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Age Groups and Haemoglobin Genotypes Influence Malaria Occurrence among Patients in Sokoto Specialist Hospital, Sokoto State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author SYL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AK and MAY managed the analyses of the study. Author ASF managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A study on the occurrence of malaria among patients of different sexes, age groups and haemoglobin genotypes was conducted among 440 patients reported in Sokoto Specialist Hospital during the period of May to December, 2019. Blood samples were collected from each of the subjects with their consent, and then, thick and thin blood films were prepared and stained. Results showed that 304/440 (69.09%) patients were positive for malaria, with no significant difference between males 150/210 (71.43%) and females 154/230 (66.96%). But a positive correlation ($P<0.05$) in malaria infection was observed among different age groups; age group 0-5 years (76.08%) showed highest prevalence, while age group >45 years (51.85%) had the lowest prevalence. Subjects with haemoglobin genotype AA were found to be significantly more ($P<0.05$) susceptible to malaria (80.13%) compared to AS (51.16%) and SS (23.81%) genotypes. To conclude, the study revealed a high prevalence of malarial infection in the study area. The results of the study would help for public education and develop strategies to prevent and control malaria in the region.

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1. INTRODUCTION

Malaria is a serious public health problem in many parts of the world. Attacks by the disease can be severe and can quickly lead to death if untreated. There is an increasing interest in research on the association of genetic indices such as ABO blood group, haemoglobin genotype and diseases. Blood group and haemoglobin genotypes have been known to predispose individuals to or protect them from various diseases or infections [1].

According to the World Malaria Report [2], malaria transmission occurs in all six WHO regions. Globally, an estimated 3.3 billion people (which is half of the world's population) are at risk of being infected with malaria and developing the disease [3]. In 2016, an estimated 216 million cases of malaria occurred worldwide (95% CI: 196–263 million) compared with 237 million cases in 2010 (95% CI: 218–278 million). About 5 million more malaria cases were estimated to have occurred globally in 2016 as compared to the total cases reported in 2015 [4].

Malaria is also on the increase compared to 2014 estimates whereby 198 million cases of malaria occurred globally in 2013 (uncertainty range 124 – 283 million) and the disease led to about 584 000 deaths (uncertainty range 367 000 – 755 000). The burden of malaria is heaviest in the WHO African regions which Nigeria is among, where an estimated 90% of all malaria deaths occur, especially among children aged less than 5 years, who account for about 75% of all deaths [2,3]. About 216 million cases of malaria occurred in the year 2010 globally, whereby 81% of the cases occurred in the African region. WHO estimated that about 655,000 malaria deaths occurred in 2015 in the world, with a total of 91% occurring in the African Region, among whom 86% were children under the age of 5 years. Thirty countries in Sub-Saharan Africa account for about 90% of global malaria deaths. Nigeria, Democratic Republic of Congo (DRC), Ethiopia, and Uganda account for nearly 50% of the global malaria deaths [3].

Most of the malaria cases in 2016 were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). About 4% of estimated cases globally were caused by *P. vivax*, but outside the African continent this proportion was 36%. *P. vivax* is the predominant parasite in the Americas (64%) and above 30% in South-East Asia and 40% in the Eastern Mediterranean regions. Most cases of malaria caused by *P. vivax* occur in the WHO South-East Asia Region (58%), followed by the WHO Eastern Mediterranean Region (21%) and the WHO African Region (10%) [4].

Malaria is a major public health problem in Nigeria where it accounts for more cases of deaths than any other country in the world. About 97% of Nigeria's population is at risk of malaria (PMI, 2016). The remaining 3% of the population live in the malaria free highlands. Malaria contributes to an estimated 25% infant mortality and 20% less than five mortality by National Malaria Eradication Programme (NMEP) [5]. This study is aimed in studying the relationship between malaria parasitaemia and haemoglobin genotype among patients attending Sokoto Specialist Hospital with a view to find out the possible ways of preventing and controlling the disease.

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted at the Haematology Department of Specialist Hospital, Sokoto. The Hospital is located in the township centre of Sokoto South Local Government area, opposite Nagarta College Sokoto. Sokoto State is one of the seven Northwestern States of Nigeria. It is located 13^0 05'N 05^0 15'E (Fig. 1) and covered an extensive geographical area of about 25, 973 square kilometer with a total population of over four million people [6]. Sokoto State shares border with Zamfara State, Kebbi State, and Niger Republic. The state consists of 23 local government areas; with its head quarter in Sokoto and its people are predominantly Hausa – Fulani whose main occupation is farming and animal husbandry [7,8].

The vegetation of the State falls under Sudan Savannah which is characterized by abundant grasses with scattered trees. The grasses are usually short 1.5 – 2 m whereas the trees are short, few and scattered; not more than 15 m tall. The mean annual rainfall is about 1000 mm. Rainfall usually starts from May and ends in September [9]. The main town of Sokoto is surrounded by Rima River in the east which serves as breeding ground for mosquitoes.

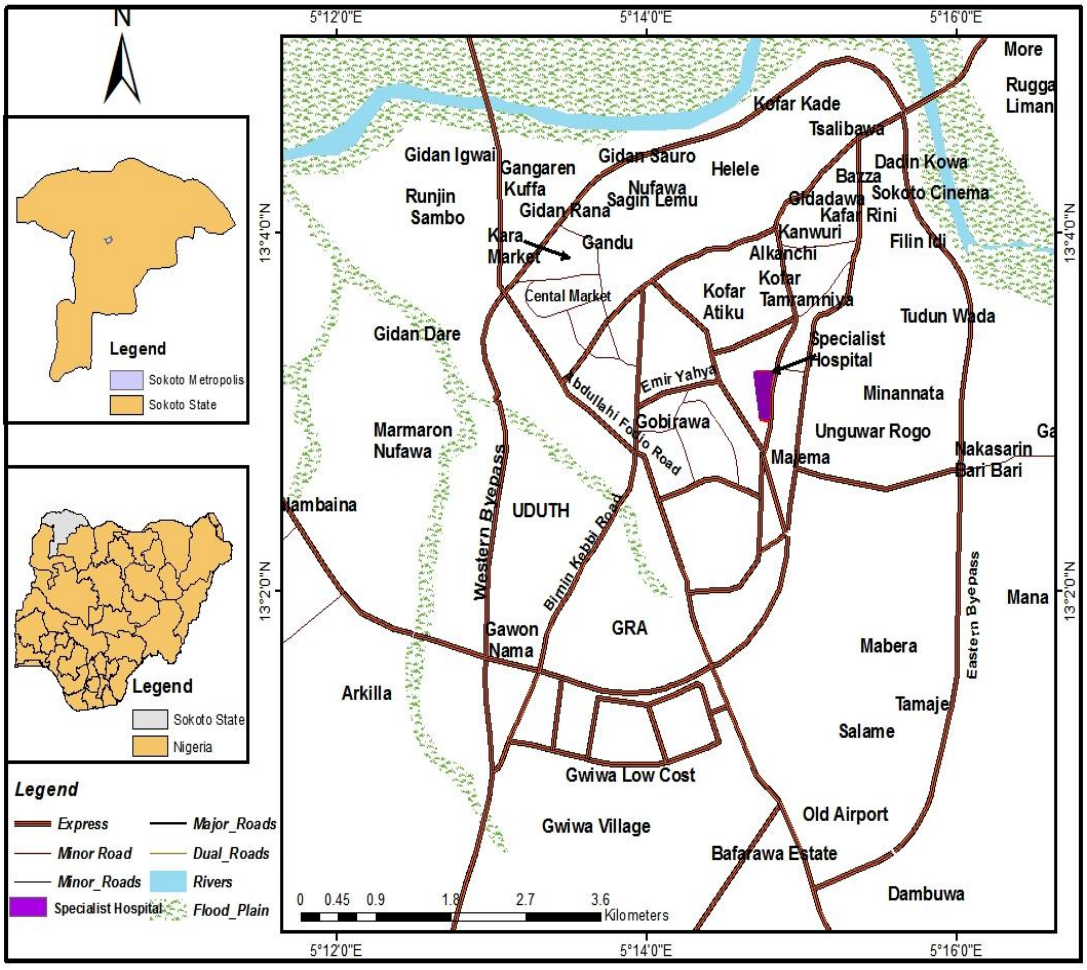

Fig. 1. Map of Sokoto and its environs showing the study area
Source: Arc GIS Generated by GIS Lab. Department of Geography UDUS, 2019

2.2 Study Population

A total of four hundred and forty patients of all ages presenting at the Hematology Department of Specialist Hospital, Sokoto during the period May to December, 2019 formed the subjects for this study. The patients with clinical signs of HIV/AIDS and other infectious diseases such as tuberculosis, hepatitis etc. were excluded from the study as their immune system would have already compromised.

2.3 Sample Collection

The blood samples were collected from each of the 440 subjects. Their age and sex were documented using a consent form before sample collection. All tubes and glass slides were labeled with patients corresponding identification number and date of collection. Each patient was assigned two tubes and glass slides; universal EDTA tube and thick and thin glass slides. Venous blood sample was then collected from each subject by standard venepuncture technique at the Department of haematology Specialist Hospital Sokoto. About 5 mL of venous blood was collected from each subject, 4 mL in a designated EDTA tube which was used for haematological analysis and the remaining 1 mL was used for preparing thick and thin films for microscopy [10].

2.4 Thick and Thin Blood Film Preparation

Blood samples collected were used to prepare thick and thin blood films according to [11] with the help of a laboratory staff of Haemotology Department of Specialist Hospital, Sokoto.

2.5 Staining Procedure and Interpretation of the Results

Thick and thin films were placed on a staining rack and flooded with 3% Giemsa stain, they were then allowed to stain for about 45 min before washing with buffered distilled water pH 7.2. The back of the slides was cleaned with cotton wool and then kept on the draining rack to air-dry for eventual microscopy. The films were microscopically examined using oil immersion (×100) objective. Species specific characteristics of human *Plasmodium* as listed by [10,11] were utilized in identifying the species of *Plasmodium* encountered.

A thick film was recorded negative if 100 microscopic fields showed no parasites. Confirmation of positive samples was done by an experienced professional at the Microbiology laboratory to ensure good, accurate result and for quality control. A plus (+) sign scheme was used in reporting the degree of parasitaemia: + for low parasitaemia of 1 – 10 parasites per 100 thick film field, ++ for moderate parasitaemia of 11 – 20 parasites per 100 thick film field and +++ for severe parasitaemia 20 – 100 parasites per thick film field [8,11].

2.6 Malaria Parasite Species Identification

Morphological features of the infecting species of *Plasmodium* in the peripheral blood smears were conducted based on guidelines for *Plasmodium* species identification Centre for Disease Control, 2015 [2,3].

2.7 Determination of Parasite Density

Thick films were used for counting malaria parasite density by white blood count (WBC) method [12,13]. The numbers of sexual forms (trophozoites) were counted against 200 WBC. The malaria parasite density was calculated using white blood cell (WBC) from full blood count result obtained by automation, using the following formula:

$$\text{Parasitaemia}/\mu l = \frac{\text{Number of asexual parasite} \times 8000}{\text{Number of WBC}}$$

2.8 Haemoglobin Genotyping

Complete blood count was performed using an automated Mythic 22 machine based on the manufacturer's instructions**.** To run the blood samples, the power supply of the machine was first switched on by pressing the ON/OFF button and then the machine was allowed to adjust to room temperature. When start-up was pressed, the system was rinsed automatically with the cleaning solution before analysis, the cleaning process lasted for about 6 min after which the cycle button turned green and the initialization menu was displayed on the touch screen of the machine. Three fields were used for identification; patient name, patient identification and sample identification. The fields were filled accordingly for all the 440 samples.

Then, the available needle for taking up blood into the machine emerged as soon as the working temperature was reached, EDTA tube containing the blood sample was then presented under the needle and the run-sample button was pressed. The needle immediately got retracted, and the results were sent for checking by the machine at the same time as the printer started. After about 2 min, the results were shown on the screen and printed by the printer.

2.9 Statistical Analysis

Data generated were analyzed using the Statistical Package for Social Sciences (SPSS). The results were presented in tables showing frequency distribution. Correlation analysis was made between gender, age and haemoglobin genotype of the subjects. Differences in the frequency distribution were statistically analyzed using Chi-square test. Differences were shown to be significant at ($P<0.05$).

3. RESULTS

3.1 Prevalence of Malaria According to Gender

Out of 440 patients, 304/ were positive for malaria (69.09%) with a total parasite density of 190320/µl. Among the examined patients, 210 were males and 230 females; and out of the 210 males examined 150 (71.43%) were positive for malaria with a mean density of 560.00/µl. Among the 230 females, 154 were positive (66.96%) for malaria infection with a mean density of 690.34/µl (Table 1).

3.2 Prevalence of Malaria According to Age Group

Among the different age groups, 0-5 years presented the highest prevalence (192/250; 76.08%) with a mean parasite density of

567.92/µL.In age group of 6-17 years, 54/82 patients (65.85%) had malaria infection with a mean parasite density of 675.56/µL; and the age group of 18-45 years showed 30/54 (55.56%) patients with malaria infection with mean parasite density of 896.00/µL. While 28/54 (51.85%) patients in the age group > 45 years had malaria infection with a mean parasite density of 640.00/µL (Table 2).

3.3 Prevalence of Malaria According to Genotype

Prevalence of malaria with respect to different genotypes of the subjects indicated that 250/312 subjects with genotype AA (80.13%) had malaria infection with a mean parasite density of 643.12/µL, while 44/86 patients with genotype AS (51.16%) had malaria infection with a mean density of 531.36/µL, and 10/42 patients with genotype SS (23.81%) had malaria with mean parasite density of 616.00/µL (Table 3).

4. DISCUSSION

The results of this study indicated malaria prevalence rate of 69.09% among the patients attending Specialist Hospital, Sokoto during the period of May to December, 2019. This suggested that there was active transmission of malaria in the study area. The high prevalence of the disease observed might be attributed to a number of factors. In Nigeria, high breeding rate of the malaria vector and high transmission rate occur throughout the year especially during the rainy season [14, 15, & 16]. It might be due to several factors such stagnant water in the drainages, relative humidity, temperature, extent of urbanization, availability of breeding grounds for malaria vectors, overcrowded human population and behavioral attitude of inhabitants in the area. Likewise, other studied have also reported high prevalence of malarial infection associated with favorable environmental conditions (both natural and man-made) which promote the breeding of mosquito vectors and transmission of the parasite [17]. Other factors such as ignorance, poverty, unsanitary environmental conditions, poor behavioral attitudes and inadequately planned socio-economic projects might have also contributed to increased disease transmission [18].

Oparaocha [19] reported 88.8% prevalence of malaria among the communities in Ikwuano Local Government area of Abia State. Aribodor et al. [17] recorded a prevalence of 76% in their investigation among people of Azia community of Anambra State. Aribodor et al. [20] reported malaria prevalence of 66.0% in their study among the people of Ndiowu community of Anambra State, Nigeria;

Table 1. Prevalence of malaria according to gender

Gender	No. of samples examined	No. of positive samples	Prevalence of malaria (%)	Parasite density/µl	Mean density/µl
Male	210	150	71.43	84000	560.00
Female	230	154	69.96	106320	690.39
Total	440	304	70.70	190320	625.20

x^2= 3.84, df=1, P= 1.0000

Table 2. Prevalence of malaria according to age group

Age	No. of samples examined	No. of positive samples	Prevalence of malaria (%)	Mean density/µL
0-5	250	192	76.08	567.92
6-17	82	54	65.85	675.56
18-45	54	30	55.56	896.00
>45	54	28	51.85	640.00
Total	440	304	62.34	694.87

x^2= 7.8147, df= 3, P= 0.000

Table 3. Prevalence of malaria according to genotype

Genotype	No. of samples examined	No. of positive samples	Prevalence of malaria (%)	Mean density/µL
AA	312	250	80.13	643.12
AS	86	44	51.16	531.36
SS	42	10	23.81	616.00
Total	440	304	51.70	596.83

x^2= 5.9915, df= 2, P= 0.0000

while Ejezie [18] recorded 85% prevalence rate and Kalu et al. [19] have reported malaria prevalence of 71.43% in their studies. The malaria prevalence recorded in this study was relatively higher than that of *Fatima* [22] who reported malaria infection prevalence of 54.5% among the children below five years of age attending General Hospital Kankia, Katsina State. Likewise, Nwaorgu and Orajaka [23] have reported relatively less prevalence of malaria (58.2%) among communities in Awka North Local Government Area, Anambra State, Southeast Nigeria.

The results of this study showed that males (71.43%) were more frequently infected than females (69.96%) with no significant difference between male and female subjects (P>0.05). This observation is in agreement with the findings of other researchers [24,23], who reported that gender does not affect the prevalence of malaria. Our findings further indicated that antibody response or immunity to malaria is not in any way affected by gender, suggesting that both males and females are equally exposed and susceptible to malarial infection. However, slightly higher prevalence of malaria infection observed in males against females in this study may be attributed to the fact that males were more exposed to malaria than females because males keep late nights outdoors more often than the females at the time when mosquitoes were more active. However, the female subjects had higher parasite density compared to the male subjects. The difference in mean density could possibly arise from the genetic makeup of individuals and qualitative and/or quantitative variation in structure and chemical composition of the receptor sites on the erythrocytic membrane of the various subjects. Pregnancy in the females might also play an important role in determining malaria susceptibility [25,26].

This study indicated that malaria parasitaemia was present in all the age groups. However, prevalence rate differed from one age group to another. The rate of malaria infection decreased with an increase in age of the subjects. This finding was in agreement with the recent World Malaria Report [2], which reported that malaria was a serious disease of childhood and Yahaya et al. [27] have reported higher incidence of malaria infection in 0-5 years of age. Children in the 0-5 years of age were most vulnerable to malaria. There was an estimated 584000 malaria deaths around the world in 2013, out of which approximately 78% were in children under five years of age [2]. While the low parasite load (567.92/µl mean density) observed in 0-5 years age group might be due to the constant maternally derived antibodies that they have in their serum from the time of birth [28,29]. The markedly increased parasite density in the 6-17 years and 18-45 years age group subjects could be attributed to the gradual loss of these maternal antibodies and the development of acquired immunity. Furthermore, increased parasitic density in adolescents and adults might be because they generally spend longer hours outside their homes both at the day and night, exposing them to mosquito bites. Considering the nature of their age, younger and elderly people are given more attention in terms of protection against the mosquito bites by staying indoors and sleeping in mosquito fortified zones such as the mosquito nets or insecticides treated room and therefore come down with low malaria parasitaemia compared to the adolescents and adults [30,31 & 32].

This study further revealed that malaria was present in all the genotypes, but the prevalence differed among the different haemoglobin genotypes. Genotype Hb^{AA} (82.24%) had the highest prevalence, followed by genotype Hb^{AS} (14.47%), while genotype Hb^{SS} (3.29%) had the lowest prevalence. This indicates that malaria infection can occur in all the three different haemoglobin genotypes. Similarly malaria parasite density varied greatly among the genotypes; genotype Hb^{AA} had the highest

parasite density, followed by genotype Hb^{AS}, while genotype Hb^{SS} had the lowest density. This was in agreement with Opara et al. [33], who reported that the degree of malaria infection was less among subjects with Hb^{AS} and Hb^{SS} than those with Hb^{AA}. Haemoglobin AA was more receptive to malaria parasite than haemoglobin AS and haemoglobin SS, probably because of the protective effect of haemoglobin S in Hb^{AS} and Hb^{SS} cell sickle in the circulation, which are removed by the spleen before the parasite can develop into schizonts. Similarly, the low prevalence observed in genotype Hb^{AS} and Hb^{SS} could be attributed to the presence of sickle haemoglobin in the red blood cells which limits the development and multiplication of the malaria parasite [33]. Eariler reports [10,34] also indicated that haemoglobin AA had the highest prevalence of the disease.

5. CONCLUSION AND RECOMMENDATIONS

Malaria prevalence rate of 66.09% is an indication of high rate of malarial infection, posing a great risk especially among the vulnerable groups. The study has also found that there was no discrimination between males and females in terms of susceptibility to the disease. Under-aged subjects were more vulnerable and at high risk of getting infection than the adults who have a reasonable immunity. Findings of this study have also shown that individuals with certain haemoglobin genotypes were predisposed to *Plasmodium falciparum* malaria infection than others. Thus the fight should be geared towards assisting the more vulnerable groups through educating them and developing strategies to prevent and control malaria.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Informed consent of the management of the Sokoto Specialist Hospital and the individual subjects was sought and obtained before the commencement of the study.

ETHICAL APPROVAL

As per university standard guideline participant ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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