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Prevalence of Malaria Infection among Children with Different Haemoglobin Types, Attending Specialist Hospital Sokoto State, Nigeria

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Abstract

This study determined the prevalence of malaria infections among children with different haemoglobin types, attending Specialist Hospital, Sokoto Nigeria. Venous blood was used to make thick and thin blood smear and stained witt Giemsa stain. Microscopic method was used to identify malaria parasite. Parasite density was determined using Total Leukocye Method. Haemoglobin genotype was determined using haemoglobin electrophoresis method. Of the total sample examined, prevalence of malaria infection was 45(56.3%). Results obtained revealed patients with HbAA having high parasite frequency, with 30(66.7%), HbSS having low parasite frequency with 8(17.8%) and HbAS having the least parasitaemia with 7(15.6%). However, due to low turnout of patients with HbAC and HbSC, results show no parasitaemia with 0(0.0%). Therefore, there is statistical significance between malaria infection and haemoglobin types ($p<0.05$). With relation to parasite density, this study shows that HbAA has the highest parasite density with 22(48.9%) for <10,000pf/µl and 8(17.8%) for >10,000pf/µl, while HbAS has the least 4(8.8%) for <10,000pf/µl and 3(6.6%) for >10,000pf/µl. There is a strong association between parasite density and haemoglobin types (p-value < 0.05). There is no statistical association between socio-demographic factors (Gender, Age and Place of residence) with malaria parasitaemia ($p>0.05$).Malaria infection among children remains a burden in Sokoto. Efforts should be made to prevent the spread of malaria by providing good drainage systems and delivering free insecticide treated mosquito nets.

Keywords: *Malaria, Infection, Children, Haemoglobin, Sokoto*

Introduction

Malaria is the most important tropical parasitic disease affecting about 247 million people each year among the 3.3 billion people at risk, resulting in nearly a million deaths, mostly children under

the age of five years (1). Nearly 90% of these deaths occur in Africa, south of the Sahara thereby making it the leading cause of under-five mortality, killing an African child every 30 seconds (2). Pregnant women and their unborn children are also particularly vulnerable to malaria, which is a major cause of prenatal mortality, low birth weight and maternal anaemia. It accounts for 40% of public health expenditure, 30 - 50% of in-patient admissions and. up to 50% of out-patient visits in areas with high malaria transmission (3). In Nigeria, malaria is endemic and stable, being a major cause of morbidity and mortality, resulting in 25% infant and 30% childhood mortality (4). It was ranked as the highest cause of death in 1978 and 1982 (5). Tragically, the health status of children under the age of five and women has remained a major barrier to Nigeria's development. It is estimated that one out of every five Nigerian children die before his or her fifth birthday (6). Beyond the impact on children and pregnant women, it affects the general population (7, 4).

The disease is the commonest cause of outpatient attendance across all age groups with about 66% of clinic attendance due to malaria (8) and thus constituting a great burden on the already depressed economy. The comparison of malaria indicators among population with different genetic backgrounds that are uniformly exposed to the same parasite strains is one approach to the study of human heterogeneities in response to the infection. Many researches have shown that genetic factors play a key role in determining resistance or susceptibility to infectious disease. Susceptibility of the human host to malaria infection has been reported to be influenced by genetic factors, which could be confounders if not taken into account in the assessment of the efficacy of interventions against malaria (9). Haemoglobin types are mutant forms of haemoglobin in a population (usually of humans), caused by variations in genetics. Some well-known haemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered haemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants (10). Haemoglobin variants occur when there are genetic changes in the specific genes, or globins, that cause changes or alterations in the amino acid. They could affect the structure, behaviour, the production rate, and the stability of that specific gene. Usually there are four genes that code for alpha globin and two genes that code for beta globin (11). Erythrocytes containing HbS and HbC may impede parasite growth and replication relative to normal red cells when subjected to low oxygen tensions. Protein targets of specific antibodies may be more rapidly exposed in HbS-containing red blood cells resulting in an enhanced immune response to infection (12). It is also possible that unkown innate protective process may up regulate the malaria-specific immune response or enhance non specific immunity to malaria.

Plasmodium *falciparum* infections can have diverse clinical presentations, even within the same person over time, (13) and this might impact the potential for onward transmission of the parasite. For example, individuals with asymptomatic infection can carry chronic parasitemia without seeking treatment and might remain infectious to mosquitoes for long periods of time. Consequently, identifying and addressing factors involved in frequent or long-lasting parasite carriage can be important to malaria elimination and control strategies that aim to reduce human-to-mosquitoe transmission. Two haemoglobinopathies, hemoglobin (Hb) S and HbC traits (AS and AC genotypes, respectively), that are frequent in sub-Saharan Africa can influence parasite carriage and transmission (14). These two haemoglobin variants have been associated with protection against severe malaria syndromes in numerous epidemiological studies (15), and mechanistic hypotheses proposed to explain this protection include impaired blood stage parasite

development (16), reduced cytoadherence of infected red blood cells (17), which is linked to the redox imbalance of haemoglobinopathies and its effects on the export of parasite-encoded proteins (18), tolerance to malaria infection related to heme catabolism by heme oxygenase-1, or accelerated acquisition of immunity (19).

Malaria is the most important tropical parasitic disease affecting about 247 million people each year among the 3.3 billion people at risk, resulting in nearly a million deaths, mostly children under the age of five years In Nigeria, malaria is endemic and stable, being a major cause of morbidity and mortality, resulting in 25% infant and 30% childhood mortality (4). It was ranked as the highest cause of death in 1978 and 1982 (20). Many researches have shown that genetic factors play a key role in determining resistance or susceptibility to infectious disease. Susceptibility of the human host to malaria infection h2as been reported to be influenced by genetic factors, which could be confounders if not taken into account in the assessment of the efficacy of interventions against malaria (21). A lot of researches on prevalence of malaria infection among children in sokoto and beyond, but to the best of my knowledge non was able to link it with Haemoglobin types of the subjects. Therefore, this work is geared to investigate the relationship between the prevalence of malaria parasite, parasite density among children with different haemoglobin genotypes in sokoto. Data obtained from this research will enhance proper treatment of children with malaria parasite in sokoto and beyond. It will also help to plan for more preventive programmes where the particular haemoglobin variants on the higher prevalence is established. This study aimed at assessing the prevalence of malaria infections among children with different haemoglobin types, attending Specialist Hospital,Sokoto state.

Materials and Methods

Study Area

The study was carried out in Specialist Hospital Sokoto, Nigeria. According to UNFPA (22), Sokoto state lies in the (longitude 11^0-13^0-50^0 East and latitude 4-6^0 North), North- Western Nigeria. Sokoto state shares boundaries with the Republic of Niger to the North, Kebbi state to the West and South, and Zamfara state to the South and East. It occupies an area of short-grass savannah vegetation in the south and thorn scrub in the north. A generally arid region that gradually merges into the desert across the border in Niger republic, it has limited rainfall from mid-May to mid-September and is subjected to the Sahara's harmattan (dry, dust-laden wind) from November to March (23). The state covers a total land area of about 25,973 square kilometers with a population of 3,702,676 (24).

Study Design

This is a cross-sectional study which involves children attending Specialist Hospital, Sokoto from the month of July to November, 2019. The blood samples for this research was collected in Peadiatric ward, after obtaining informed consent from the parents or guardians of the children, and ethical approval from Specialist Hospital Sokoto, Nigeria. The samples were used for thick

and thin blood film preparation for malaria microscopic examination. Haemoglobin genotype was also determined using haemoglobin electrophoretic pattern. A semi constructed interviewer questionnaire was administered to all consenting participants to obtain information on subjects'bio data, parents socio-demographic data and medical history. The samples were analysed in the departments of Medical Microbiology, Haematology and blood transfusion sciences, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

Ethical Approval

Ethical approval for this study was obtained from Specialist Hospital, Sokoto State (SHS/SUB/133/ VOL.1).

Study Population

A total of 80 children aged 1 year to 12 years were used in this study. Children, who were refered to the Laboratory for malaria confirmatory diagnosis in Specialist Hospital Sokoto, were recruited as the subjects.

Sample Size Estimation

The sample size for the study was determined with G-power. 3.7 software.

Informed Consent

Written informed consent was sought from the parents or guardians of each elligible participant in the study

Inclusion Criteria

Inclusion criteria for the study were as follows:

i) Willingness to give written informed consent obtained from the parents or legal guardian of the children between the age of (1-12years) of apparently healthy males and females attending Specialist Hospital Sokoto.
ii) Children not on anti malaria medication.
iii) Children visiting out patient department of Specialist Hospital, Sokoto.

Exclusion Criteria

Exclusion criteria includes

i) Parents or guardians who refuse to give their consent will be excluded from the study.
ii) Children undergoing medications.

Sample Collection

Two milliliters (2 mls) of venous blood was collected intravenously by clean venipuncture, through a media cubital fossa using a plastic 2ml syringe, from each participant and transfered into K_3EDTA bottle and then mixed gently inorder to mix blood with the anticoagulant. Each blood sample was labelled and tallied with the subjects number on the questionaires.
Thick and thin blood films were made within 30 minutes,and thin films were mixed in methanol. The blood films were stained with 10% Giemsa working solution for identification of malaria parasite and parasite density. The subjects haemoglobin genotype were also determined using haemoglobin electrophoretic pattern.

Materials and Methods

Determination of Malaria Parasite.

A) Method for making thin blood films (25).

Procedure

1. A drop of EDTA anticoagulated whole blood was placed at the end of a clean, grease free glass slide with the aid of a pasture pipette.
2. A clean smooth edged spreader was placed on glass slide ,drawn back to touch the drop of blood and the blood was allowed to extend along the edge of the spreader.
3. A spread of blood was made and about 40-50mm of thin blood film in length (two third of the slide) by inclining the spreader at an angle of 45 degree.
4. The film was air dried by waving the slide back and forth.

B) Method for making thick blood film (25)

Thick blood film was prepared by placing a drop of blood with the aid of pasteur pipette on a grease free slide.A smear was made and allowed to air dry away from contaminant.

C) **Method for staining thin and thick blood films (25)**

Procedures

1. Thick and thin blood films were air dried.
2. Thin blood films were fixed with methanol.
3. Both thick and thin blood films were placed on a staining rack and covered with 10% Giemsa working solution for 10 minutes.
4. The stained blood films were rinsed with distilled water.
5. The films will be drained and allowed to air dry.

D. Identification of malaria parasite (25).

Thick and thin blood films were prepared for microscopic identification and quantification of the plasmodium species and determination of parasite density, according to the technique outlined (25).

Procedures

1. A drop of immersion oil was placed on the tail end of the stained thin films and on the thick blood films.
2. The films were placed on the microscope stage.
3. The blood films were examined using the oil immersion (100×) objective with the condenser iris closed and the number of malaria parasites of each species and stage were recorded.
4. The number of parasites per microlitre of blood were calculated by assuming that there are 20 white blood cells per high-power field and a fixed cell count of 8,000 perμl.
5. Each film was read twice by two experienced scientists.

Haemoglobin Electrophoresis (25).

Principle

Very small samples of heamolysates prepared from whole blood were applied to the cellulose acetate paper.The haemoglobins in the sample were seperated by electrophoresis using alkaline buffer (PH 8.2-8.6). The patterns were observed for migration.

Procedure

Step 1: preparation of haemolysate

1. Anticoagulated blood was centrifuged at 2500 rpm for five minutes
2. The plasma was removed and the packed cells was washed with large volume of normal saline for five minutes.
3. The red cells was lysed by adding equal volume of distilled water, one quarter volume of toluene and one drop of 3% potassium cyanide.
4. The mixture was mixed by inversion and was centrifuged to remove the cell debris.

Step 2: electrophoresis

1. TRIS buffer was poured into the electrophoretic chamber
2. The cellulose acetate paper was pre soaked for 20-30 minutes in the buffer.
3. Excess buffer was removed by placing the cellulose acetate paper between filter paper (bloating)
4. An applicator was used to apply 0.5 -0.6ml of the specimen approximately 3cm from the cathode.
5. Abnormal controls was also placed on each strip.
6. The cellulose acetate paper was placed in the electrophoretic chamber
7. The electrophoresis was run at 450 volts for 20 minutes.
8. The cellulose acetate paper was removed .
9. The strip was washed in three changes of 5%acetic acid.
10. The strip was fixed in absolute methyl alcohol for five minutes.
11. The strip was cleared in 20% acetic acid in absolute methyl alcohol for 10 minutes.
12. The cellulose acetate paper was checked for clear migration.

Statistical Analysis

Data analysis was carried out with statistical package of social sciences (SPSS) version 23 (SPSS, Inc., Chicago, IL, USA). The prevalence of malaria infection was presented as frequencies and percentages and the parasite density was also presented as frequency and percentages. Chi-square was used to determine association between variables. P- values of < 0.05 were considered statistically significant.

Results

The results of 80 children recruited from the peadiatrics ward Sokoto State Specialist Hospital, Sokoto were analyzed. A semi constructed questionnaire was used to obtain information of the subjects and thier blood samples were analyzed for Malaria parasite and heamoglobin genotypes in the Haematology and Microbiology Laboratory, Usman Danfodiyo University Teaching Hospital,Sokoto. **Table 1** Shows the prevalence of malaria parasite among children attending Specialist Hospital,Sokoto. About 45% of the children were positive for the malaria parasite test while 35% of the children were negative for the malaria parasite.

Table1: Prevalence of malaria parasite among children

Malaria parasite	Frequency(N)	Percentage (%)
Positive	45	56.3
Negative	35	43.8
Total	80	100.0

Table 1 above shows that the prevalence of malaria parasite is (56.3%).

Table 2 Shows the distribution and relationship of different haemoglobin types among children with malaria parasite. The positive count were 30(66.6%), 7(15.6%), 8(17.8%), 0(0%), 0(0%) for AA, AS, SS, AC,SC respectively.

Table 2: Relationship of different heamoglobin types among children with malaria parasite.

Genotype	Frequency (%)	p-value
AA	30 (66.7)	
AS	7 (15.6)	
SS	8 (17.8)	

AC	0 (0.0)	0.001
SC	0 (0.0)	
Total	45 (100)	

Table 2 above shows `the relationship of haemoglobin types with malaria infection. The p values of the haemoglobin types are all statistically significance (p =0.001).

Table 3 Shows the parasite density frequencies among children with malria parasite. The frequency of parasite density <10,000pf/µl is 32 (71.1%) and 13(28.9%) for >10,000pf/µl.

Table 3: Parasite Density of Children with Malaria Parasite attending Specialist Hospital Sokoto.

Parasite Density(pf/µl)	Frequency(N)	Percentage (%)
<10,000	32	71.1
>10,000	13	28.9
Total	45	100.0

Table 4 above shows the parasite density frequency of 71% for < 10,000pf/µl and 28.9% for > 10,000pf/µl.

Table 4 Shows the association of parasite density with haemoglobin types. HbAA has the highest parasite density, with 48.9% having <10,000pf/µl and 17.8% having >10,000pf/µl.

Table 4: Association between parasite density and haemoglobin types.

Parasite Density	Genotype					Total	p- value
	AA	AS	SS	AC	SC		
< 10,000	22	4	6	0	0	32	
>10,000	8	3	2	0	0	13	0.001
Total	30	7	8	0	0	45	

Table 4 above shows the association between parasite density and haemoglobin types, the p-value of HbAA,HbAS,HbSS,HbAC,HbSC are statistically significant (p< 0.05). HbAS has a lower parasite density of 8.8% for <10,000pf/µl and 6.6% for >10,000pf/µl. HbSS also shows low parasite density of 13.3% for <10,000pf/µl and 4.4% >10,000pf/µl.

Table 5 Presents socio-demographic factors which include: age,gender, place of residence of the children, and malaria parasite distibution among children. The distribution of the subjects based on their place of residence showed that 40% of the children who were positive of malaria parasite and 42%, negative of malaria parasite were from urban areas. While 60% with positive malaria parasite and 57% negative of malaria parasite, were from rural settlements. The p-value of both age and gender were not statistically significant, (p > 0.05).

Table 5: Socio-demographic parameters and frequency of malaria parasite among children attending Specialist Hospital Sokoto.

Variable	Malaria Parasite (%)		Total	p-value
	Positive n(%)	Negative n(%)		
Age group				
1 – 4	14 (31)	6 (17.1)	20	
5 – 8	20 (44.4)	19 (54.3)	39	**0.358**
9 – 12	11 (24.4)	10 (28.6)	21	
Gender				
Male	29 (64.4)	22 (62.9)	51	**0.534**
Female	16 (35.6)	13 (37.1)	29	
Place of Residence				
Urban	18 (40)	15 (42.9)	33	**0.488**
Rural	27 (60)	20 (57.1)	47	

Table 5 above shows socio-demographic characteristic of the control and subjects. The p values of age, gender and place of residence are not statistically significant (p>0.05).

Discussion

This study tends to assess the prevalence of malaria infections among children with different haemoglobin types, attending Specialist Hospital, Sokoto. A total of 80 children reporting to

Specialist Hospital Sokoto with fever and fever related illnesses were screened for malaria and later analysed by electrophoresis to determine their genotypes. The prevalence of malaria infection among children in this study still showed that malaria is still a burden in North- Western part of Nigeria, particularly Sokoto State. In this study, of the 80 samples screened, the prevalence of malaria was 56.3%. This finding was higher when compared to previous work which reported 50.6% prevalence in Yola (26, 27). The prevalence rate in this study was quite higher indicating high level of malaria infection among children. This could attribute to the fact that the study was conducted during the raining season from the month of August and October,as a result of blockage of water due to the poor drainage systems and inadequate waste disposal and sanitation in Sokoto State. This could have led to ecological changes that might have affected mosquitoe vectors to breed for possible malaria transmission. Many endemic areas transmission is seasonal, with peak during and just after the raining season. *P. falciparum* was the only *Plasmodium* specie identified in this study, which is the most virulent and also has the greatest propensity for developing resistance (28). Malaria cases in West Africa are almost exclusively due to *P. falciparum* (29). This finding is consistent with the findings of Oladeinde *et al.* (30). *P. falciparum* cause most of the severity and death attributed to malaria which is most present in Africa South of Sahara, where Nigeria has the largest population (31).

In relation to genotype, results obtained revealed that patients with genotype HbAA had higher malaria parasitte when compared with HbAS, HbSS, HbAC and HbSC respectively. This research further observed that of the 80 children screened for malaria, 8 (10%) and 2(2.5%) had HbAC and HbSC genotypes respectively with (0.0%) having parasitaemia, 36(45%) HbSS homozygous genotypes and were less infected with 8 (17.8%) having low parasitaemia and 7 (20.0%) uninfected. HbAS were 19 (23.75%) with 7(15.6%) positive parasitaemia and 12(34.3%) uninfected. HbAA were 36 (45%) with 30(66.7%) having the highest parasitaemia, 6 (17.1%) were unaffected. This corresponds with the results obtained Eridani (32) in his study which reported the impact of the coexistence of HbA and HbS, revealing that such patients have a relatively mild clinical malaria with near-normal haematological parameters. Moreover, 80% reduced binding to MVECs was reported in HbSS infected RBCs (iRBCs). An important mechanism of resistance offered by various haemoglobinopathies is the impairment of RBC cytoadherence: which is the binding of *P. falciparum*-infected RBCs to endothelial cells of small vessels, a fundamental event in both parasite survival and malaria pathogenesis in humans. Following early reports on the evidence for altered or decreased surface expression of the malarial protein [*P. falciparum* erythrocyte membrane protein 1 (PfEMP1)] on infected erythrocytes from individuals heterozygous for HbS Increased oxidation of HbS affects actin cytoskeleton formation, thereby reducing delivery of PfEMP1 to the erythrocyte surface (33).

Parasite density of this study showed that values <10,000pf/µl are higher with 32(71.1%) , while those >10,000 pf/µl has lower percentage of 13(28.9%). The result of parasite density with relation to haemoglobin types shows that children with HbAA has the highest parasite density, whereas HbAS has the least. There is strong association between parasite density and haemoglobin types ($p< 0.05$). This finding was in accordance with the work in Yobe (34) which reported that patients with genotype AA had high parasite density and HbAS with the least parasite density. Comparing the level of parasitaemia between children with different haemoglobin types, results

indicate HbAS are less infected, signifying high protection. This coincides with the findings which revealed that, invasion of merozoite into HbAS RBCs is impaired (35), parasite growth in HbAS RBCs is hampered due to the low oxygen tension environment that recapitulates that of post capillary venules and phagocytosis of HbAS infected *P. falciparum* red blood cells (PfRBC) are enhanced at the immature ring stage of parasite development, thus reducing parasite load.

Infection rate based on gender showed that males had a higher prevalence of malaria than the females which was not statistically significant (P>0.05). There were 51 male (63.75%) and 29 female (36.25%) who participated in this study. This present study confirms with previous reports which states that, that males were more infected than females (36, 37). But differs from Ezeigbo *et al.* (38) who reported higher prevalence rate in females than their male counterparts. This varied finding may be explained Abdullahi *et al.* (39) and Okonkwo *et al.* (40), who stated that, there is no scientific evidence to prove higher prevalence being related to gender as susceptibility to malaria infection is not influence by gender. This implies that malaria infection depends on the person's exposure to infectious bites of mosquito vectors. Reduction in the prevalence rate among 1 to 4 years might be that more attention and care are given to those children and were significantly more likely using malaria preventive measures put in place such as sleeping under ITNs (Insecticide Treated Nets) than the age group 5 to 8 years who had highest prevalence rate.
However, malaria infection decreases with an increasing age as shown among age group 9 to 12 years which could be due to previous exposure to malaria infection. In areas where endemicity of *P. falciparum* malaria is stable, severe malaria most commonly occurs in children up to 5 years of age, while is less common in older children and adults because of the acquisition of partial immunity (41). Observation of high rate of malaria infection was found among those children that live in the rural areas (60%) and a lower rate in urban areas (40%). However, there is no association between malaria parasitaemia and rural or urban areas (p> 0.05). The least prevalence rate in this study was consistent with previous reports from various part of Nigeria, Olasehinde *et al.* (42) and Cheesed *et al.* (43), who 52.2% and 17.1% respectively among children that live in urban areas. There was no clear cut demarcation between the communities with stagnant water and water logged drainage, where mosquito vectors breed in both urban and rural areas. This can enhance the proliferation of *Plasmodium* species which may probably be the cause of high infection rate. Therefore, it is essential to avoid stagnant pools and poor environmental conditions.

Conclusion

The prevalence rate of malaria infection in this study still showed malaria is still a burden amongst children in Nigeria, particularly in sokoto state. Haemoglobin genotype (AS, AC, SC and SS) have a great effect in showing resistivity to malaria infection. Also HbAA has the highest parasite density while HbAS has the least. Finally, There is no association between malaria parasitaemia and socio demographic factors. Parasite density should be estimated alongside the determination of malaria parasite inorder to identify the the level of the infection. Government should increase standard of living to reduce poverty, and improve the socio-demographic condition of the populace and facilitate economic oppurtunities for general public.

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