4 %). Blood group AB however displayed the lowest percentages across all stratifications. These associations though comparable were not statistically significant ($p > 0.05$) **(Table7).**

Table 6: Relationship between the ABO blood groups and the degree of parasitemia

ABO blood Groups	LP (N = 12)	MP (N = 63)	HP (N = 65)	Total (N = 140)
	-8.60%	-45.00%	-46.40%	-100%
A	2 (1.4)	13 (9.3)	13 (9.3)	28 (20.0)
B	6 (4.3)	11 (7.9)	16 (11.4)	33 (23.6)
AB	1 (0.7)	4 (2.9)	4 (2.9)	9 (6.4)
O	3 (2.1)	35 (25.0)	32 (22.9)	70 (50.7)

N: Frequency. Chi-square value (X2) = 6.636, df = 6, p – value = 0.356. Healthy control participants ABO blood group frequency (N = 140): 'A' = 17.9%, 'B' = 22.9 %, 'AB' = 7.9 % and 'O' = 51.4 %.

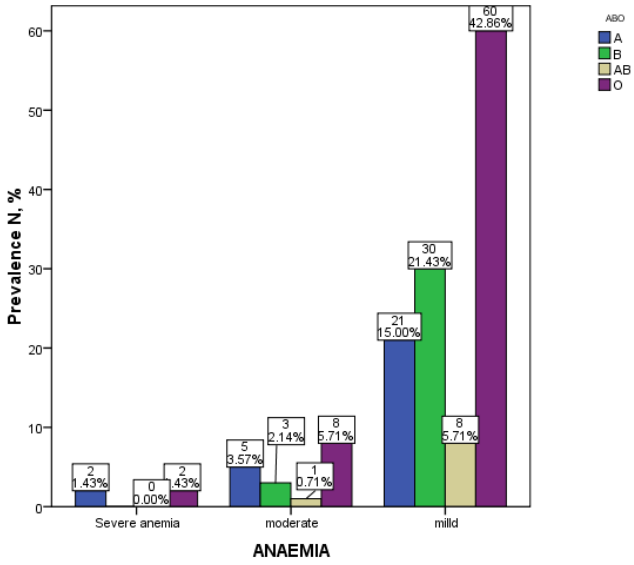

Figure 2: Frequency of ABO blood group among malaria subjects in relation to anaemia

Discussion

This study was conducted to establish the possible relationship between ABO blood groups and the risk of developing severe malaria among malaria patients of all ages. The distribution of blood groups among healthy control individuals revealed a higher percentage of blood group 'O' (51.4 %) followed by blood group 'B' (22.9 %), 'A' (17.9 %) and 'AB' (7.9 %). Matching the 140 healthy controls to 140 malaria infected cases, blood group 'O' represented 50.7 % of the cases which is lower than 51.4% represented by the control group. This is in consistence with the findings of Rowe et al., (J. A. Rowe et al., 2007) who also found a lower percentage of blood group 'O' among severe malaria infected Malian children compared with healthy controls. Conversely, a significantly lower frequency of blood group 'O' have been observed in other malaria non-endemic areas (Chavhan, Pawar, & Baig, 2011; RAI & KUMAR, 2011) which indicates the selective advantage of blood group 'O' in malaria endemic places. Blood group 'O' has been reported by several studies to confer protection against severe malaria. It has therefore been hypothesised that the prevalence of blood group 'O' would be higher in malaria endemic areas by natural selection due to its capacity to stay protected from severe malaria. This study being carried out in a malaria endemic region also confirms this hypothesis. The observations and hypotheses on the protective advantage of blood group 'O' and the less protective role of non-O blood groups are therefore in line with the ABO blood group distributions among both healthy controls and malaria cases recorded in this study. This could be further supported by the higher prevalence of blood type 'O' and lower prevalence of blood types 'A', 'B' and 'AB' globally, where malaria is more prevalent (Cserti & Dzik, 2007; Tekeste & Petros, 2010).

By gender, the mean parasite density was found to be higher in males (30502 parasites /µl of blood) than in females (30084 parasites /µl of blood). This finding is in agreement with previous findings of Akanbi et al (Akanbi, Badaki, Adeniran, & Olotu, 2010; Akanbi, Odaibo, Olatoregun, & Ademowo, 2010) who attributed this to the reason that men expose their bodies more than females when the weather is hot, thus increasing their chances of being bitten by mosquitoes more than females. Moreover, both males and females showing low levels of haemoglobin concentrations suggest the impact of parasitaemia on the haemoglobin molecules. Malaria parasites feed on haemoglobin of infected host in order to grow (Kulkarni, Suryakar, Sardeshmukh, & Rathi, 2003). The increase in malaria parasites present in males (evident by the higher mean parasite density) caused the critical reduction in their haemoglobin level. This effect had a greater impact on the males than females considering the fact that males naturally have a higher haemoglobin level (13 - 18 g / dl)

Table 7: Relationship between the ABO blood groups and platelet count

ABO blood group	Low platelet count			Normal	Total
	Severe	Moderate	Mild	Platelet count	
	N = 2 (1.40)	N = 8 (5.70%)	N = 67 (47.90%)	N = 63 (45.0 %)	N=140 (100%)
A	1(0.7)	3(2.1)	16(11.4)	8(5.7)	28(20.0)
B	0(0.0)	0(0.0)	17(12.1)	16(11.4)	33(23.6)
AB	0(0.0)	1(0.7)	6(4.3)	2(1.4)	9(6.4)
O	1(0.7)	4(2.9)	28(20.0)	37(26.4)	70(50.0)
Chi-square value (X2) = 9.786, df = 9, p – value = 0.368.					

than females (12 - 15 g / dl), a feature more attributable to the increased body exposure of males to mosquito bites than females. The mean differences of parasite densities and haemoglobin concentration together with hematocrit and red blood cell counts between both sexes were however not statistically significant ($p < 0.05$). Gender, thus did not influence the severity of malaria in this study.

The study showed a significant difference between the mean ages of healthy controls and that of malaria cases ($p = 0.001$). The healthy controls can be perceived as an adult-aged group (mean age, 38.3 ± 1.17) and the malaria cases as a young-aged group (mean age, 18 ± 1.53). It could therefore be inferred that malaria is more prevalent among children than adults. To assess how age influence parasitaemia and inferably the risk of developing severe malaria, the relationship between age groups and degree of parasitaemia was established. Prevalence of parasitaemia differed between age groups, with a very high percentage of children under five years old (20 %) recording high parasitaemia in contrast to lower percentages of high parasitaemia recorded among adults. This could be due to the increased number of exposures to mosquito bites among adults by virtue of age, a fact which is found to be coherent with similar studies (Akanbi, Badaki, et al., 2010; Kuadzi, Ankra-Badu, & Addae, 2011). These researchers attributed this to lack of protective immunity in children compared to adults who by their increased previous exposure to malaria have developed an acquired immunity to the disease stage of malaria and its severe complications thereof. The frequencies of parasitaemia found to be highest among patients less than five years of age, and decreased with increasing age respectively; 20 % among <5 years, 11.4 % among 5 – 20years, 10 % among 21 – 36 years and 2 % among 37 – 51 years. As indicated by Akanbi et al (Akanbi, Badaki, et al., 2010), the exposure of persons to mosquito bites has been reported to increase with age which confirms the relation of increased level of immunity against malaria and the decreased level of parasitaemia with increasing age. Almost all adults living in malaria-endemic areas experience repeated malaria infections (Doolan, Dobaño, & Baird, 2009). This might help in the development of antibodies against many sporozoite, liver-stage, blood-stage and sexual-stage malaria antigens that protect the adults from severe forms of malaria complications.

It was however observed in this study that patients above 52 years though older than patients within the ages of 37 – 51years presented with a comparably higher parasitaemia. This may be due to the functional degeneration of the immune system as one ages. Older age is commensurate with weakened immunity more importantly when the thymus is not producing enough T cells to respond to the numerous ill conditions. Therefore, suppressed immunity in old age renders them most vulnerable to malaria infections just as in children. It can be emphasized then, that age influence parasitaemia in relation to malaria severity.

Looking at the overall ABO distribution among malaria-infected participants, infection was more prevalent in blood group 'O' and less prevalent in blood group 'AB' (Satti, 2017; Singh, Urhekar, & Singh, 2015). The trend of high infection was then followed by blood group 'B' (15 %) before 'A' (10.71 %) in females but took a reverse trend in males. This should give a clue that parasite densities will be very high among blood group O and very low in blood group AB patients, but this wasn't so for 'O' blood groups. The high frequency of 'O' blood groups is therefore due to their high distribution in general (Anjomruz et al., 2014; Zerihun, Degarege, & Erko, 2011) Hyperparasitaemia among non-O blood groups was more prevalent among blood group 'A' and 'B' malaria patients (Fig. 1) (Panda et al., 2011; J. A. Rowe et al., 2007; Chigozie Jesse Uneke, Ogbu, & Nwojiji, 2006). A significantly high prevalence (3.6 %; p – value = 0.042) of severe malaria was observed to be among blood group 'A' malaria patients compared to the other blood groups. This indicates the higher vulnerability of blood group 'A' individuals to severe malaria and also confirms the reports by other studies that severe malaria is more implicated in blood group 'A' individuals (Deepa, Rameshkumar, & Ross, 2011; Degarege, Gebrezgi, Ibanez, Wahlgren, & Madhivanan, 2019; Pathirana et al., 2005; A. Rowe, Obeiro, Newbold, & Marsh, 1995; Zerihun et al., 2011), but contradicts earlier reports of high prevalence of severe malaria among blood group 'AB' patients in Sri Lanka (Panda et al., 2011) and blood group 'B' patients in Odisha, India (Deepa et al., 2011). High prevalence and increased risk of placental malaria in blood group O primigravid pregnant subjects has also been recorded(Gupte, Patel, & Patel, 2012).

The variability of observations made considering the different blood groups may be attributed to varied infective strains and probably to different rosetting capacity (Cserti & Dzik, 2007; Loscertales et al., 2007). Blood group 'O' showing the highest frequency (68 / 140; 48.6 %) among uncomplicated malaria cases indicates that majority of blood group 'O' patients are much more protected from severe malaria than non-O blood groups. Blood group 'A' patients again showed significantly increased susceptibility to *P. falciparum* malaria infection evidenced by the highest

mean parasite density they recorded, followed by blood group 'B' (Table 5) and agree with previous findings (Lell et al., 1999; C J Uneke, 2007), which implicate the presence of A or B antigens on rbcs and increasing frequency of malarial episodes in Brazil. Blood group 'AB' showed the lowest parasite density in this study though many previous reports suggest that individuals with blood groups 'A', 'B' and 'AB' are more susceptible to P. falciparum infection than those with blood group 'O' (Gupta & Chowdhuri, 1980; J. A. Rowe, Opi, & Williams, 2009). Similar observation has been made in Sars Cov2 infections 45. However, some previous studies have reported blood group 'O' type to be highly susceptible malaria infection (Singh et al., 2015). Conversely, no differential susceptibilities in blood groups to malaria infection were found in India (J. A. Rowe et al., 2009; Tadesse & Tadesse, 2013). This could be attributed to the smaller sample size of this study and the universally low prevalence of blood group 'AB' compared to other blood groups.

P. falciparum is a parasite of blood and so induce haematological changes. It was observed generally that, haemoglobin concentration decreases with increasing parasitaemia explaining the feeding effects of parasite numbers on the haemoglobin molecule. Blood group 'A' patients were seen to be more "anaemic" than the other blood groups because they showed lowest haemoglobin level, hematocrit and RBC counts (Table 5) owing to their higher mean parasite density (52318 parasites / µl of blood). They are thus, likely to be more susceptible to malarial anaemia, a complication of malaria infection. This finding supports that of Fischer and Boone 48 but contrary to low hematocrit in blood group O in Vivax malaria (Resende et al., 2017). Platelets counts were significantly reduced in blood group 'A' and blood group 'AB'. This may be attributed to increased rosetting reported to be greater in non- O individuals than blood group 'O (Carlson & Wahlgren, 1992; Udomsangpetch, Thanikkul, Pukrittayakamee, & White, 1995) and immune mediated platelet destruction due to hyperparasitamia as reported in vivax malaria(S. Akhtar, Gumashta, Mahore, & Maimoon, 2012). Blood group antigens A and B are trisaccharides attached to variety of glycoproteins and glycolipid on the surface of erythrocytes. These antigens are absent on blood group O erythrocytes but are also found on platelets and to a lesser extent on von Willebrand Factor (vWF) and endothelial membrane (Cserti & Dzik, 2007). These trisaccharides are thought to act as receptors for rosetting on uninfected erythrocytes binding to parasite rosetting ligands such as Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1) and sequesterin on infected red blood cells. Cserti and Dzik explained that infected RBCs adhere to uninfected RBCs via A or B antigens and also to endothelium either by binding to blood group antigens on endothelial cells or by binding to blood group antigens on platelets or vWF (Cserti & Dzik, 2007). Thereby causing clumping and sequestration of erythrocytes in small blood vessels as indicated by Barragan et al.(Barragan et al., 2000). Hence, binding of A and B antigens on platelets could be the cause of thrombocytopenia observed in blood group 'A' and 'AB'. Thus, reduction in the number of platelets in circulation could be as a result of platelets sequestration. Thrombocytopenia could also result from increased platelet destruction through an immune mechanism during when increased immune complexes generated by malarial antigens lead to sequestration of deformed platelets by macrophages in the spleen (M. N. Akhtar, Jamil, Amjad, Butt, & Farooq, 2005; Batool et al., 2019). This could also explain why 'A' blood groups showed thrombocytopenia because they showed the highest parasite densities among the blood groups. Platelet survival is reduced in severe P. falciparum malaria through increased utilization of platelets and reduced compensation by bone marrow production. The evidence is that, rosetting in blood group 'A' and 'AB' caused thrombocytopenia when compared to the higher frequency of normal platelet counts among 'O' blood groups (Table 7). Because blood type 'O' lacks A and B antigens on erythrocytes and platelets, which are not involved in binding with PfEMP1, hence, platelets are free in circulation, which is evidenced by the higher distribution of blood group 'O' patients with normal platelet counts. The iRBCs of blood group 'O' patients are not sequestered but are easily destroyed by phagocytes in circulation for elimination by the spleen. It can therefore be said that splenic destruction of iRBCs accounts for the degree of anemia observed among blood group 'O' while hyperparasitaemia and rosetting accounted for severe anemia and thrombocytopenia in non-O blood groups. Thrombocytopenia causes bleeding observed in severe P. falciparum malaria. Clumping of infected and non-infected red blood cells together with platelets may cause vaso-occlusion which induce clotting that may lead to death through disseminated intravascular coagulation (DIC), cerebral malaria, ischaemia and coma.

Conclusion

Hyperparasitaemia, thrombocytopenia and severe malarial anemia are all associated with severe malaria. Children under five years of age were significantly more vulnerable to developing severe malaria. Parasitemia was highest in 'A' blood groups while platelet count, hemoglobin together with hematocrit and red blood cell count were lowest in blood groups 'A' and 'AB'. This study therefore provides the evidences that individuals of blood group 'A' are more susceptible to malaria infection and at a comparably higher risk of developing severe malaria than other blood groups.

The study recommends based on these current findings that ABO blood group identification should be included in malaria test requests to further help in directing course of treatment and prioritization of patients for intensive care. Also, further studies should be conducted in Ghana on the impact of ABO blood groups on the risk of developing severe malaria recruiting a larger sample size to attain more statistical significance. Further studies should take a longer span including more laboratory markers and other blood polymorphisms that might have protective role against severe malaria.

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REVIEW

Analyzing Facilitators and Barriers to Telehealth in Rural North America During the COVID-19 Pandemic: A Scoping Review

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Abstract

Background: It has previously been established that many people living in rural communities experience health disadvantages and less access to medical care. Over the past decade, innovations in telehealth and other innovative models of care have been developed with the goal of overcoming these inequities for those living in rural areas.

Objective: The aim of this paper was to describe both outcomes and characteristics of studies involving telehealth in rural areas of North America during the COVID-19 pandemic.

Materials and Methods: A scoping review was undertaken. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method was utilized in order to understand the empirical and theoretical data on telehealth usage in the United States, Canada and Mexico during the COVID-19 pandemic. The following terms were utilized: 'rural health' AND 'telehealth' AND 'covid-19'. Separate searches were completed for the three included countries: 'United States', 'Canada', and 'Mexico'. PubMed and Google Scholar were utilized.

Results: The literature search revealed 1197 articles published in English between 1st January 2019 and 31st August 2022. One hundred and fifty articles were included in the review including 135 from the United States, 12 from Canada, and 10 from Mexico. Some articles were cross-collaborations between two of these countries. Among these papers, 18% (27) focused on telemedicine for mental health treatments, 14.7% (22) focused on oncology or cancer, 11.3% (17) focused on telemedicine for the veteran subpopulation, 2.7% (4) used a mixed methods approach, and 14% (21) used a qualitative approach.

Conclusion: This scoping review reveals that the current literature on telehealth in rural areas during the COVID-19 pandemic is largely descriptive. There were only a few publications that focused on comparative health outcomes using telehealth in urban and rural populations in close proximity to each other. Telehealth is well represented in published literature on inequities and innovation, but there is still limited data on health outcomes and comparisons that can be drawn cross-nationally. Further studies should aim to study longer term health outcomes for those in rural areas using telehealth as opposed to areas where telehealth interventions have not yet been adopted.

Keywords: COVID-19, North America, Rural and remote, Rural healthcare, Telehealth, Telemedicine.

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Introduction

The National Aeronautics and Space Administration (NASA) contributed to telemedicine as through its usage of telemedicine for astronauts during space travel (Kichloo et al., 2020). Since its first introduction, telemedicine's prevalence steadily increased. When compared with other countries in Europe and Asia, North America, especially the United States, uses telemedicine services at much higher rates (Oh, Park, Jo, & Kim, 2015). Interestingly, telemedicine has gained the most traction over the past decade and risen in usage during the COVID-19 pandemic (McAdam, 2022). Overall, in most geographic areas there is a trend in discussion about and even implementation of telehealth programs.

The COVID-19 pandemic has posed many challenges to global healthcare systems. Indeed, the COVID-19

pandemic has further increased both demand and reliance on telemedicine (Rush, Seaton, Li, Oelke, & Pesut, 2021). Even prior to the pandemic, there were many well-documented healthcare disparities in rural areas when compared to urban areas. However, the pandemic has exacerbated and shed light on many existing disparities in healthcare systems as a whole.

It is of growing interest to determine what factors in particular serve as the strongest or most predictive facilitators or barriers to telemedicine usage and subsequently increased access to healthcare. Some factors are intuitively associated with barriers such as any of the social determinants of health like low socioeconomic status, and reduced health literacy which is more prevalent in many rural communities.

Despite many advances in the administration and execution of telehealth services, there are still several current challenges that must be addressed. For example, 33% of rural Americans lack access to high-speed broadband internet which is necessary to support video-based telehealth visits (Holtz, Mitchell, Hirko, & Ford, 2022). Other barriers include limited experience with technology or disabilities that may prevent participation in telemedicine (Annaswamy, Verduzco-Gutierrez, & Frieden, 2020). Of course, other barriers to medicine are similar to those experienced in traditional healthcare settings such as language barriers and lack of culturally concordant patient education materials (Peters, 2020).

Given these acknowledged disparities in health between rural and urban populations, many would anticipate that existing literature should bolster our understanding of why this may be the case and how these disparities may continue to persist. Of particular interest are studies which compare pre-COVID-19 and post-COVID-19 conditions and outcomes. This can help with developing an overarching picture of this complex issue.

This paper details the current state of knowledge of telehealth facilitators and barriers across several developed North American countries, all of which have recognized disparate health outcomes between urban and non-urban populations. A thorough review of existing literature is required to address next steps for telehealth utilization especially in rural areas. This integrated review is aimed to analyze and review peer-reviewed empirical and theoretical data, inclusive of both qualitative and quantitative methodologies, on telehealth utilization in rural areas of the United States, Canada and Mexico with a specific focus on the COVID-19 pandemic. Furthermore, this paper aims to identify current gaps in our understanding of existing health disparities and how telehealth may improve to better benefit rural populations.

Materials and Methods

Design: Systematic approach following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method. The authors considered a scoping review the most appropriate review for this research question, given the broad research question, qualitative synthesis and gaps in current literature.

Eligibility criteria: Papers were included if they were:

1. published in peer reviewed journals between January 2019 and 31 August 2022;
2. written in the English language;
3. reported telehealth use in rural communities in North America and the role of COVID-19 on telehealth in these communities.

Papers written prior to January 2019 were excluded in order to focus on papers published during the COVID-19 pandemic. Original research papers, systematic reviews, and reviews were considered. Letters to the editor, opinion pieces and case studies were excluded from this review. Papers were excluded if data did not correspond to at least one of the following countries: United States, Canada and Mexico. Papers were included if they reported on telehealth use in rural health care, rural in this context was accepted if defined by the authors as rural or remote contexts relative to country of origin.

Information sources: The electronic databases PubMed, and Google Scholar were searched using Medical Subject Headings (MeSH) and key words. **Figure 1** outlines the utilized search strategy for these databases. The following terms were utilized: '*rural health*' AND '*telehealth*' AND '*covid-19*'. Separate searches were completed for the three included countries: '*United States*', '*Canada*', and '*Mexico*'.

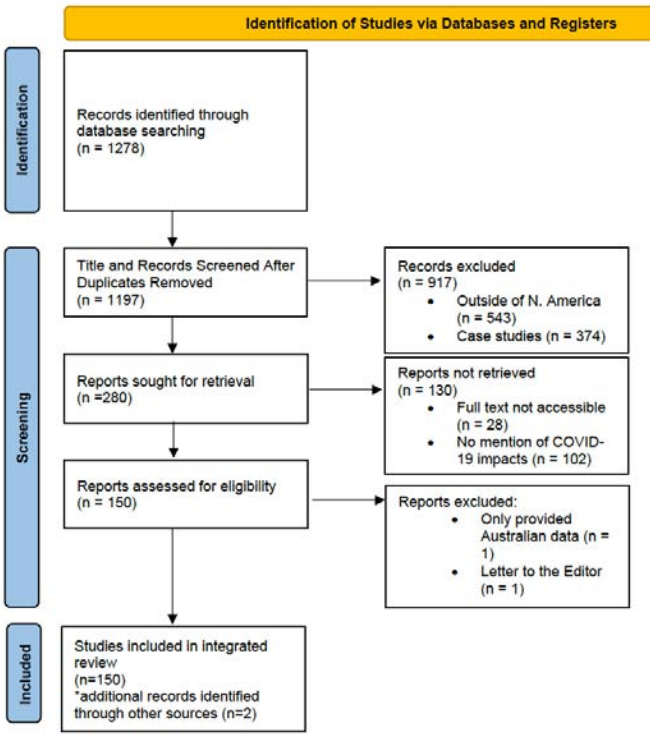

Figure 1: PRISMA Flow Diagram. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al., The PRISMA Group (2020). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Study selection: Papers were assessed for eligibility for

inclusion by the primary investigator (MW) and by utilizing the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method.

Data extraction: Data were extracted using a structured data extraction Table. The data extraction table included the following: author details; journal details; methodological approach; summary of findings; and conclusions. This was later distilled to provide overall characteristic summary tables which included methodological approach, facilitators, and barriers.

Ethics: This is a systematic review and the reviewers used publicly accessible documents as evidence. There was no requirement for an institutional ethics approval before commencing a systematic review.

Results

Characteristics of included studies: Literature searching revealed 1197 articles published in English between 1st January 2019 and 31st August 2022. One hundred and fifty articles were included in the review including 135 from the United States, 12 from Canada, and 10 from Mexico. Some articles were cross-collaborations between two of these countries. Among these papers, 18% (27) focused on telemedicine for mental health treatments, 14.7% (22) focused on oncology or cancer, 11.3% (17) focused on telemedicine for the veteran subpopulation, 2.7% (4) used a mixed methods approach, and 14% (21) used a qualitative approach **(Table 1)**.

Table 1: Methodological Approach Characteristics of Included Studies.

Design Type	f	%
Systematic Review	56	37.30
Observational Descriptive	12	8.00
Randomized Control/ Cluster	34	22.70
Qualitative	21	14.00
Mixed Methods	4	2.70
Other	23	15.30

Facilitators and Barriers: Also among these papers, 82.7% (124) address COVID-19 as a facilitator of increased telemedicine usage, 70.7% (106) indicated that higher rates of health literacy increased telemedicine usage, 56.0% (84) demonstrated a difference in uptake of telemedicine wherein urban areas used telemedicine at higher rates than rural areas **(Table 2)**. Additionally, 44.7% (67) of the papers indicated higher rates of digital literacy (namely how to use video conferencing software) resulted in more telehealth usage, and 2.7% (23) indicated other facilitators such as translated patient education materials **(Table 2)**.

These factors are not only attributed to telemedicine usage but also to other factors like satisfaction and demographic differences. In one study, it was found that telemedicine satisfaction scores were significantly higher among participants who used video conferencing (M= 4.18) compared to those who used phone alone (M = 3.79) (p= 0.031) (Rush et al., 2021). Another study also indicated that older age, rural status, lower SES, Asian race, Black race, and Hispanic/Latino ethnicity are all associated with lower rates of video-based telemedicine (Hsiao et al., 2021). A surprising finding was that urban areas, despite already having greater access to health services, utilized telemedicine at higher rates than rural areas (Chu et al., 2021).

Table 2: Facilitators and Barriers of Telehealth From Included Studies.

Facilitators & Barriers	f	%
COVID-19	124	82.70
Health Literacy	106	70.70
Urban Environment	84	56.00
Broadband Access	79	52.70
Digital Literacy	67	44.70
Other	23	2.70
*Note: facilitators include the presence of the above, barriers include the absence of the above		

Several barriers also appear to overlap. Namely, many of these studies suggest, individuals without digital literacy and reliable internet broadband access typically also have struggles with health literacy because of inability to access reliable patient education materials. It is also more common that rural populations have lower socioeconomic status and educational attainment which are broadly associated with poorer health outcomes and literacy scores.

Discussion

Through this review it was revealed that the literature on telehealth facilitators and barriers for rural and remote populations were generally qualitative or some form of review. Interestingly, there were only a few studies which utilized surveys to gauge facilitators and barriers for people living in rural areas. Potentially, this is because many telehealth interventions are happening on a government-level rather than a study-level so data from health departments may help provide a more complete picture. There were a vast amount of telehealth papers, but data comparing outcomes and significance of different barriers was relatively sparse as was data comparing telehealth usage efficacy to other interventions.

Disparities in healthcare access in remote and rural areas is an ongoing issue that is universally acknowledged. It is well documented that "health deserts", where there are inadequate or nonexistent medical facilities, are common in rural areas (Behrman, Fitzgibbon, Dulin, Wang, & Baskin, 2021). Despite many well-intentioned efforts, there remain logistical practicalities that are challenging to overcome. It is also a highly complex issue with other factors such as social isolation, resilience, economic well-being and aging potentially also playing important roles. Solutions that aim to address issues such as lack of broadband access, digital literacy and language barriers, are crucial to ensure telemedicine access is equitable for all patients (Romain, Trinidad, & Kotagal, 2022). It is also important that future research aims to address ways to reduce health disparities for the rural population.

It is also important to consider that classifications of urban, regional, or remote may be differentially applied across publications which may impact interpretations of

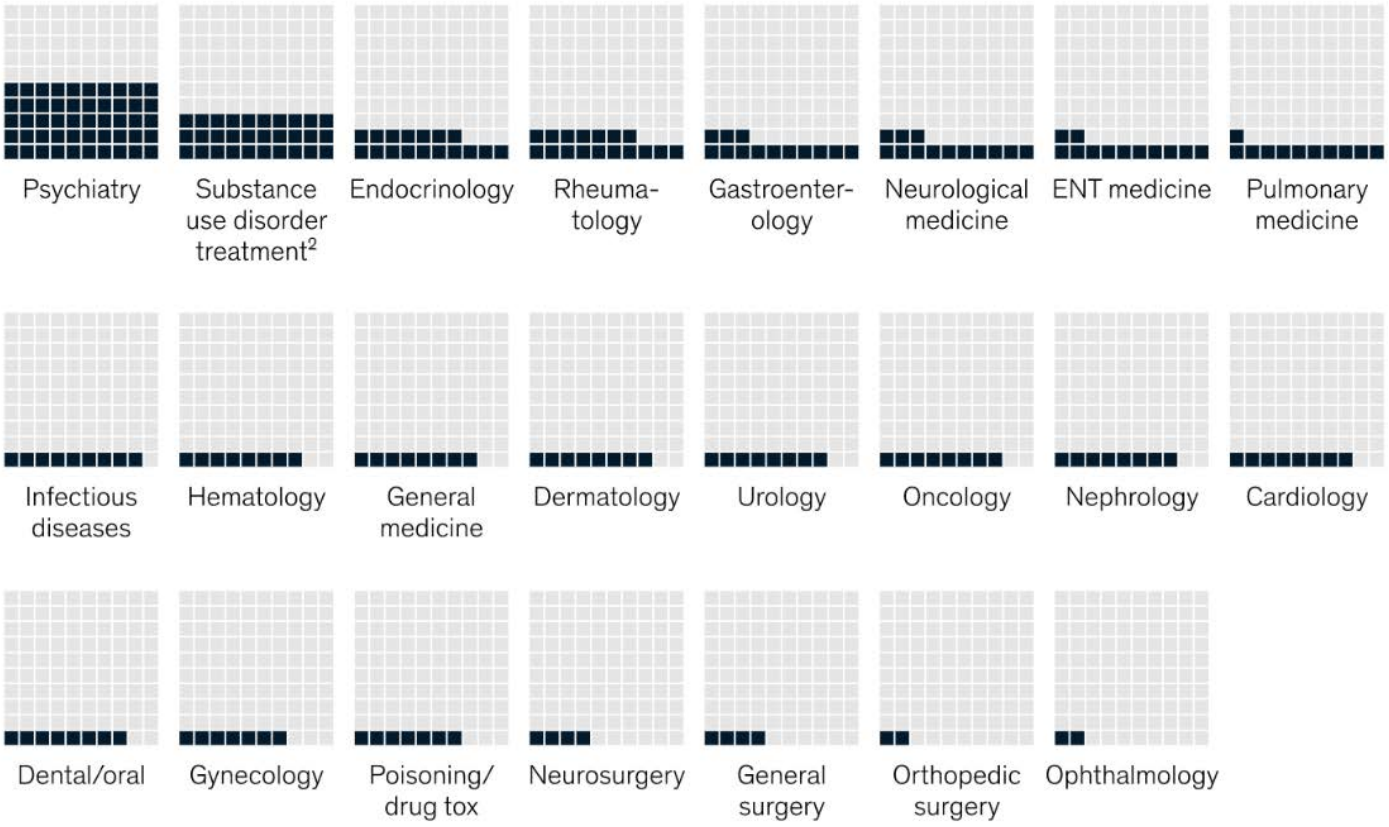

Figure 2: Substantial Variation Exists in Share of Telehealth Claims Across Specialties data from McKinsey Analysis. *Note: Substance Use disorder treatment includes addiction medicine.

data. The countries represented in this review had similar amounts of rural population percentages but differed in overall population and land mass. Furthermore, distance from health services is important for determining access, however, different countries may use different distances to classify an area as medically underserved. Likewise different countries may vary slightly in their classification of what constitutes an underserved area as anything from a region with no medical access to a region with limited medical access. Furthermore, increasing urbanization and its impacts may also be a consideration for study alongside these complex factors.

An area of particular interest in this review were differences in telehealth across North America utilizing the United States, Canada and Mexico as examples. There were many papers that focused specifically on rural America and less papers that focused exclusively on Canada or Mexico's rural areas. Therefore, it is important to consider that as more research is performed different barriers and facilitators unique to these areas may become visible. Approximately 18% of Canadians make up its rural population compared to 20% of Americans and 36% of Mexicans (Romain et al., 2022). One of the studies revealed that because of the work hours of Mexican farmers, offering telehealth services outside of typical business hours improved access) (Tulimiero et al., 2021). During the time period of 2019 to 2021, telehealth usage increased 73% in Mexico, 19.3% pre-pandemic vs. 41.2% during the pandemic in Canada, and telehealth utilization increased 78x in the United States especially during the early months of the pandemic and stabilized to a 38x spike after that (Johnson, Dupuis, Goguen, & Grenier, 2021) (Johnson et al., 2021). Many of these data points, in addition to the ones in the studies analyzed, indicate a $p<0.005$ which shows that the increase in telehealth just in these past few years is highly significant.

However, another important difference is telemedicine claims across different healthcare specialties. In Figure 2, Psychiatry, Substance Use and Addiction, Endocrinology and Rheumatology are among the top specialties for telehealth claims. This differential uptake of telehealth depending on specialty could be for a few reasons including: 1) unique challenges with diagnosing patients virtually 2) less digital literacy amongst specialists from certain specialties compared to others 3) lack of demand for telehealth for some specialties.

It is also important to consider that these countries also have different systems of health service funding and health care systems, in general. The United States utilizes Medicaid as its service for low-income individuals and families, however, many Americans utilize private insurance plans which are offered either through their employment or paid out-of-pocket. Canada has a decentralized, universal, and publicly funded health system which covers necessary care with people able to buy prescription, vision and dental coverage out-of-pocket. Mexico uses a mixture of public health insurance programs, employer-provided health insurance and out-of-pocket care. Despite this, healthcare costs are the highest in the United States and estimates state that by 2026, consumer out-of-pocket spending is slated to reach $1,650 per person or $491.6 Billion (Bestsennyy, Gilbert, Harris, & Rost, 2021). Patient access to medical services

due to different health service funding models is another factor to consider when exploring and determining health outcomes.

This review demonstrates that there were large spikes in telehealth usage during the COVID-19 pandemic across different countries in North America and that in many cases telehealth usage has remained at a higher level than pre-pandemic. Despite telehealth's promise as an innovative way to remediate health disparities in rural areas, several studies have laid out barriers to telehealth in rural and remote areas such as broadband access, business hours and language barriers. There remains much research to be performed comparing telehealth to other innovative interventions aimed at increasing access, telehealth patient education program efficacy and longitudinal comparisons of telehealth usage and health outcomes in rural and urban populations. Such studies would ultimately help the public health community's understanding of the effects of remote geography on healthcare and healthcare interventions.

There are several limitations of this review. One limitation is that it is entirely plausible that not all relevant literature has been identified. Furthermore, this is a topic of a lot of current research, so it is possible that additional relevant literature has been published as this review is performed. The review process utilized is a rigorous approach used by many researchers and our particular use of broad search terms, screening and data extraction helped to include relevant information. This review focused on North America of which Canada, the United States and Mexico were selected based on their relative similarities in rural population proportions and differences in their healthcare systems and uptake of telehealth. There are some differences in classification for Mexico, namely some that classify Mexico as Latin America. However, many of the papers focused most heavily on the United States which may serve to limit generalizability to other regions as the US healthcare system differs significantly from many other developed countries. There were very few studies that directly compared the US rural telehealth usage to Canada and Mexico which provided a challenge as this paper then needed additional analysis to be performed to delineate differences and similarities. However, this paper provides important and crucial insights and comparisons into what data is currently available and gaps in current literature.

Conclusion

Telehealth is well-represented in published literature on inequities and innovation, but there is still limited data on health outcomes and comparisons that can be drawn cross-nationally. Further studies should aim to study longer term health outcomes for those in rural areas using telehealth as opposed to areas where telehealth interventions have not yet been adopted.

Overall, this review demonstrates that there are many facilitators and barriers to telemedicine that may contribute to differential health outcomes of urban and rural populations. Future studies should aim to address which facilitators and barriers are most significant, plans for future telemedicine expansion and differences cross-culturally in telemedicine use. Such data would further knowledge on this growing field and ultimately will help healthcare systems improve healthcare access and outcomes for rural populations.

The review also exposed gaps in existing research that has been published, including a lack of studies comparing telemedicine to other approaches. There was also a lack of research informed policies in these countries to support ongoing efforts to improve healthcare accessibility.

Declarations

Registration and Protocol: The review was not registered.

Funding: There was no financial support for the review.

Competing Interest: The author declares no competing interests.

Availability of Data: For data, please reach out to the author's communication email.

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RESEARCH ARTICLE

Transfusion-related Adverse Reactions: An update from a District Hospital in Ghana

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Abstract

Background: Transfusion therapy is indispensable for effective emergency medicine practice. However, in resource-poor settings transfusion therapy is suspected to be practiced in a manner that may be contributory to morbidity and mortality among blood recipients. Yet, there exists a wide information gap, with respect to incidence of transfusion-related adverse reactions secondary to inappropriate blood prescription.

Objective: This study assessed incidence of transfusion-related adverse reactions in a district hospital in Ghana.

Materials and Methods: A hospital-based cross-sectional study was conducted from December 2020 to January 2021. Patients attending a district hospital in Kumasi, Ghana who were prescribed blood transfusion were recruited into the study after oral/written consent. Patients' demographic data, blood components and units of blood requested as well as the ward of patients were recorded. Records of patient's full blood count (FBC), body temperature, blood pressure and transfusion-related adverse reactions were extracted from their medical folders.

Results: Out of 200 patients, 169 were transfused with whole blood while 31 patients received packed red cells. Majority (48%) of the patients were transfused with ≤1 unit whereas 10% received 3 units of blood. Anaemia (Hb = 6.13 g/dl) and leukocytosis (WBC = 13.83 x 103/µL) were observed in the haematological parameters in which the MCV and lymphocyte count differed significantly in males compared to females. The overall incidence of transfusion-related adverse reactions was 47.5%. Of the 169 patients who received whole blood, more than half (53.8%) experienced more than one of the transfusion-related adverse reactions that were monitored. Finally, patients within the ages of 19-29 years had the highest incidence of transfusion reaction (30.5%) and overall, the number of units administered was significantly associated with the incidence of transfusion reaction ($p<0.011$).

Conclusion: Transfusion-related adverse reaction was common at the study setting. This could be attributed to inappropriate administration of blood and blood products. Strict adherence to World Health Organization recommendations on transfusion therapy needs to be encouraged and supervised in the study setting.

Keywords: Blood Transfusion, Blood Donors, Packed red cell, Transfusion reaction, Whole blood.

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Introduction

Transfusion of blood and its products remains a major life-saving response in the treatment of most disease conditions involving reduced blood volume. Blood and its components play vital physiological roles including, oxygen and nutrient transport, thermoregulation, transport of waste products of metabolism to various excretory organs as well as forms integral component of the body's immune system (Cheesbrough, 2005). In view of the crucial functions of blood and its components, severe blood loss or deficiency in any of its components in a person as a result of any cause may present serious clinical consequences including organ failure and death. To address the issue of reduced whole blood or blood component, and blood-related complications, blood transfusion is normally recommended (Padmakumar & Bellamy, 2011).

Increased demand for blood is a global phenomenon. In the United States, roughly 4.5 million patients receive nearly 21 million blood components through blood transfusion technique (Carman, Uhlenbrock, & McClintock, 2018). In Canada the incremental rate was 4.2% for red cell units supplied to hospitals (Ejaz et al., 2015). In Ghana, demand for blood and blood products by patients outweighs what hospitals and the national blood bank can even supply. Ghana's average collection rate is 5 - 9.9/1000, which is 0.5 to 0.99 % lower than the World Health Organization's (WHOs) minimum required (Laperche et al., 2009). Despite the life-saving benefits of blood transfusion, it may present life-threatening transfusion-related adverse reactions, when carried out inappropriately (Kumar, Thapliyal, Coshic, & Chatterjee, 2013).

Adverse transfusion reaction encompasses all forms of adverse reactions related to administration of whole blood or blood components. Adverse reactions that occur within 24 hours of post-transfusion are termed as either acute immune mediated blood transfusion reactions or acute non-immune mediated blood transfusion reactions and these may include, acute hemolysis, febrile non-hemolysis and sepsis. Delayed transfusion reactions, unlike acute transfusion related adverse reactions may occur many days or weeks post-transfusion, examples of which may include delayed hemolysis, post-transfusion purpura and transfusion-associated graft versus host disease (Sahu & Verma, 2014). A consideration which constitute the heart of the Clinical Practice Guidelines on the use of blood components emphasize that blood component therapy should only be given when the expected benefits to the patient are likely to outweigh the potential harm (Song et al., 2013). Component thresholds form an important aspect of clinical decision making to prescribe red blood cells and other blood components in transfusion therapy. A finding supported by the WHO states that thresholds should not be used as the primary basis for clinical decision making. For example, although prescribing RBCs may be of benefit when hemoglobin (HB) levels fall between 70 - 100g/L, caution should be exercised and comprehensive clinical assessment of patients' presentations should be conducted (Napolitano et al., 2009).

According to Gray and colleagues (Gray, Hart, Dalrymple, & Davies, 2008) and the Northern Ireland Regional Transfusion Committee (Northern Ireland Regional Transfusion Committee, 2006), 47 - 50% of RBCs are inappropriately transfused. Inappropriate blood component transfusion (IBCT) is known globally to cause post-transfusion morbidity and mortality, which may arise from several factors including, patient misidentification, sample labelling errors, blood group typing errors and inappropriate prescription of whole blood and specific blood components (Sawadogo et al., 2018). The incidence of IBCT has been reported to be 0.3 cases per 1000 units of blood in Burkina Faso (Sawadogo et al., 2018) and 0.24 per 1000 units in Tunisia. The inappropriate prescription of blood products exposes patients unnecessarily to the risk of transfusion-related adverse effects with significant cost implications (Boateng, Schonewille, Sackey, Owusu-Ofori, & Afriyie, 2014). Educating medical practitioners with regards to potential adverse outcomes of transfusion is reported to have reduced inappropriate transfusion by 40% (Knowles, 2011). Importantly, development of an appropriate transfusion method coupled with enhanced supervision and education of the major players (patients, nurses, doctors, medical laboratory scientist and policy makers) will ensure improved transfusion services and patient safety. However, in resource-poor settings, accurate data on incidence of inappropriate blood transfusion and its attendant transfusion-related adverse reactions is scant. The present study assessed transfusion-related adverse reactions secondary to inappropriate blood transfusion in a district hospital in Kumasi, Ashanti region of Ghana.

Materials and Methods

Study Site and participants: Asokwa municipality is one of the 43 districts in the Ashanti region of Ghana. It is located at the central part of the region with Asokwa as its capital. It has a total land area of about 25.31-kilometer square and a population of about 125, 642(Ghana, 2010). The district hospital is located specifically at Chirapatre a suburb in the Asokwa municipality. The study site is the second largest hospital in the southern part of Ashanti region. The geographical location of the hospital and its surrounding road network makes the hospital one of the busiest in Ashanti region as well as many referral points for all other clinics and for most road traffic accidents and industrial accidents. The catchment area includes the whole of Asokwa, Ahensan, Atonsu, Esreso, Gyenyase, and Kaase.

All patients (in-and-out patients) who attended the district hospital from December 2020 to January 2021, who were prescribed blood transfusion as evidenced by possession of a request form and consented to partake in the study by means of an oral or a written agreement were purposefully recruited into the study.

Study Design and procedures: This was a hospital-based cross-sectional study conducted from December 2020 to January 2021. Transfusion request information of each participating patient was extracted unto a data collection sheet. The information collected included patient name, age, sex, the indication for the request and the blood component requested (either red blood cells, platelets, plasma, packed red cells or whole blood etc.), number of blood units requested and the ward of the patient. The full blood counts (FBCs) of patients were checked if it was done before the blood was transfused. Records of patients' temperature and blood pressure were obtained from patients records. Transfusion reactions were monitored with the help of nurses and doctors during or after transfusion. Inappropriate blood transfusion was assessed by benchmarking transfusion request information of each participating patients with the National Guidelines for Clinical Use of Blood and Blood Products in Ghana.

For the purposes of this study, inappropriate blood transfusion was defined as:

(a) Prescribing blood based on low threshold of a particular blood component.

(b) Transfusing whole blood without acute blood loss with hypovolemia.

(c) Prescribing packed red cells for patients with acute blood loss.

Ethics approval and consent to participate: The study was approved by the University of Cape Coast Institutional Review Board (UCCIRB/CHAS/2020/42) and the management of the hospital. Also, patients' consent was sought for by means of verbal or written agreement after the aim of the study was clearly explained to study participants. All information extracted from medical folders was strictly kept confidential.

Data Analysis: Data was presented in percentages, averages and figures where appropriate. Statistical analysis was carried out by using Statistical Package for Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY). Comparison of categorical data was done using Student's T-test. $P \leq 0.05$ was considered statistically significant in all analyses.

Results

Demographic and Request Information: Over the 2 months study period, 200 patients received 200 units of transfused blood consisting of whole blood (167) and packed red blood cells (33). Majority (31%) of the recipients were 19-29 years old, among whom 38.7% were males and 61.3% were females. Patients in the General wards received the highest (53%) number of blood transfusions with the least being patients in the lying -in and recovery ward (6%) transfusion cases. Among the recipients, 84.5% received whole blood and 15.5% received packed red cells. On the basis of units of blood transfused, 48% of patients received ≤1 unit of blood, 42% received 2 units of blood and only 10% received 3 units of blood **(Table 1)**.

Average Body Temperature, Blood Pressure and Hematological Profile Presented by Patients: The average body temperature among the study participants was 36.79 ± 0.64°C and that of the systolic and diastolic blood pressures were 109.20 ± 10.93mmHg and 74.57 ± 11.42mmHg respectively **(Table 2)**.

Medical Conditions of Patients Receiving Blood Transfusion and the Type of Blood Requested: The common medical conditions reported were anemia, malaria, sickle cell anemia, abortion, and abortion. Among 27 anemic patients, 17/27 received whole blood while 10/27 received packed red cells. For those diagnosed with malaria (22), majority of them (20/22) received whole blood while 2/22 received packed red cells. Patients who presented conditions such as abortion, abdominal injury, gastroenteritis, chronic kidney diseases (CKD) and renal tubular acidosis (RTA) were all administered whole blood only **(Table 3)**.

Requisition of Blood Transfusion Based on HB, WBC AND PLT: Request for blood or blood components for a patient was based on the levels of hemoglobin, white blood cells and platelets count. The first category of patients was denoted as C1, which represented patients with low level of hemoglobin but normal levels of White blood cells and platelets. The C1 category consisted of 14% patients, out of which 85.7% of them received whole blood and 14.3% received packed red cells. The second category was denoted as C2, these were patients with low hemoglobin level, normal white blood cells level and increased platelets count. Only one person reported of bronchopneumonia who received a whole blood in this category. The third category denoted as C3 were patients with low hemoglobin level but high levels of both white

Table 1: Demographic and request information of patients receiving blood transfusion

Variable	Total (n = 200)	Male (n = 87)	Female (n = 113)	P-value
Age (years)	27.60 ± 16.55	28.29 ± 19.83	27.06 ± 13.55	0.605
Age group n (%)				0.008
≤ 5	21 (10.5)	13 (61.9)	8 (38.1)	
6-18	36 (18.0)	16 (44.4)	20 (55.6)	
19-29	62 (31.0)	24 (38.7)	38 (61.3)	
30-39	34 (17.0)	10 (29.4)	24 (70.6)	
40-49	37 (13.5)	9 (33.3)	18 (66.7)	
50-59	10 (5.0)	6 (60.0)	4 (40.0)	
≥ 60	10 (5.0)	9 (90.0)	1 (10.0)	
Ward				<0.001
Emergency	39 (19.5)	24 (61.5)	15 (38.5)	
General	106 (53.0)	63 (59.4)	43 (40.6)	
Labour	31 (15.5)	0 (0.0)	31 (100)	
Lying in	12 (6.0)	0 (0.0)	12 (100)	
Recovery	12 (6.0)	0 (0.0)	12 (100)	
Blood requested				0.839
Whole blood	169 (84.5)	73 (43.2)	96 (56.8)	
Packed red cells	31 (15.5)	14 (45.2)	17 (54.8)	
Units of blood transfused				0.762
≤ 1	96 (48.0)	44 (45.8)	52 (54.2)	
2	84 (42.0)	34 (40.5)	50 (59.5)	
3	20 (10.0)	9 (45.0)	11 (55.0)	
Values are presented as frequency (percentage)				

Table 2: Average body temperature, blood pressure and complete blood count of patients receiving blood transfusion

Variable	Total	Male	Female	P-value
Temperature (units)	36.79 ± 0.64	36.78 ± 0.63	36.79 ± 0.65	0.963
Blood pressure				
SBP mmHg	109.03 ± 10.93	108.89 ± 11.0	109.14 ± 10.92	0.87
DBP mmHg	74.57 ± 11.42	74.47 ± 12.01	74.64 ± 10.99	0.919
HB (g/dl)	6.13 ± 1.46	6.18 ± 1.57	6.09 ± 1.37	0.666
WBC (x 103/µL)	13.82 ± 8.44	14.78 ± 7.35	13.08 ± 9.16	0.159
RBC (x 106/µL)	3.82 ± 1.45	3.83 ± 1.41	3.82 ± 1.49	0.955
MCV (fl)	72.51 ± 8.77	74.25 ± 9.73	71.18 ± 7.73	0.014
MCH (pg)	23.66 ± 5.65	23.80 ± 6.11	23.55 ± 5.30	0.754
MCHC (g/dl)	29.59 ± 5.13	29.74 ± 6.28	29.48 ± 4.05	0.722
PLT (x 103/µL)	212.72 ± 143.49	202.28 ± 134.10	220.75 ± 150.41	0.368
GRAN (x 103/µL)	5.71 ± 3.78	5.87 ± 3.01	5.58 ± 4.30	0.58
LYM (x 103/µL)	6.29 ± 4.07	7.11 ± 4.19	5.65 ± 4.19	0.011
MID (x 103/µL)	1.95 ± 1.78	2.02 ± 1.51	1.90 ± 0.19	0.64

Values are presented as mean ± standard deviation. SBP = Systolic blood pressure; DBP = Diastolic blood pressure; HB = Hemoglobin count; WBC = White blood cells count; RBC = Red blood cells count; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; PLT = Platelet count; GRAN = Granulocyte; LYM = Lymphocytes;

Table 3: Some medical conditions of patient's receiving blood transfusion and the type of blood requested

Conditions	Type of Blood Requested		Total (n = 200)
	Whole Blood (n = 169)	Packed red cells (n = 31)	
Anaemia	17 (63.0)	10 (37.0)	27 (13.5)
Malaria	20 (90.9)	2 (9.1)	22 (11.0)
Malaria + Anaemia	13 (92.9)	1 (7.1)	14 (7.0)
Sickle cell disease	4 (25.0)	12 (75.0)	16 (8.0)
Abortion	8 (100)	-	8 (4.0)
Chronic kidney disease	5 (100)	-	5 (2.5)
Renal tubular acidosis	12 (100)	-	12 (6.0)
Cardiovascular accident	3 (100)	-	3 (1.5)
Abdominal injury	2 (100)	-	2 (1.0)
Abdominal tuberculosis	2 (100)	-	2 (1.0)
Bronchopneumonia	2 (100)	-	2 (1.0)
Gastroenteritis	2 (100)	-	2 (1.0)
Respiratory viral infection	6 (42.9)	8 (57.1)	14 (6.0)
Burns	8 (100)	-	8 (4.0)
Antepartum haemorrhage	5 (83.3)	1 (16.7)	6 (3.0)
Pulmonary hypertension	6 (100)	-	6 (3.0)
Premature rupture of membranes	11 (100)	-	11 (5.5)
Trauma	3 (100)	-	3 (1.5)
Road traffic accident	9 (100)	-	9 (4.5)
Gastrointestinal bleeding	3 (100)	-	3 (1.5)
Ectopic Pregnancy	7 (100)	-	7 (3.5)
Sepsis	5 (100)	-	5 (2.5)
Urinary tract infections	4 (100)	-	4 (2.0)
Uterine Rapture and Myomas	6 (100)	-	6 (3.0)

- = not administered to patients

blood cells and platelets. Out of the 7.5% patients in this category, 66.7% received whole blood and 33.3% received packed red cells **(Table 4)**.

Units of Blood Received: Patients with different levels of hemoglobin, white blood cells and platelets count received different type of blood. Those deemed to be anemic (Hb <7g/dL), were made up of 67.5% of those who received whole blood and 54.8% of those who received packed red cells. Patients with WBC count >10, were made up of 63.9% of those who received whole blood and 74.2% of those who received packed red cells. Of those with WBC levels between 4 - 10, 34.4% of them received whole blood and 25.8% of them received packed red cells (Table 6). Of patients with platelets count between 150 - 450, 45% of them received whole blood and 51.6% received packed red cells **(Table 5)**.

Association of Blood Transfusion Reactions in Relation to Age and Units of Blood Transfused: There was a significant association between the units of blood transfused and transfusion reactions (p = 0.011). There was no significant association between transfusion-related adverse reactions and age distribution **(Table 6)**.

Out of the 200 patients issued blood over the two months period, 95 (47.5) experienced adverse transfusion reactions

(Table 1, Figure 1). From the observed 95 transfusion-related adverse reactions, whole blood accounted for 95.79 % while packed red blood cells accounted for 4.21%. The highest (30.5%) number of transfusion-related adverse reaction was observed among people aged 19-29 age groups. There was however no statistically significant difference between age and occurrence of transfusion reaction. Of 96 patients who received ≤1 units of blood, 58.1% experienced some form of transfusion-related adverse reactions **(Figure 2)**.

Table 4: Requisition of blood for transfusion based on HB, WBC and PLT levels

Categories	Total	Type of Blood Requested	
		Whole Blood	Packed red cells
C1	28 (14.0)	24 (85.7))	4 (14.3)
C2	1 (0.5)	1 (100)	0 (0.0)
C3	15 (7.5)	10 (66.7)	5 (33.3)
C1 = Low HB, Normal WBC and Normal PLT; C2 = Low HB, Normal WBC and Increased PLT; C3 = Low HB, Increased WBC and Increased PLT. HB = Hemoglobin count; WBC = White blood cells count; PLT = Platelet count			

Table 5: HB, WBC, RBC and Platelet levels with the Type of blood requested

Parameter	Type of Blood Requested		P-value
	Whole Blood (n = 169)	Packed red cells (n = 31)	
HB (g/dL)			0.288
< 7	114 (67.5)	17 (54.8)	
7-10.	53 (31.4)	14 (45.2)	
> 10	2 (1.2)	0 (0.0)	
WBC (103/µL)			0.461
< 4.0	3 (1.8)	0 (0.0)	
4.0-10	58 (34.3)	8 (25.8)	
> 10	108 (63.9)	23 (74.2)	
RBC (106/µL)			0.012
< 4.4	123 (72.8)	17 (54.8)	
4.4-5.5	35 (20.7)	7 (22.6)	
> 5.5	11 (6.5)	7 (22.6)	
PLT (103/µL)			0.161
< 150	80 (47.3)	10 (32.3)	
150-450	76 (45.0)	16 (51.6)	
> 450	13 (7.7)	5 (16.1)	
Values are presented as frequencies (percentage). HB = Hemoglobin count; WBC = White blood cells count; RBC = Red blood cells count; PLT = Platelet count			

Discussion

This study was conducted over a period of two months at a district hospital in Kumasi, Ghana. The study assessed the incidence of transfusion-related adverse reactions among patients attending a district hospital in Ghana. Transfusion-related adverse reactions were observed to be quite common at the district hospital. Of the 200 units of blood issued, 95 transfusion reactions were reported among patients during the study period. From this study, the incidence of transfusion-related adverse reaction was found to be 47.5% which is approximately twice the incidence (21.3%) reported earlier at a teaching hospital in Ghana (Owusu-Ofori, Owusu-Ofori, & Bates, 2017).

Table 6: Association of blood transfusion reactions in relation to age distribution and units of blood transfused

Characteristics	Transfusion Reaction		P-value
	No reaction	Reaction	
Age group n (%)			0.354
≤ 5	5 (5.3)	16 (15.2)	
6-18.	19 (20.0)	17 (16.2)	
19-29	30 (31.6)	32 (30.5)	
30-39	15 (15.8)	19 (18.1)	
40-49	15 (15.8)	12 (11.4)	
50-59	5 (5.3)	5 (4.8)	
≥ 60	6 (6.3)	4 (3.8)	
Units of blood transfused			0.011
≤ 1	35 (36.8)	61 (58.1)	
2	48 (50.5)	36 (34.3)	
3	12 (12.6)	8 (7.6)	

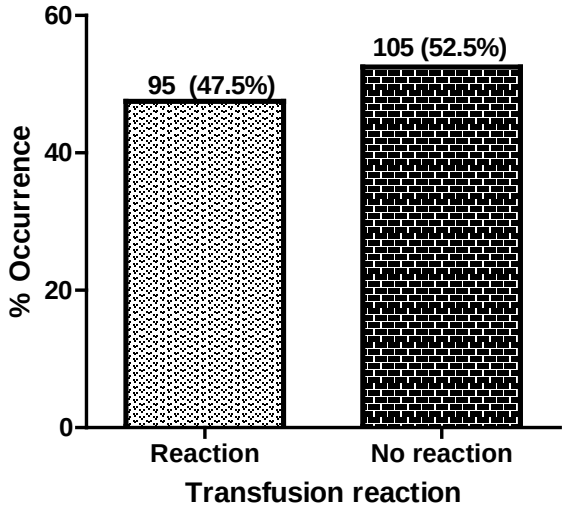

Figure 1: Percentage occurrence of transfusion reaction in patients receiving blood transfusion

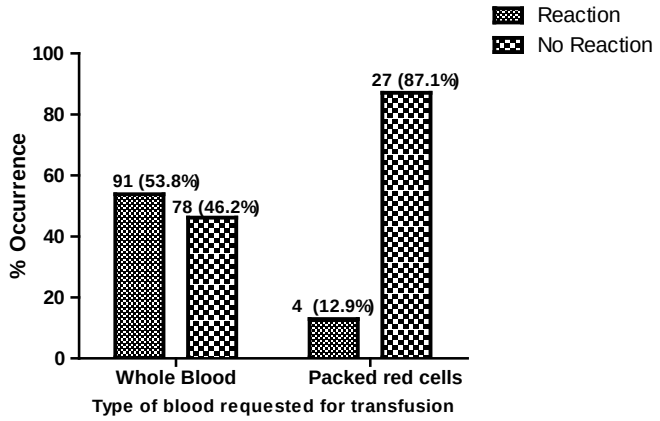

Figure 2: Occurrence of blood transfusion reaction based on the type of blood transfused

The main difference between the two findings could be attributed to the low number of participants and the short duration (two months) of this study in comparison to the later which recruited 432 in a twelve-month study. Adherence to proper protocols on the part of clinicians and

laboratory scientists could also be a major factor as the former was conducted at a teaching hospital.

Nevertheless, these results indicate high incidence of transfusion-related adverse reactions at the study setting, which could be due to inappropriate blood product prescription, hemovigilance system, blood safety operations and poor resourced laboratories (Owusu-Ofori et al., 2017). The incidence in this study was higher than those reported in other studies within other Sub-Saharan African countries. For instance, 87 per 1000 blood components resulted in transfusion-related adverse events in a tertiary Hospital in Nigeria (Arewa, Akinola, & Salawu, 2009) and more than 50 adverse reactions per 1000 blood components was also reported in a principal teaching hospital in Cameroon. However, the incidences were very low in some countries outside the Sub-Saharan region, as 0.046% was reported in a study carried out in Zimbabwe (Mafirakureva et al., 2014) and 0.049% incidence at the South African National Blood Service (SANBS) (Gounden, 2019). The Quebec Hemovigilance system in 2004 also reported an adverse transfusion incidence rate of 3.5 per 1000 blood components transfused (Robillard, Nawej, & Jochem, 2004) and 4.2 incidents per 1000 blood components was reported by a General University Hospital in Switzerland (Michlig et al., 2003). Kumar also reported an incidence of 0.5 incidents per 1000 blood components transfused in India (Kumar et al., 2013).

The need for quality and safe blood transfusion process as stipulated by WHO involves quality assurance in all operation stages: donor recruitment and selection, infection screening, blood grouping and blood storage, appropriate administration to the patients and clinical monitoring for adverse reactions (Allain, Owusu-Ofori, & Bates, 2004). In this study, whole blood transfusion accounted for the highest number of transfusion reactions (53.8%) compared to packed red blood cells making up for only 4.21%. This results was consistent with that earlier reported as 66.6% of the adverse reactions from whole blood transfusion while packed red blood cells accounted for only 20.1% of the transfusion reactions (Mafirakureva et al., 2014)These indicate a high incidence of transfusion reactions in patients receiving whole blood compared to those receiving packed red cells.

It was found that 85.7% of individuals with acute blood loss anemia, having low hemoglobin level but normal white blood cells and platelets counts were given whole blood even though only packed red blood cells were required. Moreover, for individuals with low hemoglobin level but having high white blood cells and platelets levels, 66.7% of them received whole blood and only 33.3% received packed red blood cells. These results coincide with the results obtained in another study where almost 60% of its blood requests was whole blood and for a 45% packed red blood cells requested for patients, 80% of most of these cases were given whole blood transfusion in place of packed red blood cells (Boateng et al., 2014). Similarly, (Rebibo, Hauser, Slimani, Hervé, & Andreu, 2004) reported that inappropriate blood components transfusion accounted for 137/2911 transfusion reactions. Of this number, 12/137 were cases of ABO cross matching errors. Inappropriate prescription of whole blood for acute blood loss anemia could be a major contributing factor for the high adverse transfusion reactions recorded among patients who received whole blood in this study. In addition, these adverse transfusion reactions could have been triggered by undue increment in total blood volume in circulation together with elevated levels of white blood cells and platelets since their levels were already high or normal prior to the transfusion.

In western and industrialized countries, the common transfusion practice is blood component therapy since the latter half of twentieth century (Acker, Marks, & Sheffield, 2016). Following the enactment of Safe Blood Transfusion Act 2002 by WHO, a lot of measures have been put in place over the past decades to ensure that proper screening is done to improve the quality and safety of blood samples before they are administered to recipients. For instance screening blood for infectious agents to establish blood safety has been at the forefront of transfusion medicine since the HIV/AIDS epidemics started over the past 30 years (Opare-Sem et al., 2014). Nonetheless, there is little improvement on giving the right blood components like, fresh-frozen plasma, packed red blood cells, platelet concentrates and white blood cells to address different needs of patients especially in developing countries (Dipta & Rahman, 2009). Giving whole blood to a patient who requires just a specific component is a form of inappropriate blood transfusion commonly practiced in Ghana and some Sub-Saharan African countries. This may be attributed to poor resourced laboratories and inadequate knowledge on whole blood separation techniques (Boateng et al., 2014). Blood can be used efficiently if component therapy is practiced, since one unit of whole blood can be separated into components and could be used to treat more than one patient. Conversely, administration of superfluous blood components in whole blood to a recipient who requires only a specific blood component is likely to result in complications like circulatory overload, physiological derangement, allergic reactions, acute hemolytic reaction and febrile non-hemolytic reaction (Boateng et al., 2014; Kleinman, Chan, & Robillard, 2003).

Circulatory overload normally results when too much fluid is transfused or the transfusion is too rapid for the patient, thus the fluid overload could cause pulmonary and systemic venous engorgement which is normally followed by cardiogenic pulmonary edema and acute respiratory failure (Napier, 1995). Massive transfusion especially of whole blood in anemic patients is known to trigger a number of complications, including hypothermia, hyperkalaemia, dilutional coagulopathy and citrate toxicity (Elmer, Wilcox, & Raja, 2013). In addition, high and rapid transfusion of whole blood or its components in patients with liver damage or failure often leads to hypocalcemia and hypomagnesia (Carson, Triulzi, & Ness, 2017). It is also worth noting that patients receiving whole blood transfusion have an increased risk of acquiring blood borne pathogens like malaria, syphilis, hepatitis, bacteria infections and HIV/AIDS compared to those receiving specific blood components (Adjei et al., 2006; Allain et al., 2004).

Conclusion

This study has shown that incidence of adverse transfusion reactions in Ghana, especially at resource poor facilities is still common despite advancement in transfusion practice. It is important that the Ghana Health Services and National Blood Service, and relevant bodies train clinicians in the practice of blood transfusion in Ghana. Transfusion safety can be achieved through appropriate prescription of whole blood, promoting blood component therapy, improved adverse reaction monitoring and blood product manufacturing. Also, it is advisable to strengthen our hemovigilance system and also make room for assessing the specific need of a patient before giving the appropriate blood component. Finally, Clinicians are expected to prescribe appropriate blood products or whole blood by strictly adhering to guidelines by WHO and other recognized bodies for the safety of patients.

Declarations

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Conflict of Interests: The authors declare that they have no competing interests.

List of Abbreviations: HB: Hemoglobin, IBCT: Inappropriate Blood Component Transfusion, KSH: Kumasi South Hospital, PLT: Platelets, RBC: Red Blood cells, SANBS: South African National Blood Service, SPSS: Statistical Package for Social Sciences, WB: Whole Blood, WBC: White Blood Cells, WHO: World Health Organization

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RESEARCH ARTICLE

Analysis of Seven Micronutrients in Breast Milk of Lactating Mothers from the Central Region of Ghana Using Epithermal Neutron Activation

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Abstract

Background: Breast milk contains various micronutrients which nourishes a baby with nutrition, and therefore the endorsement for exclusive breastfeeding during the first six months after birth. Such micronutrients, if in excess, can have adverse effects on the baby.

Objective: The levels of seven micronutrients in breast milk obtained from 27 lactating mothers in the Cape Coast Metropolitan area have been determined using Epithermal Neutron Activation Analysis (ENAA). This technique was used because it is suitable for performing both qualitative and quantitative multi-nutritional analyses on samples, and offers accuracies and sensitivities superior to those attainable by other methods.

Materials and Methods: During the analysis, a- 3 mm thick flexible boron was used to cut-off thermal neutrons so as to assess epithermal neutrons, thereby creating an activation energy which measured the levels of micronutrients in the breast milk. The standard reference materials used were the International Atomic Energy Agency (IAEA)-336; IAEA-407, IAEA-350 and National Institute of Standard and Technology (NIST) USA SRM 1577b. The Relative standardization method was used in the quantification of the micronutrients, with an accuracy of about 94.7 %. The micronutrients are Sodium (Na), Magnesium (Mg), Potassium (K), Calcium (Ca), Manganese (Mn), Copper (Cu) and Iodine (I).

Results: Except for iodine which had levels below the recommended dietary allowance (RDA), the remaining micronutrients had levels above the upper limit of the RDA, with Manganese being the highest.

Conclusion: The levels recorded are directly linked to the food intake of the mothers, and therefore the need for pregnant and lactating mothers to be mindful about what they eat. Children could be exposed to metabolic disorders and diseases as a result of such high levels.

Keywords: Epithermal Neutron Activation Analysis (ENAA), Hazard Quotient (HQ), Lactating Mothers, Micronutrients, Recommended dietary allowable (RDA).

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Introduction

Breast milk is the natural first food for babies. It provides their energy and nutrients, promotes sensory and cognitive development, and protects them against infectious and chronic diseases. Exclusive breastfeeding during the first 6 months of life promotes immunity and reduces infant mortality due to illnesses such as diarrhoea and pneumonia (Ochoa & Turin, 2014). Babies go through a rapid period of growth after birth and usually double in length and triple in weight within their first twelve months. Even when solid foods are introduced, breast milk remains an important source of nutrition for proper growth and development (World Health Organization, 2009). The

Academy of Nutrition and Dietetics recommends that infants consume breast milk alone during the first six months of life, and breast milk with complementary foods from 6 to 12 months. Mothers who choose not to, or are unable to breastfeed can offer infant formula in place of breast milk. The decision to breastfeed or formula feed a baby could be personal or influenced by health issues such as HIV/AIDS infection. Weighing the pros and cons of breastfeeding or formula feeding can help make a decision for the baby (WHO, 1998). Infants from 0 to 6 months should take breast milk or infant formula on demand, to help meet their nutritional requirements (Felder et al., 2018). Carbohydrates are the main source of energy for the body while protein contributes to the energy used by the body, and is essential for the building of tissues of the growing body. Fat contributes to the energy requirements of the body, and helps in the absorption of fat-soluble vitamins such as Vitamin A, D, E and K. Vitamins and minerals assist in the general protection and functioning of the body (World Health Organization, 2009).

Micronutrients are vital for the wellbeing of the human body and derived from diet. Iodine (I) is important for the production of thyroid hormones mostly concentrated in the thyroid gland (Endocrineweb, 2017). Thyroid cells combine with iodine and the amino acid tyrosine to control metabolism. The leading preventable causes of brain damage, which is iodine deficiency, can significantly lower the intelligence Quotients (IQ) of a whole population. It has been estimated that countries could prevent the loss of intellectual capacity by 10 to 15 percentage points if young children, newly born babies and pregnant mothers receive enough iodine (Caulfield et al., 2006). There is the concern that the continuous occurrence of Iodine Deficiency Disorders (IDDs) among children in Ghana may hamper the objectives of the educational reform programmes and the nation's developmental efforts. Statistics indicates that about 81,200 babies are born annually with mental impairments as a result of iodine deficiency, resulting in stunted growth and low IQs, thereby impeding their learning abilities (Ghana News Agency, 2007). Breast milk is the best source of iodine for babies, and helps in development of their brain and nervous system. Infants up to 6 months need an iodine intake of 90 micrograms per day (Andersson & Braegger, 2021).

Magnesium (Mg) promotes the health of children and adults, and is responsible for approximately 800 enzymatic functions in the human body. Babies up to the age of 6 months may require 30 mg per day. It is needed for DNA formation, helps children get a better sleep to conserve energy and aids in insulin and blood sugar regulation (Mayo Clinic, 2023).

An adequate amount of Copper (Cu) is important for the optimal functioning of the brain as its presence within certain enzymes in the brain helps form key neurotransmitters that allow brain cells communicate to one another (Svetlana et al, 2019). Babies up to the age of 6 months may require 0.20 mg per day (Food and Nutrition, 2001). The normal term infant is born with a generous store of copper in the liver making copper deficiency a rare event (Widdowson, Dauncey, & Shaw, 1974). Copper deficiency might be responsible for a resistant anemia in milk-fed infants because cow's milk is low in copper (Picciano, 1985).

Manganese (Mn) is involved in the formation of bones (Food and Nutrition, 2001) and aids in the action of some enzymes involved in carbohydrate metabolism (Lenntech, 2019). Babies up to the age of 6 months may require 0.003 mg of Mn per day (Food and Nutrition, 2001), with an unbalanced level (relatively high or low) resulting in poor brain development (Norton, 2023). Obesity, changes in hair colour, abnormal bone and cartilage function and growth retardation may be an adverse effect of Mn deficiency (Lenntech, 2019).

Sodium (Na) is an electrolyte/mineral that functions as a major ion of the extra cellular fluid and also aids nerve impulse transmission. Babies up to the age of 6 months may require 120.0 mg of Na per day (Health Supplements Nutritional Guide, 2017). A baby will have its entire sodium requirement from breast milk, and the need not to add additional salt, which is the main source of sodium, to the baby's food.

Potassium (K) works with sodium to control the body's water balance. This helps maintain a normal blood pressure, to the extent that a diet low in potassium and high in sodium will cause.

Materials and Methods

Study Area: The study is quantitative research aimed at determining the level of seven micronutrients in the breast milk of volunteer mothers from 20 communities in the Central Region of Ghana. Two health facilities located in Cape Coast and Elmina were used due to their accessibility and high utilization by patients. These facilities provide both static and outreach child welfare services, and therefore attend to mothers from towns in and around them. Cape Coast and Elmina are metropolis of the Central Region of Ghana, along the Gulf of Guinea **(Figure 1)**. The original inhabitants of the communities are mostly subsistence farmers and fishmongers (Hood, Vowotor, Nyarko, & Fletcher, 2011), with the volunteers being mainly petty traders, caterers, teachers, nurses, seamstresses, students, hairdressers, and some unemployed.

Sample Collection

Inclusion Criteria: The criteria for selection was that the mothers should have volunteered to be part of the study, should have had full term pregnancies, practicing exclusive breastfeeding and was within the first six months of the babies' lives.

The study utilised the services of trained midwifes and nurses who were service providers to these mothers, and were able to take the breast milk aseptically. Before the sample (breast milk) collection, the nipples and areolas of the breasts were cleaned with 70% methyl alcohol. The breast milk was collected by manual expression, was between 10-20 mL and delivered directly into sterile 60-

mL plastic vials labelled with codes and immediately stored on ice to prevent spoilage and contamination, as recommended by The Breastfeeding Network, 2014. The samples were transported to the storage point and stored in a freezer below -20oC. They were then transported in a vaccine carrier at the required temperature to the Ghana Research Reactor-1 (GHARR-1) facility at the Ghana Atomic Energy Commission, Kwabenya, Accra, for analysis. Multivariate Analysis was used to analyse the results. Data collection at the health facilities lasted for one month.

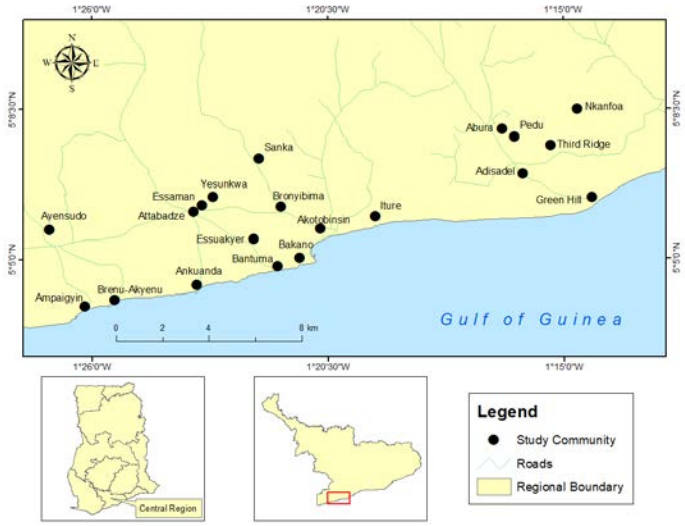

Figure 1: A map showing the towns of individual volunteer mothers used for the study

Epithermal Neutron Activation Analyses: During the analysis using ENAA **(Figure 2)**, epithermal neutrons interact with the breast milk (target nucleus) via a non-elastic collision, resulting in the formation of a compound nucleus in an excited state. The excitation energy of the compound nucleus is due to the binding energy of the neutron with the nucleus. The compound nucleus will almost instantaneously de-excite into a more stable configuration through the emission of one or more characteristic prompt gamma rays. In many cases, this new configuration yields a radioactive nucleus which also decays by the emission of one or more characteristic delayed gamma rays, but at a much slower rate according to the unique half-life of the radioactive nucleus. Depending on the particular radioactive species, half-lives can range from a fraction of a second to several years (Ali, 2000).

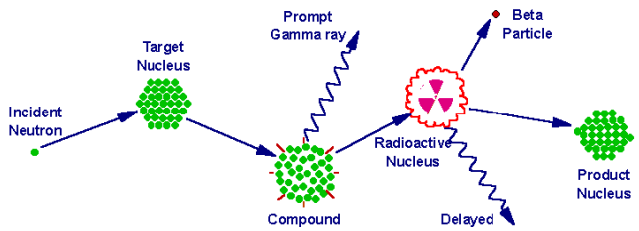

Figure 2: A process of neutron capture followed by emission of gamma ray (Vowotor et al., 2020)

Most neutron energy distributions are quite broad and consist of three principal components: Thermal, Epithermal, and Fast. The thermal neutron component consists of low-energy neutrons (energies below 0.5 eV) in thermal equilibrium with atoms in the reactor's moderator, while the fast neutron component (energies above 0.5 MeV) consists of the primary fission, yielding neutrons having much of their original energy following fission (Ali, 2000).

ENAA technique uses only epithermal neutrons (energies between 0.5 eV to about 0.5 MeV) to induce (n, gamma) reactions. In a typical unshielded reactor irradiation position, the epithermal neutron flux represents about 2% the total neutron flux (Ehmann & Vance, 1993). In reactor activation, this ENAA technique is performed by enclosing samples in thermal neutron filters such as cadmium or boron, which removes thermal neutrons from the reactor neutron spectrum. This has been applied to a variety of sample matrices including geological and biological materials (Chisela, Gawlik, & Brätter, 1986). A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions is presented in **Figure 3**.

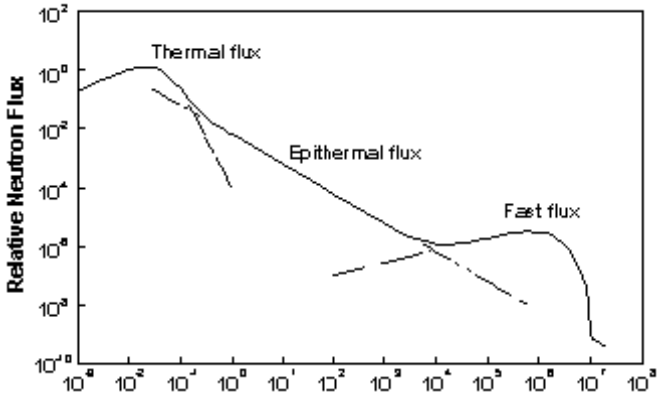

Figure 3: A typical reactor neutron energy spectrum showing the various components used to describe the neutron energy regions (Vowotor et al., 2020).

Sample Preparation and Irradiation: 500 mg of each sample (breast milk) was measured into polyethylene vials using a micrometre pipette after shaking to ensure uniformity. The polyethylene capsule with diameter 1.2 cm and height 2.35 cm containing the liquid sample was then put into a bigger polyethylene capsule with diameter 1.6 cm and height 5.5 cm (Rabbit capsule) and smoothly heat-sealed with a soldering rod. This technique is known as Double encapsulation. The irradiation vials (capsules) used were pre-cleaned by first washing with distilled water, after which they were soaked in an acidic reagent for 24 hours and then rinsed in distilled deionised water. They were then further soaked in nitric acid for 24 hours, rinsed thoroughly with distilled deionised water and air-dried in a clean fume hood.

To validate the procedure, various standard reference materials, namely IAEA-336 (trace and minor elements in Lichen), IAEA-407 (trace elements and methyl mercury in fish tissue), IAEA-350 (trace elements in tuna fish

homogenate) and SRM 1577b (Bovine liver) were prepared and packed similarly as the samples (IAEA, 2003; IAEA, 2023; EVISA, 2010a; EVISA, 2010b). The samples were transferred to the irradiation sites through a pneumatic transfer system at a pressure of 60 psi. The irradiation was categorized according to the half-life of the element of interest and carried out in the Ghana Research Reactor-1 (GHARR-1) at the Ghana Atomic Energy Commission (GAEC). The reactor operates at 15 KW at a thermal flux of 5×1011 ncm-2s-1, and uses a 3 mm thick flexible boron element to cut off thermal neutrons in order to assess only epithermal neutrons. 94.7% accuracy was achieved.

Relative Standardization: In the relative standardization method, a standard chemical (index std) of known mass, Wstd, of the element is co-irradiated with the sample of unknown mass Wsam. When the sample to be irradiated is a short-lived radionuclide, both the standard and sample are irradiated separately under the same conditions, usually with a monitor of the same neutron fluence rate, and both counted in the same geometrical arrangements with respect to the gamma-ray energy. It is then assumed that the neutron flux, cross section, irradiation times and all other variables associated with the counting are constant for the standard and the sample at a particular sample-to-detector geometry. With this assumption, the neutron activation equation reduces to equation 1 as:

$$\rho_{sam} = \frac{\left[(P_A/t_c)\right]_{sam}\left[\rho\,CDW\right]_{std}}{\left[P_A/t_c)\right]_{std}\left[CDW\right]_{sam}}$$

where (PA/tc)std and (PA/tc)sam are the counting rates for the standard and sample respectively, std and sam are the counting concentrations of the standard and element of interest respectively, Cstd and Csam are the counting factors for standard and sample, Dstd and Dsam are the decay factors for the standard and sample respectively.

Qualitative and Quantitative Analysis: The qualitative analysis determined the levels of the seven micronutrients in the breast milk samples by isolating the spectra peaks, assigning corresponding radionuclides and identifying the micronutrients present. This was done using an ORTEC EMCAPLUS Multichannel Analyzer (MCA) Emulation software. A Microsoft Window-based software, MAESTRO, was used for spectral analysis (Serfor-Armah et al, 2018). This software identifies the various photo peaks, estimates and works out the areas under them.

The quantitative analysis involves the calculation of the areas under the peaks of identified micronutrients and converting them into concentrations using an appropriate software or equation(s) (Jean & Alfassi, 1994). The counting of the induced radioactivity was done using a PC-based γ-ray spectrometer which consists of an n-type high purity Germanium (HPGe) detector (model GR2518), coupled to a computer-based Multichannel Analyzer via electronic modules, and an amplifier (model 2020, Canberra Industries Incorporated). The relative efficiency of the detector is 25%, with an energy resolution of 1.8 KeV at γ-ray energy of 1332 KeV of 60Co. The other quantitative measurements were carried out using Equation 1. The detection limits (DL) of the micronutrients and the nuclear data used to undertake the ENAA have been summarized in **Table 1**.

Results

Measured levels of the micronutrients were directly linked to the types of foods taken in by the volunteer mothers. **Table 2** shows some of the foods consumed by the mothers and the individual micronutrients they contain.

Table 2: Micronutrients contained in some foods consumed by mothers involved in the study

Micronutrient	Foods
Na	Shrimp, Soup, Ham, Vegetable Juice, Salad Dressing, Sandwiches, Dried Meats, Sauces, Breads, Canned Meats, Poultry and Seafood, Biscuits, Baked Beans, Sausage, Bacon (Healthline, 2023: a)
Mg	Wheat, Spinach, Almonds, Cashews, Peanuts, Dark Chocolate, Legumes, Vegetables (Peas, Cabbage, Green Beans, Avocado, Banana, salmon, mackerel, tuna (Steen, 2020)
K	White Beans, Potatoes and Sweet Potatoes, Beets, Spinach, Tomato Sauce, Oranges and Orange Juice. (Healthline Media, 2023)
Ca	Yogurt, Sardines, Salmon, Beans, Almonds, Some Leafy Greens like Broccoli, Parsley, Spinach, Lettuce, Cabbage, "Kontomire", etc, Milk, Lemon, and Orange. (Healthline, 2023: b)
Mn	Pineapple, Spinach, Lima Beans, Sweet Potatoes, Brown Rice, Soybeans, Corn, Turmeric (Food and Nutrition, 2021)
Cu	Organ meats (liver), Oysters, Lobster, Squid, Mushrooms, Nuts and Sesame Seeds, Cashew Nuts, Almonds, Sunflower Seeds, Leafy Greens, Dark Chocolate. (Healthline, 2023)
I	Yogurt, Iodized Salt, Shrimp, Tuna, Eggs (Healthline, 2019)

Table 1: Detection limits of the micronutrients and the nuclear data used to undertake the ENAA

Element	Radioisotope	Gamma Ray Energy (keV)	Half-life	Irradiation Time (min)	Counting Time (min)	DL (μg/g)
Na	^{24}Na	2754	15 hr	10	10	0.001
Mg	^{27}Mg	1014.4	9.46 min	10	10	0.1
K	^{42}K	1524.6	12.4 hr	10	10	0.01
Ca	^{49}Ca	3084.5	8.72 min	10	10	1.0
Mn	^{56}Mn	1810.7	2.58 hr	10	10	0.0001
Cu	^{66}Cu	1039.2	5.1 min	10	10	0.01
I	^{128}I	440.9	25 min	10	10	0.0001

Table 3: Summary of the levels of the micronutrients measured in the breast milk

	Sodium (Na)	Magnesium (Mg)	Potassium (K)	Calcium (Ca)	Manganese (Mn)	Copper(Cu)	Iodine(I)
Min	294.4000	132.4000	1359.0000	408.1000	0.0018	0.2235	0.0290
Max	2797.0000	426.7000	5225.0000	1497.0000	1.1150	1.0199	0.1445
Mean	1098.6259	309.2481	2438.0741	697.4037	0.4028	0.6944	0.0613
SD	36.4403	23.7186	176.349	35.2538	0.0384	0.0079	0.0102

Assessment According to Levels: Measured levels of the micronutrients in the breast milk are shown in **Figure 4**, and a summary of the statistics presented in **Table 3**.

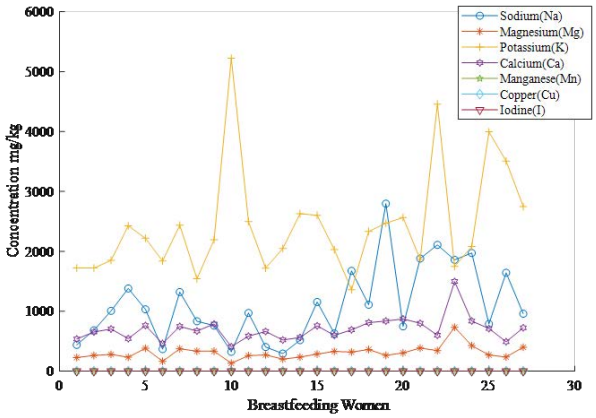

Figure 4: Measured levels of the seven micronutrients in the breast milk

The measured levels were within the following intervals: Na: 294.4-2797.0 mg/kg; Mg: 132.4-426.7 mg/kg; K: 1359.0-5225.0 mg/kg; Ca: 408.1-1497.0 mg/kg; Mn: 0.0018-1.115 mg/kg; Cu: 0.2235-1.0199 mg/kg; and I: 0.029-0.1445 mg/kg. In terms of abundance, they can be arranged in the order: K > Na > Ca > Mg > Cu > Mn > I. The low standard deviation (SD) values obtained indicate that the spatial distribution of the individual micronutrients in their breast milk was uniform.

Assessment According to Recommended Dietary Allowable: Recommended dietary allowable (RDA) is the dietary requirement for a micronutrient. It is an intake level which meets a specific criterion for adequacy, thereby minimizing risk of nutrient deficit or excess (Health Canada, 2022). Exclusively breastfed babies take in an average of 750 ml or 708.738 g per day between the ages of 1 month and 6 months (Bonyata, 2019). If the level of a micronutrient in milk per day (RDA) is expressed in mg/d, and the average intake of milk per day represented by M g/d, the level of micronutrients in the breast milk represented by N mg/g can be expressed in equation 2 as: (Vowotor et al, 2011);

$$N = RDA / M \quad \text{(Equation 2)}$$

The RDA levels and their upper limits, and those measured using data from the breast milk for the various micronutrients are shown in Table 4. Also in Table 4 are the Allowable daily intake values, ADI, calculated from the Mean Measured levels, MMC using equation 3.

$$ADI = MMC \times RDA / RDA\ level \quad \text{(Equation 3)}$$

Assessment According to Health Risk Estimation: To estimate the health effects, hazard quotient (HQ), the estimated lifetime average daily dose of each micronutrient is compared to its Reference Dose (RfD). The reference dose represents an estimated daily consumption level that is likely to be without deleterious effects in a lifetime, based on the equation (4) detailed in the US. EPA handbook (Chao-yang et al., 2018):

$$\text{The hazard quotient (HQ)} = ED/RfD \quad \text{(Equation 4)}$$

where, ED = Estimated Dose and RfD = Reference Dose.

HQ obtained for the various micronutrients are also presented in **Table 4**.

Except for Iodine, levels of the other micronutrients were found to be high compared to the calculated levels of the RDA (Table 4). Looking at the difference between the RDA values and the measured/calculated RDA values, a negative sign (–), as in the case for iodine, denotes a value below the recommended RDA, while a positive sign (+), as obtained for the rest, denotes levels above the recommended RDA. HQ < 1 suggests unlikely adverse health effects, whereas HQ > 1 suggests the probability of adverse health effects (Liu et al., 2018). HQ values > 1 have been highlighted. **Table 5** lists the side effects in consuming these micronutrients in excess quantities.

Pearson correlation was calculated and presented on **Table 6**. It showed a normal distribution in the levels of micronutrients consumed. The variables are continuous and exhibited a linear relationship. Though 95%

Table 4: RDA, their Upper limits and levels calculated (mg/kg), as well as HI calculated for the micronutrients for the breast milk

Micronutrient	Na	Mg	K	Ca	Mn	Cu	I
RDA	120.0	30.0	400.0	200.0	0.003	0.20	0.09
RDA Upper Level	169.3	42.3	563.4	281.7	0.0042	0.28	0.13
Mean Measured Level	1098.6259	309.2481	2438.0741	697.4037	0.4028	0.6944	0.0613
Average Daily Intake from Level	778.71	219.32	1730.97	495.14	0.29	0.5	0.04
Difference Between the RDA & Measured RDA	+ 658.71	+ 189.32	+ 1330.97	+ 295.14	+ 0.287	+ 0.3	– 0.05
HQ	6.49	7.31	4.33	2.48	95.9	2.48	0.47
RDA Values – (Andersson & Braegger, 2021).							

Table 5: The Seven micronutrients and the side effect when consumed in excess quantities

Element	Adverse Effect of Excessive Consumption
Na	Dark, yellow urine with a strong smell is a very common sign in babies with high sodium intake, weight gain, child is unusually thirsty for water, blood pressure increases, arterial blood vessels increase in thickness, predisposing the child to cardiovascular disease (Sinrich, 2019).
Mg	Large doses might cause too much magnesium to build up in the body, causing serious side effects including an irregular heartbeat, low blood pressure, confusion, slowed breathing, coma, and death (WebMD, 2023a).
K	Diarrhoea, arrhythmias, lethargy and abdominal distention are the most common manifestations of high potassium levels in babies (NHS, 2022).
Ca	Symptoms and signs of neonatal hypercalcemia include anorexia, reflux, gastroesophageal nausea, vomiting, lethargy or seizures or generalized irritability, and hypertension (Dysart, 2019).
Mn	High levels can wreak havoc on your baby's immature metabolic systems (WebMD, 2023b)
Cu	Vomiting, abdominal pain, sleep disorder, weakness, and damage to the liver and kidney. Dr. Paul Eck found that elevated tissue Cu is associated with homosexual desire (Nolan, 1983)
I	Low or High iodine causes thyroid gland inflammation, including goiter (an enlarged thyroid gland) and thyroid cancer ((Alexander, et al., 1961);(Delange & Bürgi, 1989); NIH, 2022).

Table 6: Correlation coefficients of the levels of the seven micronutrients in breast milk

	Na	Mg	K	Ca	Mn	Cu	I
Na	1						
Mgr^2	0.465* 0.01	1					
Kr^2	0.035 0.861	−0.284 0.152	1				
Car^2	0.459* 0.016	0.905** 0.000	−0.304 0.124	1			
Mnr^2	0.250 0.208	0.836** 0.000	−0.214 0.285	0.733** 0.000	1		
Cur^2	0.832** 0.000	0.545** 0.003	0.192 0.337	0.531** 0.004	0.389* 0.045	1	
Ir^2	−0.171 0.393	0.002 0.993	−0.175 0.384	0.020 0.920	−0.371 0.057	−0.269 0.174	1
*Correlation is significant at the 0.05 level (2-tailed)							

confidence level was used to ascertain the strength of their relationship, there are other strongly correlated micronutrients with high coefficients of determination, hence cannot be ruled out (Pallant, 2020).

Discussion

An important source of micronutrients is our foods, processed water and groundwater discharges (Kelly & Moran, 2002). Accordingly, it is important to characterize each distinct source and determine its contribution to mother and baby. Correlation coefficients between the seven micronutrients and their respective significance at 95% significance level in the breast milk are in Table 6. The interpretation of the strength of the correlation coefficients usually depends on the researcher as suggested by the guidelines from Rumsey 2010. In this study, values between are considered strong correlations. As the significance of rho is strongly influenced by the sample size, smaller sample sizes do not reach statistical significance as compared to larger sample sizes which may even be statistically significant at small (weak) correlations (Pallant, 2020). Even though 4 correlations were strong, 3 were statistically significant. This means that we can exude 95% confidence that the correlation between Ca and Mg is strongly correlated with a coefficient of 0.905.

As expected, there was a moderately strong correlation between Na and Mg, as the main source of Na is salt. Salt is one of essential commodities mined and exported from the study area and used for seasoning in most of the foods consumed by participants in this study. Some participants said they normally eat bread made from wheat (rich in Mg and salt as in Table 2) for breakfast. Other salted foods rich in Mg and consumed by participants are soups made from vegetables, seafood (salmon, mackerel, tuna), beans and salted roasted peanuts with banana.

There was a strong correlation of 0.905 between Na and Ca. This can be attributed to the intake of salted foods rich in Ca such as sardine, salmon, beans and leafy greens, present in most mothers' daily diets like yam and palava sauce. Chocolate which is rich in Cu is made from Cocoa. Ghanaians generally consume a lot of cocoa products which are typically spiced with salt. Other foods rich in Cu like lobsters, squid, oysters and organ meats such as liver and kidneys are also seasoned with salt before used for meals. This can explain the correlation between Na and Cu.

Mg showed a strong correlation of 0.905, 0.836 and 0.545 with Ca, Mn and Cu respectively. Mg and Ca-rich foods like the legumes (gari and beans) and soups made from seafoods are heavily consumed by the mothers.

Manganese is an essential micronutrient which participates in the action of many enzymes, lack of which causes testicular atrophy, and excess is toxic (Encyclopaedia

Britannica. 2023). It exhibited a strong correlation of 0.733 and 0.389 with Ca and Cu respectively. Foods like beans and spinach are a good source of Mn and Ca. Seafoods such as oysters, lobster and squid are rich in Cu and usually used in preparing stews which are taken with brown rice, rich in Mn. The correlation of 0.531 between Ca and Cu may come from the consumption of green leafy vegetables such as broccoli, parsley, spinach, lettuce, cabbage, Kontonmire and the likes. Mothers usually use these vegetables in preparing nutritious sauces.

Conclusion

Diseases affecting people in their later years of lives may be as a result of foods served them due to lack of education on nutrition by their mothers in their childhood. From Table 4, only iodine had levels in the normal RDA range, with the remaining micronutrients recording high levels. With such high levels, mothers will be exposing their children to metabolic disorders and unexplained diseases in their future lives without knowing the cause. Na is found in salt, which is used virtually in all foods. Its accumulation in the body therefore starts right from childhood, and may be the cause of the unexplained hypertension in early adulthood.

Declarations

Acknowledgment: The authors wish to express their sincere appreciation to the Ghana Health Service (GHS) and the directors of the two health facilities for allowing the study to be carried out there. Appreciation also goes to the volunteer mothers, nurses and technicians who collected data, and the staff of Ghana Atomic Energy Commission at Kwabenya-Accra, for their assistance in running the tests and analysing of the samples.

Ethical Approval and Consent to Participate: The Ghana Health Service Ethical Review Committee gave us the approval to carry out this study, and issued us a certificate with number GHS-ERC-02/05/15 as a document for their approval. Breastfeeding mothers participated voluntarily, and were accorded the needed respect, dignity and confidentiality as samples taken were a part of their body issue.

Conflict of interest: The authors declare that they have no competing interests.

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