# Assessment of Malaria Prevalence and Haemoglobin Genotypes among Patients Attending Selected Hospitals in the Three Senatorial Districts of Kaduna State, Nigeria

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**Abstract:** Malaria is a significant public health problem, especially in developing countries including Nigeria. It has caused the morbidity and death of millions of people; especially pregnant women and children under the age of five years in sub-Saharan Africa. The aim of this study was to assess the prevalence of malaria and haemoglobin genotypes among patients attending selected hospitals in the three senatorial districts of Kaduna State, Nigeria. Blood samples were collected from 300 consenting participants and screened for malaria parasites using microscopy. Relevant information was obtained by administration of structured questionnaire. Associations were determined using Chi-square, and $P \leq 0.05$ was considered significant. The prevalence of malaria was higher in General Hospital Kafanchan (30%) which is located in Kaduna South Senatorial District, compared to the Hospitals in Kaduna North and Kaduna Central Senatorial Districts ($P=0.062$). The age group ≤10 (31.3%) had the highest malaria prevalence, the least prevalence was found in the age group ≥41 (9.1%). The difference was statistically significant ($P=0.029$). The educational status and occupation of participants were not significantly associated with malaria ($P<0.05$). The high prevalence of malaria in the age group ≤10 may be associated with lower immunity to malaria. Malaria interventions should therefore pay special attention to this group. The percentage of *Plasmodium falciparum* malaria was higher among persons with HbAA than those with HbAS, HbAC and HbS.

**Keywords**: Blood, Haemoglobin, Kaduna, Malaria, Prevalence,

## INTRODUCTION

Malaria is a life threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. It is a preventable and curable disease (WHO, 2018a). Malaria remains one of the major health concerns where approximately half of the world's population is at risk (Norahmad *et al*., 2016). In particular, young children, pregnant women, and non-immune visitors to malaria endemic areas are at greatest risk of severe or fatal illness (Bloland, 2001). Nigeria is currently malaria endemic country with its entire population at risk of contracting malaria, and a whopping 76% of this population at high risk (WHO, 2015).

In 2017, it was reported that nearly half of the world's population was at risk of malaria (WHO, 2018a). Countries with higher proportions of their population living in poverty (less than US$ 1.25 per person per day) have higher mortality rates from malaria (Ananya, 2013). Nigeria accounted for 93, 446 and 92, 699 deaths of children less than five years old due to malaria in 2015 and 2016 respectively (UNICEF, 2018). Nigeria is currently a malaria endemic country with its entire population at risk of contracting malaria, and a whopping 76% of this population at high risk (WHO, 2015). Malaria and the costs of treatment trap families in a cycle of illness, suffering and poverty. Since year 2000, malaria has cost sub-Saharan Africa US$ 300 million each year for case management alone and it is estimated to cost up to 1.3 per cent of GDP in Africa (UNICEF, 2018). People living in malaria endemic countries are subject to frequent bites of infected female mosquitoes; this can lead to accumulation of different parasitic genotypes within infected individuals (Nguetse *et al*., 2017).

Haemoglobin S (HbS) has become a stable polymorphism within malaria endemic regions, associated with a limited life expectancy among homozygous individuals who suffer from sickle cell disease, and an extended life expectancy of heterozygous individuals who are more likely to evade malaria. HbAS is widely known to confer significant protection from severe and uncomplicated malaria (Verra *et al.,* 2007) although underlying mechanisms are not precisely defined. The objective of this study was to assess the prevalence of malaria and haemoglobin genotypes among patients attending selected hospitals in the three senatorial districts of Kaduna State, Nigeria.

## MATERIALS AND METHODS

### Study Area

The study was conducted in selected general hospitals within the three Senatorial Districts of Kaduna State, Nigeria. Blood samples were collected from patients in Hajiya Gambo Sawaba General Hospital Zaria (Kaduna North Senatorial District), Barau Diko Teaching Hospital (Kaduna Central Senatorial District) and General Hospital Kafanchan (Kaduna South Senatorial district). Kaduna State lies at latitude 10°20′ north and longitude 7°45′ east and covers an area of 45,711.2 km$^2$. According to the National Population Commission, the 2006 census puts the population of Kaduna State at 6,113,503 (Demographics, 2021). It accounts for 4.3% of Nigeria's total population. Kaduna lies in the savanna ecological belt. It experiences a rainy (wet) season between April and October and a harmattan (dry and dusty) season between November and March. The area experiences an average annual rainfall of 1099 mm and average daily temperature of 28°C (Aliyu *et al.,* 2017).

### Study Design

The study was a cross-sectional study that lasted for six months (May to October 2018)

### Ethical Clearance

Ethical clearance was obtained from the ethical committee of Kaduna State Ministry of Health as well as Barau Diko Teaching Hospital.

### Inclusion Criteria and Exclusion Criteria

All febrile patients presenting symptoms of malaria that were directed to the laboratory for malaria parasite (MP) test and gave consent were included. All patients directed to the laboratory for laboratory tests other than malaria test and those who did not give consent were excluded.

### Sample Size

The sample size was determined using a prevalence of 22.4% (Aliyu *et al.,* 2017) and the following formula as described by Naing *et al.* (2006):

$$n=\frac{Z^2p(1-p)}{d^2}$$

n= number of samples

p=prevalence of previous study =22.4%=0.224

z=standard normal distribution at 95% confidence limit=1.96

d=absolute desired precision of 5%=0.05

z=1.96

$$n=\frac{1.96^2 * 0.224(1-0.224)}{0.05^2}$$

$$n=\frac{3.8416 * 0.224 * 0.776}{0.0025}$$

n= 267 samples

For proper distribution, a total of 300 blood samples (100 blood samples from each hospital within the senatorial districts) were collected for this study.

### Administration of Consent forms and Structured Questionnaire

Consent forms and structured questionnaire were administered to consenting individuals who met the inclusion criteria. This was used to obtain bio-data and other information relevant to this research.

### Sample Collection and Preparation of Blood Films

Venipuncture technique was employed for blood sample collection by a trained laboratory technician.

Thick and thin blood films were prepared immediately after the samples were collected, according to the procedure outlined by Cheesbrough (2009). A drop of each blood sample was placed in the center of a grease-free clean glass slide, and spread immediately using a smooth edged slide spreader to make a thin film. The thin films were allowed to air dry before being fixed with methanol. The thick films were made by transferring a drop of blood to another clean slide and spread in such a way that it was possible to see (but not read through) newsprint, it was then allowed to dry properly. The blood films were lysed with water and stained using 10% Giemsa working solution for 30 minutes. After staining the blood films, they were allowed to air-dry (Cheesbrough, 2009).

**Examination of stained blood film slides**

The stained blood films were examined under the microscope using immersion oil and 100X objective lens after focusing. Presence of ring forms, trophozoites or gametocytes of *Plasmodium falciparum* was recorded. A blood smear was considered negative if no parasite was seen after 10 minutes of search or examination under 100X high power fields of microscope.

**Determination of haemoglobin genotype**

Cellulose acetate method of haemoglobin electrophoresis was carried out on all malaria positive samples as follows: A drop of blood from malaria positive blood sample was placed on a clean white tile and mixed with three drops of water to lyse the red blood cells. With the aid of an applicator, the haemolysate was placed on a cellulose acetate paper. This was followed by electrophoresis in Tris buffer solution for 15 minutes at electromotive force of 250v. Haemolysates from blood samples of Hb AS and AC were run as controls (Egesie *et al.,* 2008).

**Statistical Analysis**

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 21. Chi-square was used to determine associations. $P$ value $\leq 0.05$ was considered as significant.

## RESULTS

The prevalence of malaria obtained in each of the hospitals is shown in Figure 1. From all the 300 samples screened, prevalence of 25%, 30%, and 16% were obtained in the hospitals located within Kaduna North, Kaduna South and Kaduna Central respectively (p=0.062). Prevalence of malaria based on age group of participants in the selected hospitals is shown in Table 1.The highest malaria prevalence (33.3%) was recorded in the age group ≤10, followed by the age groups 31-40 (31.3%), 11-20 (24.7%), and 21- 30(17.2%). The age group ≥41 had the lowest prevalence (9.1%). The difference was statistically significant (p=0.029). Table 3 showed that the highest prevalence (30.6%) according to educational status was found among those with no education, this was followed by the prevalence of 28.6% among those who only passed through adult literacy, primary school education (27.0%), secondary school education (26.6%) and tertiary education (17.2%). The lowest prevalence was found among those who had non-formal education (9.1%), however, the association was not statistically significant (p=0.378). Table 3 showed the distribution of malaria based on occupation of participants. Civil servants had the least prevalence (17.1%). Traders had a prevalence of 24.5% followed by those who were unemployed (24.3%), farmers (20.0%) and artisans with 18.2%. The highest prevalence (36.4%) was recorded among those involved in other forms of occupations. Participants with haemoglobin genotype AA had the highest prevalence of malaria (73%), followed by those with haemoglobin genotypes AS (23%), AC (3%) and SS (1%) (Figure 2).

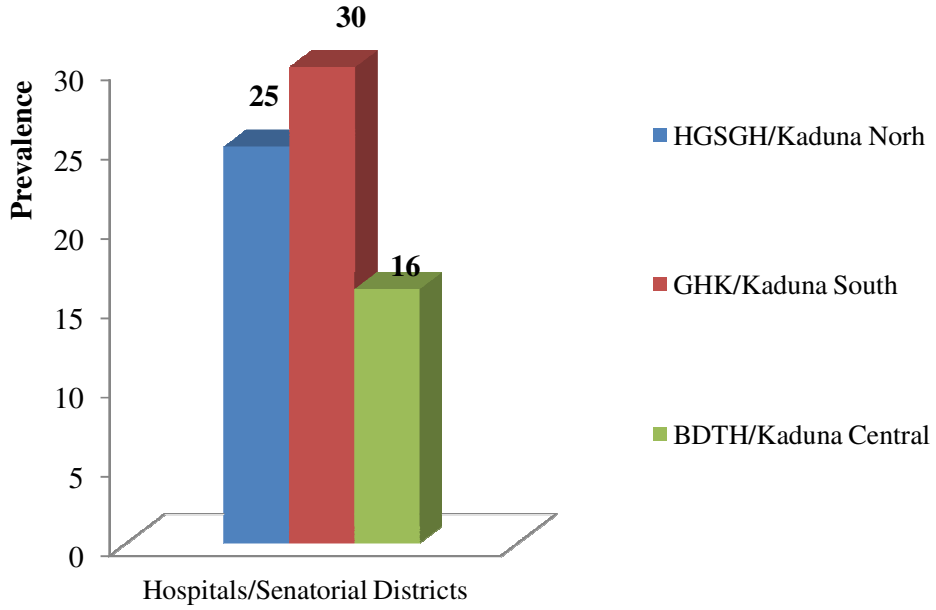


**Figure 1. Prevalence of malaria in the selected hospitals**
P=0.062, Chi square=5.572, df=2
Key: HGSGH= Hajiya Gambo Sawaba General Hospital, GHK= General Hospital Kafanchan, BDTH= Barau Diko Teaching Hospital

**Table 1: Prevalence of malaria based on age of participants**

| Age group (years) | No. Ex | Microscopy | | $\chi^2$ | P value |
|---|---|---|---|---|---|
| | | No. + | % Prevalence | | |
| ≤10 | 75 | 25 | 33.3 | 10.807 | 0.029* |
| 11-20 | 73 | 18 | 24.7 | | |
| 21-30 | 87 | 15 | 17.2 | | |
| 31-40 | 32 | 10 | 31.3 | | |
| ≥41 | 33 | 03 | 9.1 | | |

Key: No.=Number, Ex=examined, +=positive, *=significant

**Table 2. Prevalence of malaria according to educational status of participants**

| Educational status | No. Ex | Microscopy | | $\chi^2$ | P value |
|---|---|---|---|---|---|
| | | No. + | % Prevalence | | |
| Primary | 74 | 20 | 27.0 | 5.317 | 0.378 |
| Secondary | 79 | 21 | 26.6 | | |
| Tertiary | 93 | 16 | 17.2 | | |
| Adult literacy | 7 | 2 | 28.6 | | |
| Nil | 36 | 11 | 30.6 | | |
| Non-formal | 11 | 1 | 9.1 | | |

Key: No.=Number, Ex=examined, +=positive

**Table 3. Distribution of malaria based on occupation of participants**

| | | Microscopy | | | |
|---|---|---|---|---|---|
| **Occupation** | **No. Ex.** | **No. +** | **% Prevalence** | **$\chi^2$** | **P value** |
| Civil servant | 35 | 6 | 17.1 | 2.092 | 0.836 |
| Trader | 49 | 12 | 24.5 | | |
| Artisan | 11 | 2 | 18.2 | | |
| Farmer | 5 | 1 | 20.0 | | |
| Unemployed | 189 | 46 | 24.3 | | |
| Others | 11 | 4 | 36.4 | | |

Key: No.=Number, Ex=examined, +=positive

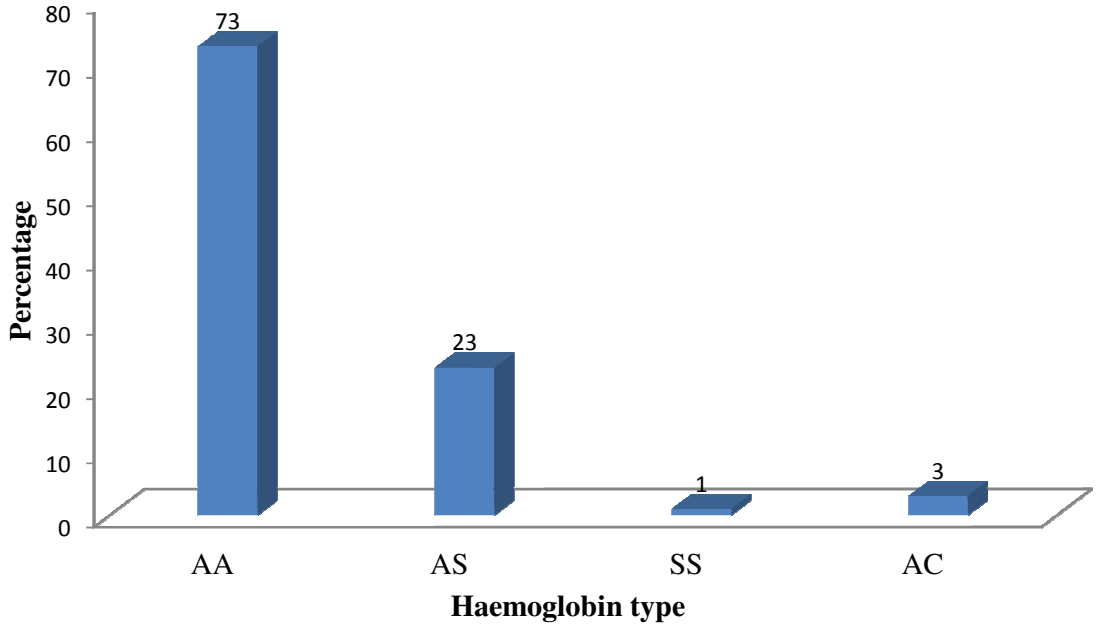


**Figure 2: Haemoglobin electrophoresis pattern of malaria positive participants in the study area**

## DISCUSSION

This study found higher prevalence of malaria among study participants in General Hospital Kafanchan compared to the other hospitals, this might be tied to higher level of rainfall in that area of study compared to others (Yunusa *et al.*, 2017). This is in agreement with the findings of Bajoga *et al.* (2019) which reported higher malaria prevalence in Local Government Areas found in Kaduna South. High rainfall has been associated with increase in the number of breeding sites of *Anopheles* mosquito, which is the major vector in malaria transmission. In many places malaria transmission is seasonal, with the peak during and just after the rainy season (WHO, 2018a). According to Confalonieri *et al.* (2007) periods of unusually high rainfall, altered humidity or warmer temperatures could result in modified distribution and duration of malaria, as well as increased transmission; even in areas where control is strong.

This study showed higher malaria prevalence in children less than 10 years old. This is in agreement with the findings of Geleta and Ketema (2006) who reported that the incidence of *Plasmodium falciparum* positive cases was higher among the children age groups (mostly <10 years).

This also agrees with the findings of Valerian *et al.* (2013) who reported a high prevalence of malaria parasitaemia and anaemia among in-patient children less than five years old. The high prevalence in the age group ≤10 years might be because majority of the children in the group have not yet developed immunity to malaria and are thus susceptible (Metanat , 2015; WHO, 2018b, CDC, 2019).

The results of this study revealed that 73% of the malaria positive participants had the haemoglobin genotype AA (HbAA), which was the highest among the other genotypes [HbAS (23%)] and HbAC (3%)]. This is similar to the report of Albiti and Nsiah (2014).The prevalence of participants with HbAS and HbAC were quite low compared to HbAA. This suggests that individuals with HbAS and HbAC are probably able to resist malaria better than HbAA. This is in agreement with the findings of Archer *et al*. (2018). According to Williams *et al*. (2005), HbAS was 50% protective against mild clinical malaria, 75% protective against admission to the hospital for malaria, and almost 90% protective against severe or complicated malaria. The level of susceptibility to malaria has been reported to be higher in individuals with HbAA when compared with those with HbAS and HbAC, thus, the high frequency of HbAC and HbAS in malaria endemic areas has been attributed to a decrease in malaria morbidity and mortality in malaria endemic areas (Archer *et al*., 2018). The protective role displayed by HbAC and HbAS in malaria infection is as a result of reduced cytoadhesion of infected red blood cell to microvasculature and impaired rosette formation as a result of the presence of abnormal PfEMP1 antigen on HbAC and HbCC (Fairhurst *et al.,* 2012).

Earlier studies had associated low oxygen tension in HbAS RBCs with impairment in the invasion and growth of *Plasmodium falciparum* parasites in the HbAS red blood cells (RBCs) which causes infected RBCs to sickle under low oxygen tension and lead to their premature destruction in the spleen, thus, reducing parasitaemia and providing protection. In a more recent study by Archer *et al*. (2018), it was reported that resistance to *Plasmodium falciparum* in sickle cell erythrocytes is driven by oxygen dependent-growth inhibition. It was shown that low oxygen (1% oxygen concentration) indeed stalled the growth of *Plasmodium falciparum* and no DNA replication was evident at that oxygen concentration. Rosette formation (binding of *Plasmodium falciparum* infected RBCs) which is thought to lead to microcirculatory obstruction in cerebral malaria was found to be impaired in *P. falciparum* infected HbAS RBCs under deoxygenated conditions; this may be due to increased sickling of the RBCs in deoxygenated condition or reduced expression of erythrocyte surface adherence protein (Cholera *et al.,* 2008). Decreased rosette formation and the resulting decreased circulatory obstruction might contribute to protection against severe malaria in HbAS. The protective effect of HbC may result from a reduced ability of *Plasmodium falciparum* to grow and multiply in RBCs containing HbC (Fairhurst *et al.,* 2005). HbC exerts its protection through a specific effect on cytoadherence, mediated by the altered display of surface expressed parasite proteins (Brittain *et al.,* 2007). It has been reported that subjects with HbSS appear to be less susceptible to developing malaria but are highly susceptible to the catastrophic consequences of malaria particularly severe anaemia if they do become infected with the parasite (McAuley *et al*., 2010).

## CONCLUSION

The high prevalence of malaria in the age group ≤10 may be associated with lower immunity to malaria in this age group. Malaria interventions should therefore pay special attention to this group. The high frequency of HbAA genotype in malaria positive participants implies that people having the aforementioned haemoglobin type are less protected from malaria than those having HbAS, HbAC and HbSS.

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