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Original Article

Public Health Section

Double Jeopardy in Malaria Infected Children with Common Haemoglobinopathies: A Cross-sectional Study in Malaria Mesoendemic Districts of Ghana

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ABSTRACT

Introduction: Malaria and conditions associated with common haemoglobinopathies constitute a health threat to young children in sub-Saharan Africa. Malaria is known to exert some influence on common haemoglobin variants while haemoglobinopathies also exacerbate malarial infection especially in children as a result of anaemia and other conditions.

Aim: To assess the views of parents/guardians on malaria and haemoglobinopathies and to determine the extent to which common haemoglobin variants influence malaria parasitaemia among children in the acute stage of the infection.

Materials and Methods: This cross-sectional study was conducted in seven districts in the forest zone of Ghana between June 2018 to July 2018 and involved 342 malaria-parasitaemic subjects aged 5 years and below. Questionnaires were administered to elicit responses on malaria prevention and sickle cell knowledge from the parents/guardians/caretakers accompanying the children to seek healthcare. Follow-up responses on medication and treatment outcome were collected during and after treatment. Venous blood samples were collected for malaria test, sickle cell investigations and identification of common haemoglobinopathies. After treatment, malaria tests were conducted using microscopy. The Chi-square test was used to compare categorical data between groups, and the Independent t-test used to compare means between groups.

Results: Out of total 342 subjects, 220 respondents (64.3%) identified fever as the most classical symptom of malaria, whilst 167 (48.8%) consistently used Insecticide Treated Nets (ITNs). More so, 146 respondents (42.7%) strongly agreed that Sickle Cell Disease (SCD) is a curse, and 182 respondents (53.2%) strongly agreed that SCD is inherited with 225 (65.8%) strongly agreeing that SCD children die before their teen ages. Common haemoglobin variants identified were Haemoglobin A and C bands (HbAC) 17 (5%), Normal Foetal Haemoglobin (HbAF) 7 (2%), Haemoglobin A and S bands (HbAS) 86 (25.1%), Haemoglobin S and C bands (HbSC) 4 (1.2%) and Haemoglobin S (homozygous) band (HbSS) 9 (2.6%). One month post-treatment, 10 (58.8%) of HbAC subjects, all 9 (100%) of HbSS and all 4 (100%) of HbSC subjects were still parasitaemic but with significantly low mean parasite density (495±1744).

Conclusion: Knowledge on malaria among the parents/guardians/caretakers was satisfactory, but usage of bed nets was poor, warranting the need for targeted health education of the rural folk. Though parasite densities had significant reductions, all subjects with HbSC and HbSS phenotypes remained parasitaemic after 4 weeks of treatment, suggesting a double jeopardy for individuals with SCD. Routine newborn screening in all healthcare facilities in Ghana could be a proactive step in malaria case management.

Keywords: Anaemia, New born screening, Parasite density, Plasmodium, Sickle cell disease

INTRODUCTION

In 2018, 228 million people across the globe were diagnosed with malaria, with Africa getting a 93% allocation at a time malaria's global mortality stood at 4,05,000 [1]. Children, especially those 5 years of age and below, bear the greatest burden of the disease [2]. Malaria poses a serious threat to the health of young children and so does Sickle Cell Disease (SCD) and other haemoglobinopathies due to their prevalence in Africa [3]; and because of their notoriety for increased mortalities, the prevalence had been placed somewhere between 50% and 90% [4].

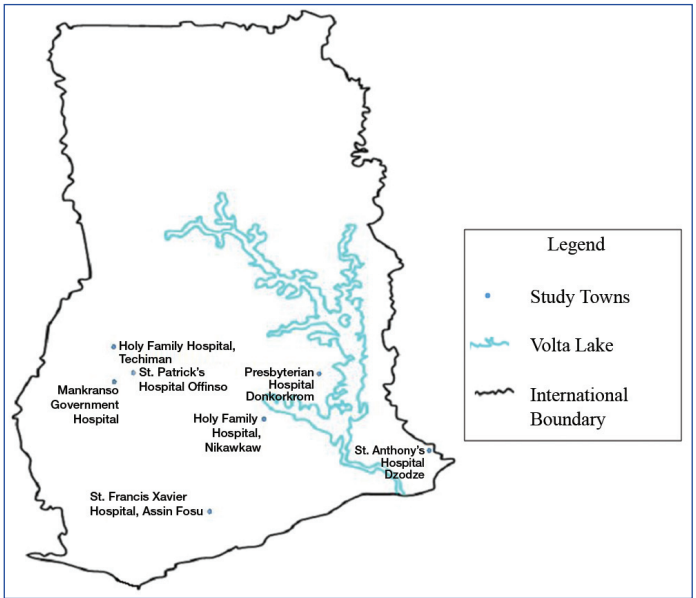
In sickle cell haemoglobinopathy, there is a point mutation in the β-globin chain. This leads to the substitution of glutamic acid with valine at the sixth position of the peptide chain [5]. Altogether, the haemoglobin variants, Hemoglobin S (HbS) (β6Glu→Val) and Hemoglobin C (HbC) (β6Glu→Lys) have carrier prevalence of up to 30% in some African countries south of the Sahara [6]. Up to 3% of all children born annually on the African continent have SCD [3], while other haemoglobinopathies have global prevalence of 25-30% [7]. Common abnormal structural globin variants in Ghana and West Africa are Haemoglobin A and C bands (HbAC), Haemoglobin A and S bands (HbAS), Haemoglobin S (homozygous) band (HbSS), Haemoglobin S and C bands (HbSC) [8].

In Ghana, malaria is hyperendemic in most regions, although there are areas that are mesoendemic and hypoendemic and malaria prevalence among infants is approximately 28% [6] and a 2% prevalence of sickle cell birth annually [9]. Because in-depth sickle cell screening, to detect common haemoglobinopathies, is not a routine procedure in many healthcare facilities in Ghana, most children with the condition are not detected and as a result may probably die young [9]. The implication is that the combination of malaria infection and sickle cell crisis could best be described as double jeopardy for young children. The present study, therefore, aimed to determine the extent to which common haemoglobin variants influence malaria parasitaemia in children in the acute stage of the infection. Also to measure haemoglobin and white cell levels, identify common structural haemoglobin variants and the malaria parasite species present and to determine the malaria parasite densities in study subjects. It also sought the views of parents/guardians on malaria preventive practices and perceptions surrounding malaria and sickle cell infections.

MATERIALS AND METHODS

The cross-sectional study was carried out in seven districts in the forest zone of Ghana between June 2018 and July 2018. The study sites

were: Eastern Region (Holy Family Hospital, Nkawkaw in the Kwahu West Municipality, and Presbyterian Hospital, Donkorkrom in Kwahu Afram Plains North District); Central Region (St. Francis Xavier Hospital, Assin Fosu in Assin North Municipal); Ashanti Region (St. Patrick's Hospital, Offinso in the Offinso Municipality, and Mankranso Government Hospital in Ahafo Ano South District); Bono East Region (Holy Family Hospital, Techiman in Techiman Municipal) and Volta Region (St. Anthony's Hospital, Dzodze in Ketu North District). The study sites are shown in [Table/Fig-1] with more details in [Table/Fig-2].



[Table/Fig-1]: Map of Ghana showing the study sites.

Study sites	Region	Co-ordinates	Digital address
Holy Family Hospital, Techiman	Bono East	7.612°N 1.955°W	BT-0014-5751
St. Patrick's Hospital, Offinso	Ashanti	6.989°N 1.690°W	A7-0020-5679
Mankranso Government Hospital	Ashanti	6.821°N 1.863°W	AY-0004-2521
St. Francis Xavier Hospital, Assin Fosu	Central	5.698°N 1.281°W	CR-0007-7636
St. Anthony's Hospital, Dzodze	Volta	6.232°N 1.001°E	VY-0004-3113
Presbyterian Hospital, Donkorkrom	Eastern	7.048°N 0.078°W	EP-0002-8289
Holy Family Hospital, Nkawkaw	Eastern	6.547°N 0.774°W	EJ-0007-7332

[Table/Fig-2]: Study sites, geographical coordinates and Ghana postdigital address.

Study area and zone: The study areas [Table/Fig-1] lie within the malaria mesoendemic zone of the tropical rainforest [10].

Ethical approval and consent: Approval (CHRPE/KNUST/KATH/AP/433/17) was obtained from the Committee on Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology, Kumasi and Komfo Anokye Teaching Hospital, Kumasi, Ghana, before the commencement of the study. Written permission to carry out the study was also obtained from the Administrators/Medical Directors of the respective health facilities. A team was also setup at each site mandated to explain the nature of the study to key hospital figures and staff followed by oral presentations at the Outpatient Departments (OPD) where the study aims, objectives as well as risks and benefits were explained (in the Akan and Eʋe languages). Informed consent was obtained from the parent/guardian of each subject before commencement of the study. The data collected from subjects were coded and placed in a secured cabinet for confidentiality.

This study analysed human venous blood specimens, for malaria parasitaemia, common haemoglobinopathies and selected haematological parameters. Cluster probability sampling technique was used to enroll children who regularly reside in the districts of the study.

Sample size calculation: The total population of individuals in the study areas was 978,483 while the percentage of children aged 5 years and below was 13.8% [11]. From a total population of 978,483 for the seven districts, 13.8% translated to 135,030. This was the total site population of the group targeted. By calculation using Slovin's formula [12], the appropriate sample size was approximately 399.

According to Slovin's formula,

$n=N/(1+N\times e^2)$

{where, n is the sample size; N is the population size; e is the significance level (0.05 for 95% confidence interval)}

N=135030 (i.e., 13.8% of 978483)

$n=135030/1+135030\times0.05\times0.05$

n=398.8

n≈399

Inclusion and Exclusion criteria: Subjects aged 5 years and below, subjects tests positive for malaria and willing to take health facility approved malaria regimen were included in the study. Malnourished children, healthy children, older children and those whose guardians refused to consent were excluded from the study.

Malaria prescreening test was conducted on 849 participants. After preliminary tests, 342 (40.3%) subjects were enrolled as they tested positive for malaria. Malaria post-treatment testing was also conducted.

Sample collection: Preinclusion venous blood samples were collected by trained phlebotomists in June 2018. After treatment of subjects, venous blood samples were again collected in July 2018. Data on the preinclusion collection is shown in [Table/Fig-3].

Variables	n, %
Daily turnout	
Sunday	46 (5.4%)
Monday	154 (18.1%)
Tuesday	162 (19.1%)
Wednesday	121 (14.3%)
Thursday	174 (20.5%)
Friday	107 (12.6%)
Saturday	85 (10.0%)
Turnout by District	
Techiman	111 (13.1%)
Offinso	191 (22.5%)
Mankranso	86 (10.1%)
Assin Fosu	113 (13.3%)
Dzodze	93 (11.0%)
Donkorkrom	118 (13.9%)
Nkawkaw	137 (16.1%)
Malaria parasitaemia (n=342)	
Rapid Diagnostic Test (RDT)	336
Microscopy	342

[Table/Fig-3]: Preinclusion data on turnout and malaria screening (N=849).
Data are presented as absolute figures of potential subjects with corresponding percentages

Unique codes generated on the questionnaire for each participant were used to label the malaria RDT kits and microscope slides. Study staff was taken through Good Laboratory Practice (GLP) before commencement of study at the respective sites. Venous blood samples were collected from 849 children who showed interest in the study out of which 342 met all the inclusion criteria at the seven selected malaria-endemic districts and municipalities. Pretreatment venous blood samples were taken from the participants before

malaria chemotherapy. The facilities used the national treatment guideline for the treatment of uncomplicated malaria {Artesunate-Amodiaquine (AA)/Artemether-lumefantrine (AL)/Dhydroartemisinin-Piperaquine (DHAP) as the active ingredients}. Because cure was expected between 14 to 28 days following drug administration [13], second blood samples were collected from the 342 subjects on day 28. Standard Operating Procedures (SOPs) for venipuncture developed by BD® Diagnostics (REF: CLSI H3-A6) were adhered to by trained phlebotomists. Venous blood sample from each subject was collected into a 4 mL tube prelaced with Ethylenediaminetetracetic Acid (EDTA) and transported to St. Patrick's Hospital, Offinso for laboratory processing and analysis. Blood spots meant for molecular work (parasite identification) were analysed at the Parasitology Department of Noguchi Memorial Institute for Medical Research, Legon, Accra.

Test Procedure

Rapid diagnostic testing: CareStart™ HRP-2/pan pLDH test kit was used to qualitatively detect parasites of *Plasmodium* species from the blood of subjects. This screening test, based on immunochromatographic technique, has both Histidine Rich Protein-2 (HRP-2) and parasite Lactate Dehydrogenase (pLDH) as antigens embedded in the cassette [14]. The testing was carried out on the field and interpreted, as positive or negative, according to manufacturer's instructions. Known malaria positive and negative blood samples, from previously validated and authenticated malaria positive and negative cases, were run alongside each test kit batch to serve as controls.

Malaria microscopy: Thick and thin blood smears were prepared on the same glass slide for each subject using the method described by Norgan AP et al., [15]. Known malaria positive and negative blood samples were included with each stained batch to serve as controls. To further enhance testing quality, experienced microscopists confirmed all slides, both positive and negative.

Haematology analysis: The Complete Blood Count (CBC) analysis was performed with Mindray Auto Haematology Analyser, BC 5300 (Shenzhen Mindray Biomedical Electronics, PR China). The parameters of interest with regards to the study (haemoglobin levels and white blood cell counts) were culled out and filed for malaria parasite density estimation and statistical analysis.

Parasite density estimation: For each subject, white blood cell counts obtained from the complete blood cell count and the number of asexual forms of the parasite counted against 200 leucocytes were utilised. Parasite densities (parasite/µL of whole blood) were then calculated as follows: Parasite Density (PD)= (Number of parasites counted/WBC) × WBC count/ µL [16].

Sickle cell test: The sodium metabisulphite technique as described by Old J et al., was used to determine haemoglobin S in whole blood specimen [17]. Positive samples were identified under the microscope as sickle-shaped red blood cells. Negative samples did not have the sickle shape but had the biconcave red blood cell shape intact.

Hb electrophoresis: The guideline suggested by Kotila TR [18] was used with modification. Migrations exhibited by the control samples were used to classify the samples into appropriate phenotypes as suggested by Adu P et al., [19].

Deoxyribonucleic Acid (DNA) extraction: Filter papers (Whatman 903®, GE Healthcare Ltd., Cardiff, UK) were used to collect whole blood samples for DNA extraction. Venous blood samples of study subjects were blotted on the labelled filter papers, air-dried, placed singly in polyethylene envelopes and labelled again. The filter paper cards were stored with silica gels in a cool, dry, secured cabinet. DNA extraction was done on the dried filter paper blots collected from subjects. For each sample, 3 punches of 3 mm diameter each were used. The extractions were done using QIAmp DNA Blood Mini Kit (Qiagen, Germany) following manufacturer's instruction.

PCR analysis: Nested Polymerase Chain Reaction (PCR) was done to determine the *Plasmodium* species as described by Fuehrer HP and Noedl H [20]. The cycling conditions Nest 1 were 94°C for 3 minutes; 35 cycles of 94°C for 30 seconds, 55°C for 1:40 minutes and 72°C for 1 minute; 72°C for 5 minutes. The cycling conditions Nest 2 were 94°C for 3 minutes; 35 cycles of 94°C for 30 seconds, 58°C for 1 minute and 72°C for 1 minute; 72°C for 5minutes. For the nest II, four separate reactions were prepared using the respective species-specific primers. These took care of the four most common species of malaria parasites involved in clinical malaria, namely, *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax*.

Analysis of PCR product: The Nest II PCR products were run on a 2% agarose gel stained with Sybr safe dye, at 120 v for 45 minutes. The PCR products were then visualised under ultraviolet light transillumination. The results were photographed using a paranoid camera. Based on the bands shown, the species presence was determined.

Questionnaire Administration

Details of each participant such as age, gender and place of abode were collected on questionnaires which were administered after written voluntary consent for participation had been obtained. As a cross-section of the parents/caretakers was either illiterates or semi-illiterates, the local Akan/Eʋe translations of the contents of the questionnaires were utilised for communication. The questions were made clear to each interviewee while the interviewer recorded the responses on the questionnaire. Those who could read and write completed the questionnaires themselves. The questionnaires were administered to determine the possibility of exposure to the malaria vector as well as the possibility of administration of any chemotherapeutic agents. Socio-demographics as well as the opinions of the parents/guardians were also collected and documented. Multiple choice and open-ended responses were sought and where relevant, Likert scale format (strongly agree, agree, not sure, disagree, strongly disagree) was adopted in questionnaire construction to measure attitudes and opinions about malaria and sickle cell. Telephone contacts of respondents were written down on each questionnaire for follow-up purposes as it also enabled respondents to provide well thought-through responses which would not have been possible in completely anonymous questionnaire [21].

STATISTICAL ANALYSIS

Data generated were stored in MS Excel and analysed with Statistical Package for Social Sciences (SPSS) version 25.0. Categorical data were expressed in numbers and percentages while continuous variables were expressed in means±SD. The Chi-square test was used to compare categorical data between groups while the Independent t-test was used to compare means between groups. For all comparisons, p-value <0.05 was considered to be statistically significant.

RESULTS

A total of 342 children took part in the study. Subjects' age ranged from 0 to 60 months, with 193 males and 149 females.

[Table/Fig-4] shows that there was no statistically significant difference between the male and female subjects, making the two groups demographically similar. However, absolute figures consistently indicates that, in randomised sampling, more males than females had malaria across the study sites.

The data presented in [Table/Fig-5] shows that there were statistically significant demographic differences (p-value <0.001) between the male and female parents/guardians/caretakers in all parameters except occupation and marital status.

[Table/Fig-6] indicates that there was statistically significant difference (p<0.003) in the mean scores of knowledge on sickle cell deaths

Variables	Total (n,%) (N=342)	Male (n,%) (N=193)	Female (n,%) (N=149)	p-value (Independent t-test)
Age (months)				
0-12	34 (9.9)	21 (10.9)	13 (8.7)	0.401
13-24	130 (38.0)	66 (34.2)	64 (43.0)	
25-36	100 (29.2)	56 (29.0)	44 (29.5)	
37-48	57 (16.7)	36 (18.7)	21 (14.1)	
49-60	21 (6.1)	14 (7.2)	7 (4.7)	
Study site				
Mankranso	32 (9.4)	23 (11.9)	9 (6.0)	0.245
Techiman	40 (11.7)	27 (14.0)	13 (8.7)	
Offinso	83 (24.3)	46 (23.8)	37 (24.8)	
Assin Fosu	48 (14.0)	28 (14.5)	20 (13.4)	
Dzodze	43 (12.6)	22 (11.4)	21 (14.1)	
Donkorkrom	52 (15.2)	25 (13.0)	27 (18.1)	
Nkawkaw	44 (12.8)	22 (11.4)	22 (14.8)	
Use of mosquito net				
Yes	167 (48.8)	91 (47.1)	76 (51.0)	0.750
No	113 (33.0)	65 (33.7)	48 (32.2)	
Not sure	62 (18.1)	37 (19.2)	25 (16.8)	
Duration of sickness				
1-6 days	217 (63.5)	118 (61.1)	99 (66.4)	0.578
1-2 weeks	101 (29.5)	60 (31.1)	41 (27.5)	
More than 3 weeks	24 (7.0)	15 (7.8)	9 (6.0)	
Does the child get sick frequently?				
Yes	67 (19.6)	35 (18.1)	32 (21.5)	0.440
No	275 (80.4)	158 (81.9)	117 (78.5)	
Medication taken prior to visit?				
Yes	68 (19.9)	41 (21.2)	27 (18.1)	0.473
No	274 (80.1)	152 (78.8)	122 (81.9)	
Medication preference				
Orthodox	294 (86.0)	164 (85.0)	130 (87.2)	0.548
Herbal	48 (14.0)	29 (15.0)	19 (12.8)	
Splenomegaly*				
Yes	75 (21.9)	40 (20.7)	35 (23.5)	0.540
No	267 (78.1)	153 (79.3)	114 (76.5)	
Source of drinking water				
Borehole	82 (24.0)	42 (21.8)	40 (26.8)	0.660
Pipe	235 (68.7)	135 (69.9)	100 (67.1)	
Stream	3 (0.9)	2 (1.0)	1 (0.7)	
Well	22 (6.4)	14 (7.3)	8 (5.4)	

[Table/Fig-4]: General characteristics of study subjects.
*Nurses at the various study sites used palpation to determine splenomegaly

Variables	Total (N=342)	Male (N=20)	Female (N=322)	p-value (Independent t-test)
Mean age (years)	33.79±11.29	35.05±2.99	33.71±11.61	<0.001
Age group (years)				
<20	45 (13.2)	0	45 (14)	<0.001
20-29	74 (21.6)	0	74 (23)	
30-39	130 (38)	18 (90)	112 (34.8)	
40-49	55 (16.1)	2 (10)	53 (16.5)	
50-59	30 (8.8)	0	30 (9.3)	
≥60	8 (2.3)	0	8 (2.5)	
Occupation				
Unemployed	36 (10.5)	0	36 (11.2)	0.058
Formal	39 (11.4)	5 (25)	34 (10.6)	
Informal	267 (78.1)	15 (75)	252 (78.2)	
Religion				
Christianity	242 (70.8)	16 (80)	226 (70.2)	<0.001
Islam	89 (26)	3 (15)	86 (26.7)	
African Traditional Religion (ATR)	10 (2.9)	0	10 (3.1)	
Others	1 (0.3)	1 (5.0)	0	
Education				
None	96 (28.1)	3 (15.0)	93 (28.9)	0.001
Primary	66 (19.3)	4 (20.0)	62 (19.3)	
Junior High School (JHS)/Middle School Leaving Certificate (MSLC)	94 (27.5)	1 (5.0)	93 (28.9)	
Senior High School (SHS)	34 (9.9)	3 (15)	31 (9.6)	
Tertiary	52 (15.2)	9 (45)	43 (13.4)	
Marital status				
Single	49 (14.3)	1 (5.0)	48 (14.9)	0.548
Married	274 (80.1)	19 (95.0)	255 (79.2)	
Separated	9 (2.6)	0	9 (2.8)	
Divorced	1 (0.3)	0	1 (0.3)	
Widowed	9 (2.6)	0	9 (2.8)	

[Table/Fig-5]: Characteristics of parents/guardians/caretakers.
Data are presented as absolute figures of parents/guardians/caretakers of subjects with corresponding percentages. p-value <0.05 considered significant

Variables	Total (N=342)	Male (N=20)	Female (N=322)	p-value (Independent t-test)
Classical symptoms of malaria				
Bitterness in mouth	22 (6.4)	3 (15)	19 (5.9)	0.998
Dizziness	5 (1.5)	0	5 (1.6)	
Fever	220 (64.3)	12 (60)	208 (64.6)	
Headache	59 (17.3)	2 (10)	57 (17.7)	
Insomnia	1 (0.3)	0	1 (0.3)	
Joint Pain	2 (0.6)	0	2 (0.6)	
Shivering	24 (7)	2 (10)	22 (6.8)	
Tiredness	5 (1.5)	1 (5)	4 (1.2)	
Pallor	4 (1.2)	0	4 (1.2)	
Malaria caused by blood parasite				
Strongly agree	204 (59.6)	17 (85)	187 (58.1)	0.150
Agree	44 (12.9)	1 (5)	43 (13.4)	
Not sure	12 (3.5)	1 (5)	11 (3.4)	
Disagree	26 (7.6)	0	26 (8.1)	
Strongly disagree	56 (16.4)	1 (5)	55 (17.1)	
Sickle Cell Disease (SCD) is a curse				
Strongly agree	146 (42.7)	7 (35)	139 (43.2)	0.053
Agree	12 (3.5)	0	12 (3.7)	
Not sure	6 (1.8)	2 (10)	4 (1.2)	
Disagree	28 (8.2)	2 (10)	26 (8.1)	
Strongly disagree	150 (43.9)	9 (45)	141 (43.8)	
Sickle Cell Disease (SCD) is inherited				
Strongly agree	182 (53.2)	11 (55)	171 (53.1)	0.998
Agree	14 (4.1)	1 (5)	13 (4)	
Not sure	19 (5.6)	1 (5)	18 (5.6)	
Disagree	15 (4.4)	1 (5)	14 (4.3)	
Strongly disagree	112 (32.7)	6 (30)	106 (32.9)	
Sickle Cell Disease (SCD) children die before their teen ages				
Strongly agree	225 (65.8)	8 (40)	217 (67.4)	

Agree	30 (8.8)	1 (5)	29 (9)	0.003
Not sure	27 (7.9)	1 (5)	26 (8.1)	
Disagree	21 (6.1)	3 (15)	18 (5.6)	
Strongly disagree	39 (11.4)	7 (35)	32 (9.9)	

[Table/Fig-6]: Knowledge level of parents/guardians on malaria and Sickle Cell Disease (SCD).
Data are presented as absolute figures of parents/guardians/caretakers of subjects with corresponding percentages. p-value <0.05 considered significant

between the groups. On the contrary, both groups had similar knowledge levels in classical symptoms of malaria, cause of malaria, sickle cell inheritance and whether on not SCD was a curse.

[Table/Fig-7] displays the summary of diagnostic tests conducted on the subjects. A total of 342 subjects were parasitaemic by microscopy, whilst only 336 tested positive by Rapid Diagnostic Test (RDT). Discordant microscopy-RDT results/speciation were subsequently confirmed and resolved by PCR.

Method of screening/Diagnosis	Number
Microscopy	342
Rapid diagnostic test	336
Polymerase chain reaction	342

[Table/Fig-7]: Malaria parasitaemia among sick children in the study sites.

Data presented in [Table/Fig-8] indicates that sickle cell had a prevalence of 28.9% across the study groups. Among the subjects with haemoglobinopathy, the sickle cell trait (HbAS) had the highest prevalence (25.1%) among the subjects and across the study areas.

Tests	n, %
Sickle cell status	
Negative	243 (71.1%)
Positive	99 (28.9%)
Prevalence of haemoglobin phenotypes	
HbAA	219 (64.1%)
HbAF	7 (2%)
HbAC	17 (5%)
HbAS	86 (25.1%)
HbSC	4 (1.2%)
HbSS	9 (2.6%)

[Table/Fig-8]: Prevalence of sickle cell and haemoglobin phenotype among subjects.
Hb: Haemoglobin; HbAA: Haemoglobin A (homozygous) band; HbAC: Haemoglobin A and C bands; HbAS: Haemoglobin A and S bands; HbSS: Haemoglobin S (homozygous) band; HbSC: Haemoglobin S and C bands

[Table/Fig-9] shows that sickle cell positivity was most prevalent (26.3%) in the Offinso study area and least prevalence in the Dzodze study area (8.1%).

	Sickling	
Site	Positive (n,%)	Negative (n,%)
Mankranso	9 (9.1)	23 (9.5)
Techiman	13 (13.1)	27 (11.1)
Offinso	26 (26.3)	57 (23.5)
Assin Fosu	12 (12.1)	36 (14.8)
Dzodze	8 (8.1)	35 (14.4)
Donkorkrom	16 (16.2)	36 (14.8)
Nkawkaw	15 (15.2)	29 (11.9)

[Table/Fig-9]: Prevalence of sickle cell in relation to study site.

The data in [Table/Fig-10] depicts that there was a statistically significant difference (p-value=0.019) between the groups with *Plasmodium falciparum* monoinfection and those with mixed infection with regard to sickling status. Phenotypically, however, the groups were similar.

Test	*Plasmodium falciparum* (n,%)	*Plasmodium falciparum*-Plasmodium ovale mixed infection (n,%)	p-value (Independent t-test)
Sickling			
Positive	98 (30.3)	1 (5.3)	0.019
Negative	225 (69.7)	18 (94.7)	
Haemoglobin electrophoresis			
HbAA	203 (62.8)	16 (84.2)	0.119
HbAC	15 (4.6)	2 (10.5)	
HbAF	7 (2.2)	0	
HbAS	86 (26.6)	0	
HbSC	4 (1.2)	0	
HbSS	8 (2.5)	1 (5.3)	

[Table/Fig-10]: Sickle cell status and haemoglobin variants in relation to species of Plasmodium.
p-value <0.05 considered significant

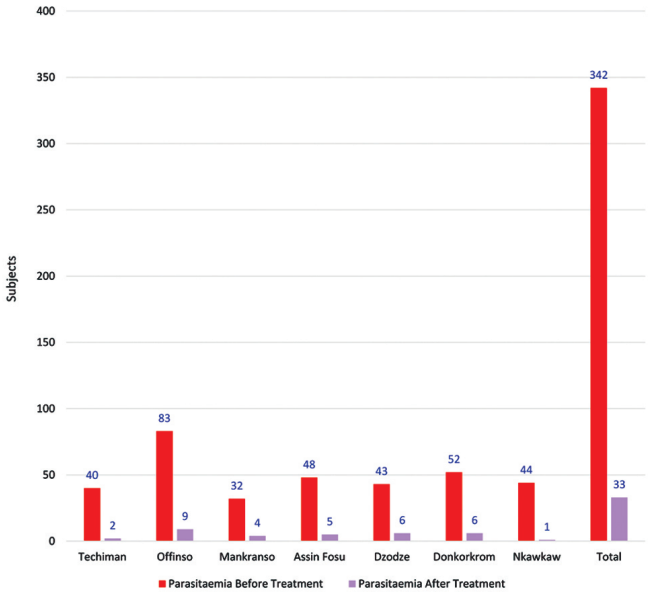
The data presented in [Table/Fig-11] shows that across the study sites, all subjects with the sickle cell trait (HbAS) were completely cured of malaria. On the other end of the spectrum, all subject with HbSS (9/9) and HbSS (4/4) were parasitaemic before and after treatment. Subjects with normal haemoglobin (HbAA) and those with HbAC were varied in treatment.

Treatment	n	Normal	Common haemoglobinopathies				Haemoglobinopathy %
		HbAA	HbAC	HbAS	HbSS	HbSC	
Before treatment							
Techiman	40	25	2	11	1	1	4.3%
Offinso	83	54	3	24	2	0	8.5%
Mankranso	32	21	2	8	1	0	3.2%
Assin Fosu	48	33	3	10	1	1	4.4%
Dzodze	43	32	3	8	0	0	3.2%
Donkorkrom	52	34	2	11	4	1	5.3%
Nkawkaw	44	27	2	14	0	1	5.0%
Total	342	226	17	86	9	4	33.9%
After treatment							
Techiman	2	0	0	0	1	1	0.6%
Offinso	9	5	2	0	2	0	1.2%
Mankranso	4	1	2	0	1	0	0.8%
Assin Fosu	5	0	3	0	1	1	1.4%
Dzodze	6	4	2	0	0	0	0.6%
Donkorkrom	6	0	1	0	4	1	1.8%
Nkawkaw	1	0	0	0	0	1	0.3%
Total	33	10	10	0	9	4	6.7%

[Table/Fig-11]: Haemoglobin phenotypes of parasitised subjects before and after treatment.
Data are presented as absolute figures of normal and variant haemoglobins of subjects with corresponding percentage on study site basis. n is the number of subjects;
Hb: Haemoglobin; HbAA: Haemoglobin A (homozygous) band; HbAC: Haemoglobin A and C bands; HbAS: Haemoglobin A and S bands; HbSS: Haemoglobin S (homozygous) band; HbSC: Haemoglobin S and C bands

[Table/Fig-12] indicates that there were varying degrees of parasitaemia across the study districts with Offinso having the least absolute subject figure reduction (9/83). Nkawkaw had the most absolute subject figure reduction (1/44).

[Table/Fig-13] depicts that there was significant positive effect of chemotherapy on parasite clearance. The mean post-treatment leucocyte level (7.12±2.37) and parasite density (495±1744) were significantly lower than pretreatment levels in the group (p-value <0.001), whereas the mean haemoglobin level significantly (p-value <0.001) increased from 9.32±2.06 to 11.72±1.54.



[Table/Fig-12]: Bar graph showing absolute parasitaemia of subject's pretreatment and post-treatment against study sites.

Parameter	Pretreatment (n, %)	Post-treatment (n, %)	p-value (paired t-test)
Hb level (g/dL)			
<12	306 (89.5)	163 (47.7)	<0.001
≥12	36 (10.5)	179 (52.3)	
WBC group (x 10^9/μL)			
<4	0	9 (2.63)	<0.001
4-10	155 (45.3)	285 (83.33)	
>10	187 (54.7)	48 (14.04)	
Quantification of malaria parasites (Plus system)			
No mps	0	309 (90.40)	<0.001
1+	128 (37.45)	16 (4.70)	
2+	128 (37.45)	17 (4.90)	
3+	74 (21.6)	0	
4+	12 (3.5)	0	
Parasites/200 WBC (Mean±SD)	162±58	8±29	<0.001
Parasite Density (Mean±SD)	9529±5806	495±1744	<0.001
Haemoglobin (Mean±SD)	9.32±2.06	11.72±1.54	<0.001
White Blood Cell (Mean±SD)	10.81±4.00	7.12±2.37	<0.001

[Table/Fig-13]: Haematological parameters and parasite quantification before and after treatment.
Data are presented as mean±SEM and as proportion with corresponding percentages in parenthesis. Anaemia (Hb<12.0 g/dL) according to WHO. Leucopenia (WBC<4.0×10^9/L), Leucocytosis (WBC>10.0×10^9/L). p-value <0.05 considered significant

DISCUSSION

Malaria has devastating effects on health and development, with everyone in Ghana being at risk of the infection [22]. The study areas fall within the endemic malarious zone [Table/Fig-1]. Worst still, it had been opined that children with the co-morbidity of malaria and SCD in a malarious zone have poorer prognosis and clinical outcomes [23]. The aims of the study were to assess the views of parents/guardians/caretakers on malaria and haemoglobinopathies and to determine the extent to which common haemoglobin variants influence malaria parasitaemia among children in the acute stage of the infection. With a parasitaemia prevalence of 40.3%, the turnout of 849 febrile children and their parents/guardians/caretakers was encouraging enough to achieve the overall aim of the study [Table/Fig-3]. The responses were satisfactory for malaria knowledge as they cumulatively averaged 68.4% [Table/Fig-6]. There were misconceptions, though, with regards to haemoglobinopathy-related questions. Subjects with SCD fared poorly in relation to malaria prognosis.

In the present study, among the infected children, there was male gender dominance [Table/Fig-4]. The percentage of male subjects was 56.4 (n=193) relative to the female 43.6% (n=149). Other similar studies conducted among young children have, likewise, found male dominance among the study subjects [24-26]. This could be due to the observation that the male gender is a significant predictor of malarial infection [27,28].

It was observed that most of the respondents (parents/guardians/caretakers) were female [Table/Fig-6]. Few male parents/guardians/caretakers accompanied their febrile infants to seek healthcare. Malik EM et al., and Dumbaugh M et al., have reported similar observations. This may be due to gender-linked, culturally-defined roles common in many settings [29,30]. A greater proportion (64.3%) of the guardians responded that fever was a classical symptom of malaria [Table/Fig-6]. Headache was the second highest symptom known (17.3%) with joint pains being the least (0.6%). Infact fever is interchangeable with malaria among rural folks in Ghana [31]. The present study corroborated findings in other studies on the frequency of fever and other subjective clinical symptoms in malariology [32], although fever was not a specific marker for malaria [33]. Most of the males (85%) and females (58.1%) guardians strongly agreed that malaria was caused by a blood parasite [Table/Fig-6]. However, 7.6% and 16.4% of the guardians respectively disagreed and strongly disagreed, that malaria was caused by a germ in the blood. However, this was lower than the 92.2% of respondents who knew that malaria was caused by mosquito in a study conducted by Owusu EDA et al., at the Kwahu Government Hospital, Eastern Ghana [34].

There are a lot of genetic polymorphisms that influence the structure and, by extension, the production of β- and α-chains of haemoglobin which are linked with protection from *Plasmodium falciparum* infection. The protection offered depends on the haemoglobinopathy in question, although it is greatest with respect to severe malaria and to some extent uncomplicated malaria [35]. The prevalence of the sickle cell status in the present study was 28.9% [Table/Fig-8]. This finding was in agreement with Aboagye S et al., who documented a prevalence range in between 10% and 40% for sub-Saharan Africa [36]. Majority of the subjects in the present study had HbAA, 5% had HbAC, 2% had HbAF, 25.1% had HbAS, 1.2% had HbSC and 2.6% had HbSS phenotypes [Table/Fig-8]. Authors reported phenotypes and not genotypes because of the shortcomings of alkaline cellulose acetate paper electrophoresis [19]. The percentage haemoglobin phenotype findings from the present study agreed with another similar study [37]. Altogether, subjects with SCD were 13 out of 342 (3.8%). Other sickle cell-positive subjects without SCD were 86 out of 342 (25.1%) [Table/Fig-11]. Of the infected children, 243 out of 342 (71.1%) did not have the S haemoglobin. The low percentage of infection in subjects with SCD relative to those without SCD (25.1%) was tantamount to the observation by Okuonghae HO et al., which found that *Plasmodium falciparum* parasites were detected in 9% of subjects with SCD compared with those without SCD (29%) [38]. Likewise, Aluoch JR in Kenya and Awotua-Efebo O et al., in Nigeria observed a lower prevalence among infants with SCD relative to those without SCD [39,40]. The conclusion is thus plausible that, from a mechanistic level, children with SCD may have a significant level of protection from malaria. It has also been noted that children who have the sickle cell trait are protected against malaria, probably because *P. falciparum* parasites fail to thrive in erythrocytes with HbS [41]. It could be that *P. falciparum* parasite-infected red blood cells get removed, by default, during an immune response [42]. It is therefore logical that when intracellular concentrations of HbS are raised, as pertains in the homozygous form, greater protection is envisaged. Such deduction is in line with studies that investigated the mechanism surrounding HbS in malaria protection [43].

The high concentration of foetal haemoglobin in the red cells of SCD infants is also opined to further confer malaria protection [43]. This is further buttressed by the finding of about 30% of the subjects who

had *P. falciparum* infection alone being sickling positive, and 5.3% of those with *P. falciparum* and *P. ovale* mixed-infection being sickling positive [Table/Fig-10]. *P. falciparum* and *P. ovale* mixed-infection were confirmed by conventional PCR [Table/Fig-7]. Approximately 26.6% with *P. falciparum* infection were AS, with 3.7% of subjects having SCD (1.2% SC and 2.5% SS) after HB electrophoresis. However, none of the subjects with *P. falciparum* and *P. ovale* mixed-infection in this study were AS or SC, with 5.3% being SS. This could suggest that abnormal haemoglobin variant heterozygosity may not favour mixed malaria species infection. Species-wise, it is not uncommon to encounter *P. falciparum* and *P. ovale* as the only species among the Ghanaian populace in the forest zone [44].

The present study also found that after treatment, 10/226 (4.4%) of the HbAA subjects and 10/17 (58.8%) HbAC subjects were still parasitaemic [Table/Fig-11] agreeing with a study of 1070 children from Afigya Sekyere District of Ashanti Region, Ghana, which found that subjects with HbAA and HbAC do not differ in their protection against malaria [45], although other studies observed that the AC genotype fared better than do the AA genotypes with regards to malaria infectivity [8]. All the HbSS (9/9) and all the HbSC (4/4) subjects in the study remained positive after treatment, suggesting a double jeopardy for HbSC and HbSS haemoglobin variants [Table/Fig-11], even though parasite densities were very significantly reduced (495±1744, p-value <0.001). They may have to be re-treated. Cure for malaria was expected between day 14 and day 28 following chemotherapy with nationally approved regimens [13].

However, the continued microscopy-confirmed parasitaemia 28 days post-treatment [Table/Fig-12] may be a pointer to dosage non adherence, incomplete treatment, treatment failure, re-infection, relapse or recrudescence. Authors also suggested that, per our study, it could be due to HbSS/HbSC haemoglobinopathies. To the best of our knowledge, there is no similar local longitudinal study to affirm or discount the HbSS/HbSC findings per post-treatment. Of the 86 HbAS subjects, none was positive after treatment [Table/Fig-11], agreeing with another similar study which showed that individuals with this haemoglobin variant fared better than the others [46]. Moreover, the finding of a significantly (p-value <0.001) reduced parasite density after treatment with 309 (90.4%) of subjects having no malaria parasites in peripheral blood [Table/Fig-13] indicated that the treatment given was effective in clearing the parasites.

The higher prevalence of HbSS than HbSC [Table/Fig-8] was comparable to the findings by Asare E et al., who reported a 55.7% HbSS relative to 39.6% HbSC among SCD patients presenting at Korle Bu Teaching Hospital, Ghana [47]. Ohene-Frempong K et al., also found that 55% of children born with SCD in Ghana were homozygous (HbSS) [48]. Among the subjects positive for sickling, most (26.3%) were from Offinso followed by Donkorkrom, Nkawkaw, Techiman, Assin Fosu, Mankranso and Dzodze in descending order [Table/Fig-9]. The reasons for the high sickling prevalence for Offinso may be varied; it could be due to the district's large contribution of study participants. It could also be due to other reasons yet to be determined. A call, therefore, for a paradigm shift and a policy geared towards universal newborn sickle cell screening in all healthcare points would be effective in curtailing the drastic effects of this and other haemoglobinopathies [49].

Approximately, 43% and 4% of the guardians, respectively, strongly agreed and agreed that SCD was a curse [Table/Fig-6]. Contrastingly, only 4.3% of respondents responded that SCD was a curse in the study by Owusu EDA et al., [34]. Majority (57.3%) however knew that SCD was inherited while 37.1% disagreed that it was inherited. Also, 65.8% strongly agreed that children with SCD died before they got to their teen ages, an observation that agreed with another similar study [50]. Evidence abounds now, however, that there is reduced mortality, improved survival rate and longevity for SCD patients [51].

From the present study, there was a demonstration of anaemia (Hb <12 g/dL) in 89.5% of the infants before treatment [Table/Fig-13]. This was consistent with the study by Sakzabre D et al., and Bawah AT et al., who had similar findings which they attributed to malaria parasitaemia resulting from factors such as destruction of infected erythrocytes and the increased clearance of both parasitised and non parasitised erythrocytes, an observation further supported by the finding of a significantly elevated mean haemoglobin level (p-value <0.001, 11.72±1.54) after treatment of the infection [51,52].

Limitation(s)

The study has limitation in the analytical method used for identification of common haemoglobinopathies. In the absence of High-Performance Liquid Chromatography (HPLC) and genetic testing techniques, some variant haemoglobins and thalassaemias could not be identified. However, alkaline cellulose acetate electrophoresis was able to identify most of the main haemoglobinopathies common to individuals in the West African sub-region, strengthening the findings and deductions here in.

CONCLUSION(S)

It was observed that most of the parasitised subjects had normal haemoglobin, and that about a third were sickling positive, suggesting some malaria protection for subjects with sickle cell haemoglobinopathy. None of the subjects with *P. falciparum* and *P. ovale* mixed-infection were HbAS and HbSC, with the assumption that abnormal structural globin variant heterozygosity may not favour mixed malaria species infection. After treatment, it was found that subjects with normal haemoglobin phenotypes as well as those with the sickle cell trait were cured of malaria. The study determined that subjects with HbSC and HbSS phenotypes remained parasitaemic after 4 weeks of treatment, suggesting a double jeopardy for individuals with SCD. It was also found that misconceptions about SCD still existed among the rural folk, necessitating regular health education.

Although the knowledge of parents/guardians/caretakers on malaria was satisfactory, there is the need for further targeted health education in order to ensure timely reporting of cases to health facilities and to erase deep-seated perceptions on malaria and haemoglobinopathies. Authors also recommend that it is time routine newborn screening was made mandatory in all healthcare facilities in Ghana.

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